



**East Pye Solar
Environmental Statement
Volume 3: Appendix 8.3 - Great Crested Newt Survey
Report**

Revision 2

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Schedule of Changes

Reference	Pages	Description of Changes	Reason for Revision
East Pye Solar Environmental Statement, Volume 3: Appendix 8.3 - Great Crested Newt Survey Report (Revision 2)	1	Updates to survey results summary	Required updates following from 2026 survey.
	2	Updates to paragraph 1.1.3	
	5	Updates to paragraph 2.1.2.	
	7 & 8	Updates to paragraphs 2.4.3, 2.6.2, 2.6.4, and 2.6.5	
	14 - 27	Updates to Table 3.6: Summary of HSI and eDNA Survey Results Within a 250m buffer of the Site Boundaries	
	28	Updates to paragraph 4.1.3.	
	29	Additional paragraphs 4.1.7, 4.1.8, 4.1.9 added and Table 3.7: Survey Results Summary added.	
	30	Updates to paragraphs 4.2.1, 4.2.2, and 4.2.3.	
	33	Updates to paragraph 4.5.2.	
	39 & 40	Additional pond information added to Appendix A.	
	42-46	Updated HIS information added to Appendix B.	
	47	Additional laboratory results added to Appendix C.	

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Non-technical Summary

Stantec was commissioned by East Pye Solar Limited to undertake an environmental DNA (eDNA) survey for great crested newt *Triturus cristatus* (GCN) to inform the design evolution of the Development Consent Order (DCO) process for East Pye Solar (also referred to as the 'Scheme').

In addition to the 249 ponds previously mapped and included for consideration within the previous GCN survey effort, and additional 16 ponds have been included within this report. These relate to 8 newly created drainage areas associated with the Long Stratton Bypass development, 7 additional pond locations provided by Landowners, and a (dry) basin recorded by surveyors during the 2026 surveys. Further information is provided within **Table 3.6**.

Out of the 265 ponds identified within the Order Limits or within 250m of the Order Limits boundaries. This updated Version 2 document incorporates the results of surveys undertaken in May and June 2026, including 48 ponds surveyed or assessed for the first time and 37 previously inconclusive or dry ponds revisited. The following results were received/recorded:

- 38 returned positive eDNA results (including five within the Site);
- 65 returned negative eDNA results (including fifteen within the Site);
- 11 returned inconclusive eDNA results (including six within the Site);
- 52 ponds were not surveyed as they were dry (including eight within the Site);
- 6 ponds are no longer present (including one within the Site);
- 78 ponds were considered positive for GCN on a precautionary basis;
- 7 were confirmed positive for GCN via desk study; and
- 8 were confirmed negative for GCN via desk study.

The Scheme layout primarily impacts suboptimal arable farmland habitats for GCN and avoids impacts upon unsuitable aquatic habitats, additionally, existing suitable terrestrial habitats such as woodland, hedgerows, lowland fen, scrub are largely avoided and incorporated within the Scheme design. There may be potential minor direct impacts upon terrestrial habitats suitable for GCN during the construction (and potentially decommissioning phases) such as the removal of short hedgerow sections to accommodate internal haul routes/access tracks.

The construction phase of the Scheme will include the provision of extensive areas of suitable terrestrial grassland/woodland/scrub habitats suitable for GCN, and the enhancement of existing pond habitats within the Site, thereby providing much increased opportunities for the species (and amphibians in general), ensuring that the favourable conservation status of GCN within the Site is maintained.

The GCN population within the Order Limits is considered to be of value at **Local** level importance.

Scheme parameters have been designed to avoid permanent impacts to suitable terrestrial habitats within 50m of positive GCN ponds and works will be undertaken in-line with an agreed mitigation strategy.

Without suitable mitigation/avoidance measures being adopted, there would be a need a derogation from the Conservation of Habitats and Species Regulations 2017 (as amended), whereby an appropriate licence would be required from Natural England for works to proceed. If required, consultation will be conducted with Natural England to determine the most appropriate licensing route specific to the Scheme.

1 Introduction

1.1 Background

Introduction

- 1.1.1 Stantec was commissioned by East Pye Solar Ltd to undertake GCN surveys in relation to an application to be made to the Secretary of State under Section 37 of the Planning Act 2008 (as amended), seeking a DCO for the Scheme on land located south of Norwich and north of Harleston (the Order Limits), see further details below, see further details below. The GCN “Survey Area” additionally includes a 250m buffer around the Order Limits boundaries¹.
- 1.1.2 This report provides details of surveys undertaken to inform proposals and includes a desk study, Habitat Suitability Index (HSI) assessment and eDNA survey to determine the presence/likely absence surveys and includes an evaluation of the importance of the Site for GCN.
- 1.1.3 Surveys commenced in 2024 with 19 waterbodies surveyed and continued in 2025 during which time a further 105 waterbodies were surveyed. This updated document includes the results of additional surveys undertaken in May and June 2026, with an additional 48 ponds surveyed directly or assessed for the first time, plus revisiting a further 37 which were assessed in 2025 and 2026 and originally found to be inconclusive/dry.

Scope of report

- 1.1.4 The objectives of the report are to:
- Present the methodology used and identify any constraints encountered during the GCN surveys;
 - Present the results of the ecological desk study;
 - Present the results of the HSI assessment for all accessible ponds and other potentially suitable waterbodies;
 - Present the results of the eDNA surveys;
 - Provide a summary of the potential for the Scheme to support opportunities for GCN; and
 - Based on the results of the desk study and surveys, assess the geographical importance of the Order Limits for GCN.
- 1.1.5 This report provides an assessment of the current status of GCN at accessible ponds within 250m of the Order Limits, and a subsequent assessment of the presence/likely absence of GCNs within the Order Limits. This will be used to

¹ Due to Order Limits amendments some surveyed ponds are now located outside of this 250m survey buffer zone, however, for the purposes of transparency, the survey results of these ponds have been retained within this report and associated figures.

identify any potential ecological constraints associated with the Scheme that may risk contravention of legislation or policy.

1.1.6 The assessment is based on the following sources of information:

- A desk-based assessment of the surrounding habitats to identify potential aquatic and terrestrial habitat within and up to a 250m radius of the Order Limits, records of GCN pond surveys, review of mitigation licences for amphibians, a study of GCN class licence returns, and local biodiversity records centre species records to 2km from the Order Limits boundary;
- A review of whether the Order Limits falls into a District Level Licence DLL scheme area and if so, what colour zones it is within;
- A HSI assessment of all accessible ponds within 250m of the Order Limits boundary (ARG UK, 2010);
- An environmental DNA (eDNA) assessment (Biggs *et al.*, 2014) of accessible ponds that are found to hold water at the time of the survey.

1.1.7 This assessment has been prepared with reference to best practice guidance published by the Chartered Institute for Ecology and Environmental Management (CIEEM, 2017; 2018) and as detailed in British Standard 42020:2013 Biodiversity - Code of Practice for Biodiversity and Development (BSI, 2013).

1.1.8 Pond locations and survey results plans are provided within **Figure 1**, photographs provided within **Annex A**, the HSI results in **Annex B**, and eDNA laboratory analysis results in **Annex C**, and details of relevant legislation in **Annex D**.

1.2 Order Limits Context and Scheme Description

1.2.1 The Order Limits are located within the administrative areas of Norfolk County Council and South Norfolk Council. Order Limits are the maximum extent of land anticipated to be acquired and/or used for the construction, operation and maintenance, and decommissioning phases of the Scheme. A description of the Order Limits can be found in **ES: Chapter 3 The Order Limits [EN0110014/APP/6.1.3]**.

1.2.2 The location of the Order Limits and surrounding landscape is shown in **Figure 1**.

The Scheme

1.2.3 The Scheme comprises the construction, operation and maintenance, and decommissioning of a solar photovoltaic (PV) electricity generating station with a total capacity exceeding 100 megawatts (MW) and associated development including a Battery Energy Storage System (BESS), up to three 132 kV Project Substations and up to three 400kV Project Substations, Grid Connection Infrastructure and a new National Grid Substation.

1.3 Relevant legislation

- 1.3.1 GCNs and their habitats are protected under the Conservation of Habitats and Species Regulations 2017 (as amended) and the Wildlife and Countryside Act 1981 (as amended). A more detailed description of this legislation is provided in **Annex D**.
- 1.3.2 Activities affecting GCN can be licenced by Natural England. These include mitigation licences that apply to development or other work. The licences allow for derogation from the strict provisions within the Conservation of Habitats and Species Regulations 2017 (as amended), subject to the application of the 'licensing tests' and specifically that there is no satisfactory alternative, and the development of an appropriate method statement including secured mitigation measures that ensure there will be no adverse impact on the species.

2 Methodology

2.1 Study Area

- 2.1.1 The Study Area incorporated the Order Limits and accessible ponds within a 250m radius, see **Figure 1** for sampling station locations.
- 2.1.2 All waterbodies with suitable habitat and the potential to support GCN were subject to eDNA surveys in June 2024, June 2025 and June 2026 (depending on access permissions); see **Table 3.5** for pond specific survey dates. The exceptions to these were where there was:
- Restricted access;
 - Where waterbodies were dry at the time of survey; and/or
 - Due to DCO order limits changes, ponds were not located within 250m of the order limits boundary at the time of survey.

2.2 Desk study

- 2.2.1 A desk study was conducted to obtain data relating to GCN within the Order Limits and a 2km radius. Records were acquired from Norfolk Biodiversity Information Service (NBIS). It was considered that GCN records received within the last decade (2015 – 2025) present a more reliable confirmation of presence, however, to provide context, all records from the year 2000 have been considered within this report.
- 2.2.2 Additional contextual information was compiled from publicly available data sources:
- MAGIC (<http://www.magic.gov.uk>) – the Government’s on-line mapping service. Information was sought regarding: the presence of statutory designated nature conservation sites with GCN as a qualifying feature; class licence return data, results from surveys used to inform DLL licensing, and extant or historic European Protected Species Mitigation Licence (EPSML) for GCN;
 - Ordnance Survey mapping and publicly available aerial photography to determine any features such as: ponds and other waterbodies within 250m of the Order Limits, and woodland, tree lines and hedgerows;
 - Natural England DLL scheme to determine what impact zone the Order Limits falls in and if the Site is suitable for the scheme.

2.3 Habitat Suitability Index (HSI) Assessment

- 2.3.1 Ponds and potentially suitable waterbodies identified within the desk study, supplemented through site walkover, were assessed for their potential to support GCN using the standardised HSI methodology (ARG UK, 2010). The HSI is a methodology that assesses habitat suitability and quantity using ten suitability indices.

2.3.2 These indices are:

- Factor 1 - Geographic location;
- Factor 2 - Pond area;
- Factor 3 - Permanence;
- Factor 4 - Water quality;
- Factor 5 - Shading;
- Factor 6 - Waterfowl;
- Factor 7 - Fish;
- Factor 8 - Pond count;
- Factor 9 – Terrestrial habitat; and
- Factor 10 - Macrophytes.

2.3.3 Each of these indices, assessed by either desk study or field survey, is given an individual value from 0 to 1 and the HSI is calculated by taking a geometric mean of these indices values, giving an overall score from 0 through to 1.

2.3.4 The results of the HSI calculation were compared to categorised HSI scores used by the National Amphibian and Reptile Recording Scheme to identify the suitability of a pond for GCN. The five categories are summarised in **Table 2.1** below.

2.3.5 The HSI method is recognised as not sufficiently precise to conclude that ponds with a high score will support newts, or that any pond with a low score will not. Instead, HSI is a tool for consistently evaluating potential rather than providing a substitute for GCN presence or assumed absence surveys.

Table 2.1: HSI categories

Pond Suitability for GCN	HSI Score
Poor	Below 0.5
Below average	0.5-0.59
Average	0.6-0.69
Good	0.7-0.79
Excellent	Above 0.8

2.4 Environmental DNA surveys (Presence/likely absence)

2.4.1 The eDNA water sampling followed the methodology set out in Natural England's 'Technical advice note for field and laboratory sampling of Great Crested Newt DNA' (Biggs *et al.*, 2014), which is provided as an appendix of the research report published by DEFRA into environmental DNA testing for GCN.

- 2.4.2 The sample locations were selected to include the entire pond margin (where accessible) targeting on areas with vegetation suitable for newt egg laying activities. The sampling procedure was as follows:
- 20 samples of 30ml each were taken from the perimeter of each pond at locations equally spaced apart (where accessible);
 - The ladle was stirred gently in the pond before the sample was retrieved in order to mix the water column, with care being taken not to stir up the sediment;
 - All 20 samples collected were emptied as they were collected into a Whirl-Pack bag, which was then sealed and mixed for a minimum of 10 seconds;
 - Using a sterile pipette, 15ml of the sample was transferred to six sterile tubes containing ethanol. Each tube was then shaken for 10 seconds to homogenize the water;
 - Samples were then stored at 2-4°C in a refrigerator until dispatched to the laboratory for analysis.
- 2.4.3 Samples were analysed following Natural England's published protocol (Biggs *et al.*, 2014). The 2024 and 2025 sampling kits were provided to NatureMetrics and 2026 sampling kits provided to SureScreen for laboratory analysis. The change in laboratory supplier is not considered to have a material impact on the results, as the same published sampling and analytical protocol was followed.

2.5 Geographic Evaluation

- 2.5.1 Where sufficient baseline data was available, the Order Limits ecological importance for GCN has been evaluated broadly following guidance issued by the Chartered Institute of Ecology and Environmental Management (CIEEM, 2018) which ranks the nature conservation importance of a site according to a geographic scale of reference: international, national, regional (East Anglia), metropolitan, county (Norfolk), local (South Norfolk); and of importance at the zone of influence of the Site only. In evaluating the nature conservation importance of the Order Limits, the following factors were considered: nature conservation designations; species/habitat rarity; naturalness; fragility and connectivity to other habitats.

2.6 Limitations

Data validity and limitation

- 2.6.1 It is important to note that a lack of desk study GCN records for a defined geographical area does not necessarily mean that the species is not present; the area may be simply under-recorded.
- 2.6.2 15 ponds located within the wider 250m survey buffer zone were not available to access, however, desk-study data was available for these, suggesting that 7 of these are positive for GCN and 8 negative for GCN. Whilst 10 of these are considered relatively historic, and dated to 2019 and 2020, it is considered that these still present a reliable confirmation of presence/absence. This is further corroborated by five further ponds (Ponds 21, 142, 149, 173 and 188) which have

historic desk study data, and have been subject to updated eDNA surveys, with the results all matching those of the desk study results (one of which dated back to 2006 desk study data).

- 2.6.3 When conducting eDNA surveys it is possible for the results returned from the laboratory to be inconclusive. The reasons for this are usually due to degradation of the sample (for example due to exposure of the sample to high temperatures) or PCR inhibition where compounds in the sample inhibit the PCR reaction. Such compounds could include organic material (sediment or animal waste), tannins from plant debris, run off from agricultural land, from water chemistry or sediment, or simply a low concentration of DNA in the sample. It is also possible to receive an inconclusive result where there is insufficient DNA in the water sample to provide a clear positive result.

Site specific limitations

- 2.6.4 In 2024 and 2025, of the 249 ponds that were located within the defined Survey Area, 97 ponds were not surveyed due to access not being agreed upon for the surveys, because the ponds were otherwise inaccessible e.g. for health and safety reasons, because the ponds were not located within 250m of the Order Limits boundary at the time of survey, or because the ponds were not present online mapping and only discovered during CRC extended habitat surveys undertaken in late 2025. Therefore, the status of GCN within these ponds is unknown. Whilst it is considered unlikely that GCN are present within all of these ponds (or indeed that all ponds are present/hold water), for the purpose of further ecological assessment, unless otherwise stated due to desk study results, a precautionary principle will be adopted and assumed that GCN are present within these ponds.
- 2.6.5 During the 2024 and 2025 surveys, 51 ponds were found to be dry, meaning a full survey could not be carried out. Of these, five were determined to no longer exist, having either been filled in or were seasonal swales that may sometimes hold shallow flood water during the winter months. In 2026, of the ponds inspected, 34 were dry (of which 18 were inspected for the first time). Ponds that frequently dry out during the survey period (Spring/Summer) are considered to be of low suitability for GCN. Drying at this time of year would prevent breeding from taking place successfully, as any eggs and larvae present could not survive. As such, these ponds are considered of low suitability to support breeding GCN. Although they may hold potential to support newts in the long term if enhancement occurs and management methods change, for the purpose of this assessment, dry ponds are not being considered as suitable habitats for GCN.
- 2.6.6 Data from GCN surveys may be valid for up to four seasons depending on the nature of the impacts of the proposals and the proximity of proposed works to ponds. Where supporting a mitigation licence application, guidance is available on the validity of data and its age within the Survey Guidance table (Instructions tab) of the Natural England mitigation licence method statement document². Therefore, the data presented here is considered appropriate to inform the Environmental Impact Assessment (EIA) process.

² [gcn-method-statement.xlsm \(live.com\)](https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/61444/gcn-method-statement.xlsm)

3 Results

3.1 Desk study

- 3.1.1 The data search returned GCN records from 63 locations since the year 2000 and a single historic EPSML issued by Natural England from within a 2km radius of the Order Limits. All records from the year 2000 onwards are listed in **Table 3.1**.
- 3.1.2 The records of confirmed GCN locations are spread throughout the search area, with two located within Sub-Site 4B. There are, however, several populations in close proximity to the Order Limits boundary including clusters of records to south-west of Sub-Site 4B, Wood Green to the east of CRC4, to the south-east of Long Stratton, and in the wider environment clusters of records are present around Wacton Common and Fritton Common. There is some habitat connectivity between these records and the Order Limits boundaries, however, connectivity severance is presented by a variety of roads (including the recently constructed Long Stratton Bypass), watercourses (such as the Hempnall Beck) and urban settlements.
- 3.1.3 The EPSML indicated on MAGIC maps is associated with a site in Seething 1.6km north-east from the Order Limits. Of those ponds surveyed between 2017 and 2019 to inform the District Level Licensing (DLL) scheme within 2km of the Order Limits, three were found to support GCNs; one near Long Stratton (110m south-west of sub-Site 4B), one near Fritton (282m east of CRC04) and one near Kirkstead Green (712m east of parcel 9).
- 3.1.4 The Order Limits falls within both Green and Amber zones of the Natural England DLL scheme for Norfolk³, which means that GCN populations, habitats and dispersal routes are present in the wider area and developers have the option of using DLL if required. There is also a Red Zone centred on the village of Fritton which is located approximately 220m south of Sub-Site 5B. This indicates the presence of a population of GCN, important on a regional scale at that location.
- 3.1.5 A summary of the results is presented in **Tables 3.1** and **3.2**.

Table 3.1: Summary of data search results

Distance from Site & Orientation	Date of most recent record	Description
Located within sub-Site 4B	2006	Present. Likely associated with Pond 21.
Located within sub-Site 4B	2006	Present. Likely associated with Pond 142.
13m, west of sub-Site 7D.	2024	eDNA score: present. Likely associated with Pond 107.
20m west of CRC04.	2006	Present. Likely associated with Pond 189.

³ Further information regarding the Norfolk and Suffolk DLL GCN risk zones can be found here:
<https://www.data.gov.uk/dataset/e6c4e491-4ce6-4d88-8e57-e028ae5a075a/great-crested-newt-risk-zones-norfolk-suffolk>

Distance from Site & Orientation	Date of most recent record	Description
27m south of sub-Site 4B.	2020	Two GCN present from six surveys. Likely associated with Pond 20.
33m south-west of sub-Site 4B	2006	GCN present. Likely associated with Pond 199.
88m west of sub-Site 4B	2006	GCN present. Likely associated with Pond 199.
99m west of sub-Sie 4B	2020	GCN records for 2019 and 2020. Maximum count of six. Likely associated with Pond 142.
102m west of sub-Site 4B	2020	Four records, between 16/04/2015 and 23/04/2020, maximum count: six. Likely associated with Pond 189.
104m west of CRC04.	2006	Present. Likely associated with Pond 190.
112m west of CRC04	2015	Torch survey, two adults present. Likely associated with Pond 190.
155m west of CRC04	2020	Four records, between 16/04/2015 and 05/05/2020. Maximum count: two. Likely associated with Pond 190.
173m south-west of sub-Site 4B	2020	GCN present, maximum count from six surveys: 19. Likely associated with Pond 199.
180m south of CRC9	2023	100's of juveniles recorded. Likely associated with Pond 173.
197m west of CRC04	2020	Two records, between 16/04/2015 and 23/04/2020, maximum count: six. Record now located beneath the Long Stratton Bypass A140.
199m west of sub-Site 4B	2020	GCN present. Likely associated with Pond 33.
215m west of CRC04	2006	Record located to the west of the Long Stratton Bypass A140.
244m east of CRC04	2020	Two positive GCN records between 2006 and 2020. Likely associated with Pond 188.
300m south-east of CRC04	2012	GCN present, maximum count of five adults.
315m west of CRC04	2006	GCN present.
320 west of CRC04	2015	GCN present – torchlight survey.
332m east of CRC04	2019	GCN present.
340m south-east of CRC04	2014	GCN present.
350m east of CRC04.	2001	Four records received of surveys on eight ponds, five ponds positive with a maximum of sixteen GCN recorded.

Distance from Site & Orientation	Date of most recent record	Description
358 west of CRC04	2020	Five records between 2006 and 2020, maximum count of four. Record located to the west of the Long Stratton Bypass A140.
381m south-east of CRC04	2018	Seven records between 2000 and 2018, maximum counts of thirty adults.
390m south-east of CRC04	2009	GCN present, maximum count of thirty adults.
395m south-east of CRC04	2015	GCN present, maximum count of eight adults.
424m north of sub-Site 1B	2014	GCN present.
425m east of CRC04	2018	Two records between 2016 and 2018. Maximum of five GCN recorded.
495m east of CRC04.	2015	GCN present, maximum of two adults recorded.
500m east of sub-Site 10E	2009	One GCN recorded.
545m east of CRC04	2015	Three adult GCN recorded beneath logpile.
554m south of sub-Site 5B	2014	GCN present.
555m south of CRC9	2023	One juvenile recorded.
560m east of CRC04	2015	GCN present.
608m west of CRC04	2006	GCN present.
662m south-east of Site 9	2009	One male recorded.
712m east of Site 9	2019	GCN present.
745m south-west of LSR2	2010	GCN pair.
762m south-east of Site 9	2011	Six records between 2009 and 2011, maximum of twelve adults recorded.
821m south of sub-Site 5B	2014	GCN present.
868m north-east of Site 9	2013	Two records, maximum of three adults recorded.
885m north-west of sub-Site 3B	2017	GCN present; two separate records both in 2017.
874m south of sub-Site 5B	2015	GCN present.
903m north of sub-Site 10C.	2009	One male recorded.

Distance from Site & Orientation	Date of most recent record	Description
920m south of sub-Site 5B	2001	Three records between 1983 and 2001. Maximum of five adults recorded.
961m south of sub-Site 5B	2017	Three GCN records between 2016 and 2017. Maximum of four recorded.
968m south of sub-Site 5B	2016	GCN present.
970m north-west of sub-Site 3B	2019	Three adult GCN and eggs.
972m north of sub-Site 2C	2015	GCN present.
989m south of sub-Site 5B	1989	Three adult GCN present.
998m south of sub-Site 5B	2017	Five GCN records between 2015 and 2017, maximum count of six.
1.0km south of sub-Site 5B	2023	Three adult GCN and eggs recorded.
1.02km south of sub-Site 5B	2018	Five records between 2014 and 2018, maximum of five adults.
1.03km north-west of sub-Site 3B	2019	10 adult GCN and eggs.
1.04km south of sub-Site 5B	2023	Adults and eggs recorded.
1.06km south of sub-Site 5B	2015	GCN present.
1.06km north of sub-Site 10E	2015	GCN present.
1.1km south of sub-Site 5B	2023	Adult GCN and eggs recorded.
1.1km south of sub-Site 5B	2018	Five GCN records between 2014 and 2018, maximum count of twelve adults.
1.34km west of CRC04	2001	GCN present.
1.42km south-west of Site 3	2007	GCN present, maximum of eight adults.

Table 3.2: GCN mitigation licences within 2km of the Site boundary

Licence number	Distance and orientation	Notes
EPSM2009-524	1.6km north-east of sub-Site 10E.	Licence allows damage/ destruction of a resting place.

- 3.1.6 There is a single statutory designated site (Fritton Common Site of Special Scientific Interest (SSSI)) within 2km that lists GCN within the qualifying criteria and this is associated with the DLL red Zone (see above). In addition, a single non-statutory designated site (Seething Observatory County Wildlife Site (CWS)) also list GCN within the citation; see **Table 3.3** below.

Table 3.3: Statutory/non-statutory sites where GCN are a Qualifying Criteria

Site	Distance & Orientation	Description
Fritton Common SSSI	0.54km east of sub-Site CRC04	Large grassy commons under traditional management by light cattle grazing. The site forms damp acidic grassland. Several natural ponds are present, and these support well-developed marginal and aquatic vegetation. Ancient, isolated oak and ash trees carry a wide range of epiphytic lichens including some locally scarce species. The ponds are shallow and show great diversity with some being only seasonally wet. The marginal vegetation is generally well developed and is distinctly zoned. The deeper ponds, with permanent water, contain a variety of water plants. The abundance of water plants supports a diverse assemblage of invertebrate species. The ponds are also breeding sites for GCN.
Seething Observatory CWS	0.74km W of sub-Site 10B.	A damp, basic grassland comprising part of Seething Airfield and Seething Observatory. The grassland in the southern field is unmanaged and rank; this part of the Site lies over heavy clay soils enclosed by ditches and a wire fence. The sward varies over the Site, depending on a level of disturbance and soil water content. There are limited patches of false oat-grass, creeping buttercup <i>Ranunculus repens</i> , and black medick <i>Medicago lupulina</i> . The small eutrophic pond to the west of the observatory car park is known to support GCN.

Other amphibian species

- 3.1.7 The data search returned records of three other amphibian species within 2km of the Site; common toad *Bufo bufo*, smooth newt *Lissotriton vulgaris* and common frog *Rana temporaria* dating from 1953 to 2015. Only one, a record of a single common frog found in terrestrial habitat, was from the past ten years (see **Table 3.4** below). The historic records within 2km of the Order Limits were six additional records of common frog *Rana temporaria*, thirteen historic records of common toad and eleven historic records of smooth newt.

Table 3.4: Summary of other amphibian species data search results

Distance from Site & Orientation	Date	Description
1.27km north of sub-Site 10E.	22/05/2015	Common frog. Found during clearance works and moved to suitable area within enhancement zone on site.

3.2 Field survey

Habitats overview

- 3.2.1 36 ponds are present within the Order Limits, largely associated with field boundary hedgerows/woodlands and occasionally as relatively isolated ponds within arable field parcels. Ponds have varying potential to support GCN (see HSI results in **Annex B**)
- 3.2.2 Terrestrial habitats within the Order Limits are predominantly arable farmland (with occasional areas of heavily grassed pastoral farmland), which is considered sub-optimal and unsuitable to support GCN terrestrial populations. Linear habitats such as grassland field margins, boundary hedgerows, pockets of linear woodland/scrub, and fen habitats (see CRC7) are present within the Order Limits, these habitats are relatively limited and only represent a small percentage of the Order Limits, however, may potentially provide suitability for terrestrial GCN (and other more widespread amphibians).

Habitat Suitability Index and eDNA Survey

- 3.2.3 A summary of the HSI and eDNA results is presented in **Tables 3.5** and **3.6** below. Photographs, full HSI calculations and eDNA laboratory reports and are presented in **Annexes B & C** respectively. A visual representation of the survey results is provided within **Figure 1**.

Table 3.5 Summary of HSI and eDNA Survey Results Within the Site Boundaries

Pond Number	Survey Date	Land Parcel	Grid reference	Description	HSI Result	Survey Result
2	01/06/2024	7L	TM270039 5423	Pond positioned at the edge of a arable field with surrounding grassland field margin.	Excellent	Negative eDNA result
3	18/06/2025	7K	TM269999 5465	The pond was located on the edge of an arable field, with nearby grassland.	Below average	Negative eDNA result
14	01/06/2024	7C	TM230269 5327	Pond located along the edge of a managed arable field, partially enclosed by a hedgerow.	Good	Negative eDNA result
21	01/06/2024	4B	TM204749 3679	Field pond within boundary hedgerow and associated scrub. Desk study confirms GCN presence in 2006.	Excellent	Positive eDNA Result
26	19/05/2025	CRC7	TM229729 4709	Pond was not fully accessible due to vegetation but the areas that could be viewed were dry.	Not surveyed	Dry
42	01/06/2024	10E	TM308549 6435	Located within broadleaved woodland, this pond is densely shaded, with duckweed present.	Average	Negative eDNA result
43	01/06/2024	8B	TM259719 7395	A field pond with very low water levels at the time of survey. The pond was heavily vegetated with reed and rush.	Good	Positive eDNA Result
44	12/06/2025	8A	TM260329 6295	Pond was dry at the time of survey; it lies within a woodland and is located adjacent to a wet ditch which may provide seasonal hydrological connection.	Not surveyed	Dry
45	18/06/2025	7K	TM271169 5587	Pond is located at the edge of an arable field, within hedgerow and surrounding trees. The pond is moderately shaded.	Below average	Inconclusive
46	01/06/2024	7I	TM258199 5629	A field edge pond within a boundary hedgerow, adjacent to a minor road. Tree cover provides partial shading.	Good	Negative eDNA result
47	01/06/2024	7K	TM267919 5167	Pond located within woodland on farmland, The pond is heavily shaded.	Good	Negative eDNA result

Pond Number	Survey Date	Land Parcel	Grid reference	Description	HSI Result	Survey Result
48	01/06/2024	7J	TM264009 5378	A pond situated at the edge of woodland, subject to heavy shading by surrounding tree canopy.	Good	Negative eDNA result
50	24/06/2025	7C	TM230259 5795	Located in broadleaved woodland, this pond is heavily shaded with dense surrounding vegetation. Aquatic and marginal plants are abundant.	Average	Inconclusive
51	12/06/2025	7C	TM224119 5707	Pond basin with minimal standing water at the time of survey, possibly due to seasonal drying.	Below average	Inconclusive
53	01/06/2024	7E	TM244189 5601	This pond lies within the field margin, enclosed by hedgerow and overhanging trees. Moderate shading.	Average	Negative eDNA result
54	18/06/2025	7E	TM244169 5385	This pond lies within the field margin, enclosed by hedgerow and overhanging trees. Moderate shading.	Poor	Inconclusive
55	17/06/2025	7F	TM239259 6370	Long, rectangular-shaped pond within a small woodland boundary of arable field.	Poor	Negative eDNA result
56	18/06/2025	10C	TM296699 5651	Pond with steeply sloping sides, surrounded by mature trees resulting in dense shade.	Below average	Positive eDNA Result
57	01/06/2024	10C	TM297439 5634	A small pond located along a field boundary with extensive aquatic plant cover.	Excellent	Positive eDNA Result
58	13/06/2025	10B	TM307789 4902	The pond was dry at the time of survey, though vegetation within the basin indicates seasonal water retention.	Not surveyed	Dry
59	13/06/2025	10C	TM300359 5402	This pond, located within a small woodland clearing was dry during the visit.	Not surveyed	Dry
60	18/06/2025	9	TM279359 8033	Pond within woodland, containing shallow, still water with limited aquatic vegetation.	Poor	Negative eDNA result
62	01/06/2024	3	TM241939 1606	Field edge pond with limited marginal and aquatic vegetation.	Excellent	Positive eDNA Result
63	01/06/2024	4A	TM199619 4549	A field-based pond surrounded by scattered mature trees providing partial shade.	Good	Negative eDNA result

Pond Number	Survey Date	Land Parcel	Grid reference	Description	HSI Result	Survey Result
64	12/06/2025	4A	TM201199 4545	A pond with very shallow water, heavily vegetated with sedge. Likely unsuitable for breeding GCN.	Poor	Inconclusive
65	01/06/2024	5A	TM224119 4006	An artificial farm pond on agricultural land, with surrounding vegetation restricting physical access.	Good	Negative eDNA result
66	12/06/2025	5A	TM224419 4210	Located within broadleaved woodland, this pond was completely dry during the survey.	Not surveyed	Dry
67	25/06/2025	5B	TM229879 4072	Pond located next to woodland and arable fields.	Good	Inconclusive
156	16/06/2025	1A	TM167108 9008	A pond adjacent to an arable field, hydrologically linked to a nearby ditch. The pond may receive runoff or drainage water.	Poor	Negative eDNA result
157	11/06/2025	1B	TM171948 8826	Pond within woodland, trees providing significant shading.	Not surveyed	Dry
160	11/06/2025	2C	TM189238 9520	Pond within woodland, trees providing significant shading.	Not surveyed	Dry
167	17/06/2025	3	TM241509 1795	An isolated pond in the middle of a managed arable field. Few surrounding trees.	Good	Negative eDNA result
191	25/06/2025	CRC4	TM212349 2451	Former field margin/corner which had previously flooded due to the storage of a large area of manure. Since removal the area is not holding water and no pond structure is present.	Not surveyed	Non existent
211	16/06/2025	2B	TM189198 8802	The pond was located in woodland area and was heavily shaded.	Poor	Negative eDNA result
212	16/06/2025	2B	TM189458 8908	The pond was located in woodland area and was heavily shaded. There was a nearby area of grassland.	Not surveyed	Dry

Table 3.6 Summary of HSI and eDNA Survey Results Within a 250m buffer of the Site Boundaries

Pond number	Survey Date	Closest Land Parcel	Grid Reference	Description	HSI Result	Survey Result
1	N/A	10A	TM297469 4332	No access available during 2025 and 2026 survey.	Not surveyed	Positive (Precautionary)
4	01/07/2025	10C	TM299469 6116	Dry long pond with associated ditch and surrounding trees.	Average	Negative eDNA result
5	22/05/2026	7F	TM248679 6414	This is a shallow roadside pond, with partial shading and emergent marginal vegetation.	Excellent	Positive eDNA Result
6	N/A	7F	TM243079 6625	No access available during 2025 and 2026 survey. Desk study records from 2024 confirm negative eDNA records from this pond.	Not surveyed	Confirmed negative by desk study
7	22/05/2026	7F	TM248879 6447	.Dry shallow pond.	Not assessed	Dry
8	30/06/2026	CRC8	TM249469 3623	No access available during 2025 and 2026 survey. Inspected from the roadside only in 2026, to generate a HSI data.	Average	Positive (Precautionary)
9	20/06/2026	6	TM253379 3282	A small lake with bankside scrub and marginal emergents.	Average	Negative eDNA result
10	17/06/2025	3	TM244609 0913	Occasionally damp but not deep enough to collect eDNA samples. Unsuitable for GCN breeding purposes.	Not surveyed	Dry
11	01/06/2024	9	TM284639 8373	Farmland water storage pond, steep banks, limited access due to scrub, adjacent woodland	Excellent	Positive eDNA Result
12	26/06/2025 and 20/06/2026	7C	TM225049 5383	A linear pond (probably a moat or similar) within a small block of woodland, dry in both 2025 and 2026.	Not assessed	Dry

Pond number	Survey Date	Closest Land Parcel	Grid Reference	Description	HSI Result	Survey Result
13	26/06/2025	7C	TM220019 5269	Dry pond within woodland. Desk study records from 2019 confirm negative eDNA records from this pond.	Not surveyed	Confirmed negative by desk study
15	N/A	7C	TM225909 6059	No access available during 2025 and 2026 survey.	Not surveyed	Positive (Precautionary)
16	NA	7F	TM240589 6602	No access available during 2025 and 2026 survey.	Not surveyed	Positive (Precautionary)
17	N/A	7F	TM239669 6654	No access available during 2025 and 2026 survey. Desk study records from 2024 confirm negative eDNA records from this pond.	Not surveyed	Confirmed negative by desk study
18	17/06/2026	7D	TM235019 6697	This is a small pond within the garden of a traditional cottage.	Good	Positive eDNA Result
19	17/06/2026	7D	TM234739 6722	No access available during 2025 survey and a landowner directly adjacent confirmed that this pond is dry. It was found to be dry in 2026 when directly inspected.	Not assessed	Dry
20	NA	4B	TM204369 3672	No access available during 2025 and 2026 surveys. 2020 desk study records confirm GCN presence within this pond.	Not surveyed or assessed	Confirmed positive by desk study
22	19/06/2025 and 22/05/2026	7B	TM228419 4709	Long, shallow pond situated under a willow with considerable shading. Considered to be unsuitable for breeding GCN.	Below average	Negative eDNA result
23	N/A	5B	TM229059 4224	No access available during 2025 and 2026 survey.	Not surveyed	Positive (Precautionary)
24	N/A	5B	TM229119 4235	No access available during 2025 and 2026 survey.	Not surveyed	Positive (Precautionary)

Pond number	Survey Date	Closest Land Parcel	Grid Reference	Description	HSI Result	Survey Result
25	N/A	CRC7	TM234619 4541	No access available during 2025 and 2026 survey.	Not surveyed	Positive (Precautionary)
27	19/06/2025 and 22/05/2026	7B	TM228999 4715	This pond is very shallow and dominated by dense sedge. In 2025 it was considered to be likely unsuitable for breeding GCN. In 2026 the emergent vegetation was very dense and open water was absent, and without future management it is unlikely to be suitable for breeding.	Not assessed	Dry
28	19/06/2025 and 20/06/2026	7B	TM223929 4566	Large nutrient enriched, silty pond with prominent algal growth.	Poor	Negative eDNA result
29	19/06/2025	CRC7	TM229109 4691	A still pond with poor water movement, located in a wooded area. Significant leaf litter and organic debris cover the pond floor and surface.	Poor	Negative eDNA result
30	30/06/2025 and 20/06/2026	4A	TM205019 4881	Drainage basin surrounded by grassland. Adjacent to the A140. This was dry in both 2025 and 2026, and only likely to support open water on a temporary basis after rain.	Not assessed	Dry
31	30/06/2025 and 20/06/2026	4A	TM205159 4916	Created as part of the roundabout construction. Large swale present but normally dry in summer and only likely to support open water on a temporary basis after rain	Not assessed	Dry
32	30/06/2025	4A	TM206339 4986	A large managed pond used for fishing.	Poor	Negative eDNA result
33	N/A	4B	TM201259 3784	No access available during 2025 and 2026 survey. 2020 desk study records confirm GCN presence within this pond.	Not assessed	Confirmed positive by desk study

Pond number	Survey Date	Closest Land Parcel	Grid Reference	Description	HSI Result	Survey Result
34	11/06/2025 and 20/06/2026	1C	TM163798 8660	Pond set within woodland strip at the edge of an arable field.	Below average	Negative eDNA result
35	16/06/2025 and 17/06/2026	1C	TM164898 8401	Dry pond located in arable field margin.	Not assessed	Dry
36	16/06/2025	1C	TM164908 8389	Pond surrounded by grassland field margin, within arable field.	Poor	Negative eDNA result
37	27/06/2026	1B	TM167668 9810	Pond within the garden of a traditional cottage.	Average	Positive eDNA Result
38	27/06/2026	1B	TM167938 9807	Pond within the garden of a traditional cottage.	Average	Positive eDNA Result
39	07/05/2026	1B	TM168288 9568	Large field pond.	Below average	Negative eDNA result
40	27/06/2026	1B	TM168348 9825	Small garden pond with a liner, and with dense vegetation throughout and little open water.	Below average	Positive eDNA Result
41	01/06/2024	1B	TM168528 9807	Within treeline between field and gardens. GCN eggs present.	Good	Positive eDNA Result
49	01/07/2025	7K	TM266429 5284	Located within broadleaved woodland, this pond is subject to dense shading.	Not surveyed	Dry
52	01/06/2024	7F	TM240759 5924	Pond located within an arable field but in close proximity to a woodland block.	Good	Negative eDNA result
61	26/06/2025 and 28/06/2026	3	TM243039 1219	Pond situated within a field margin, with an artificial inflow pipe visible, likely providing water input. Dry in both 2025 and 2026.	Not assessed	Dry

Pond number	Survey Date	Closest Land Parcel	Grid Reference	Description	HSI Result	Survey Result
68	N/A	10E	TM307909 6304	No access available during 2025 and 2026 survey.	Not surveyed	Positive (Precautionary)
69	N/A	10E	TM307249 6362	No access available during 2025 and 2026 survey.	Not surveyed	Positive (Precautionary)
70	01/06/2024	8B	TM262469 7647	Pond located on the edge of a field.	Good	Positive eDNA Result
71	01/07/2025	8B	TM262299 7369	This feature was a ditch within woodland which may periodically flood, completely dry during the survey. Not considered suitable for breeding GCN.	Not surveyed	Dry
72	21/05/2026	7L	TM273629 5353	Dry pond set within woodland.	Not assessed	Dry
73	28/06/2026	7K	TM271729 5975	Small pond set within woodland.	Below average	Negative eDNA result
74	17/06/2025	7F	TM247689 6304	The pond was found to no longer exist as it had been filled in.	Not surveyed	Non existent
75	26/06/2025	7H	TM259629 4938	Heavily shaded pond located in woodland.	Below average	Inconclusive
76	30/06/2026	7I	TM257909 5631	Small pond within a field corner surrounded by scrub.	Below average	Positive eDNA result
77	12/06/2025 and 30/06/2026	7G	TM256879 5424	Small pond along a field boundary and surrounded by scrub.	Below average	Negative eDNA result
78	10/07/2025	7J	TM261969 5631	Pond located within a small woodland, heavily shaded.	Below average	Negative eDNA result
79	28/06/2026	7K	TM267689 5604	Small pond set within a hedgerow.	Below average	Negative eDNA result

Pond number	Survey Date	Closest Land Parcel	Grid Reference	Description	HSI Result	Survey Result
80	27/06/2025	7J	TM263019 5477	A pond hydrologically linked to an adjacent ditch.	Poor	Positive eDNA Result
81	28/06/2026	7K	TM259679 4945	. This is aA small pond set within a woodland.	Not surveyed	Negative eDNA result
82	28/06/2026	7I	TM261379 5685	Pond within a small woodland with adjacent arable fields with associated ditch, which is higher and feeds the pond.	Below average	Positive eDNA Result
83	28/06/2026	7K	TM268309 5650	A large pond dominated by duckweed.	Below average	Negative eDNA result
84	27/06/2025	7J	TM264869 5344	Woodland pond, heavily shaded.	Below average	Negative eDNA result
85	N/A	7D	TM233269 5832	No access available during 2025 survey.	Not surveyed	Positive (Precautionary)
86	26/06/2025 and 28/06/2026	7E	TM238079 5585	A heavily shaded pond located within dense woodland. Aquatic and marginal vegetation is absent. Dry in both 2025 and 2026.	Not assessed	Dry
87	N/A	7A	TM216219 5877	No access available during 2025 and 2026 survey.	Not surveyed	Positive (Precautionary)
88	25/06/2025	7C	TM217879 5631	A heavily shaded pond located within dense woodland. Aquatic and marginal vegetation is abundant.	Below average	Negative eDNA result
89	NA	7A	TM216229 5866	No access available during 2025 and 2026 survey.	Not surveyed	Positive (Precautionary)
90	N/A	7C	TM218419 5853	No access available during 2025 and 2026 survey.	Not surveyed	Positive (Precautionary)
91	25/06/2025	7C	TM218179 5651	Field pond with surrounding trees.	Average	Negative eDNA result

Pond number	Survey Date	Closest Land Parcel	Grid Reference	Description	HSI Result	Survey Result
92	N/A	7A	TM211829 5752	No access available during 2025 and 2026 survey.	Not surveyed	Positive (Precautionary)
93	21/05/2026	7C	TM227299 5351	Woodland-edge pond surrounded by trees with duckweed. .	Average	Negative eDNA result
94	21/05/2026	7C	TM221039 5389	Woodland pond..	Below average	Negative eDNA result
95	N/A	7C	TM 22611955 41	No access available during 2025 and 2026 survey.	Not surveyed	Positive (Precautionary)
96	N/A	7C	TM227259 5825	No access available during 2025 and 2026 survey.	Not surveyed	Positive (Precautionary)
97	24/06/2025 and 21/05/2026	7C	TM223909 5389	A dry pond located within dense woodland. Tree canopy provides near-total shading. Dry in both 2025 and 2026.	Not assessed	Dry
98	21/05/2026	7C	TM226259 5364	Field edge pond, dry in 2025 but wet in 2026.	Below average	Negative eDNA result
99	26/06/2025	7C	TM227559 5358	A dry pond located within dense woodland. Tree canopy provides near-total shading.	Not surveyed	Dry
100	21/05/2026	7C	TM220909 5502	A heavily shaded pond located within dense woodland. Aquatic and marginal vegetation is absent.	Poor	Positive eDNA Result
101	N/A	7C	TM222609 5396	No access available during 2025 and 2026 surveys. 2020 desk study records confirm that following from four GCN presence/absence surveys, the pond did not support GCN.	Not surveyed	Confirmed negative by desk study

Pond number	Survey Date	Closest Land Parcel	Grid Reference	Description	HSI Result	Survey Result
102	N/A	7C	TM223189 5373	No access available during 2025 and 2026 surveys. 2019 desk study record confirm that the pond did not support GCN eDNA.	Not surveyed	Confirmed negative by desk study
103	N/A	7F	TM247109 5963	No access available during 2025 and 2026 survey.	Not surveyed	Positive (Precautionary)
104	21/05/2026	7E	TM241169 5793	No access available during 2025 survey and dry in 2026.	Not assessed	Dry
105	07/05/2026	7D	TM235319 6603	Pond set within garden of traditional cottage, shaded and with only sparse marginal vegetation.	Average	Positive eDNA Result
106	N/A	7D	TM236829 6574	No access available during 2025 and 2026 survey.	Not surveyed	Positive (Precautionary)
107	N/A	7D	TM238119 6553	No access available during 2025 and 2026 survey. 2024 desk study records confirm GCN presence within this pond.	Not surveyed	Confirmed positive by desk study
108	N/A	7D	TM237269 6577	No access available during 2025 and 2026 survey.	Not surveyed	Positive (Precautionary)
109	N/A	7C	TM219629 6024	No access available during 2025 and 2026 survey.	Not surveyed	Positive (Precautionary)
110	01/06/2024	10A	TM299609 4842	Located in a scrubby woodland patch, this pond has moderate duckweed coverage and receives low light levels due to surrounding tree and shrub growth.	Average	Negative eDNA result
111	N/A	10B	TM298629 5036	No access available during 2025 and 2026 survey.	Not surveyed	Positive (Precautionary)
112	07/05/2026	10C	TM297769 5982	Dry roadside pond, surrounded by scrub,	Poor	Negative eDNA result

Pond number	Survey Date	Closest Land Parcel	Grid Reference	Description	HSI Result	Survey Result
113	07/05/2026	10C	TM296349 5265	Woodland edge pond, adjacent to an arable field.. eDNA tested in 2026 only.	Average	Positive eDNA Result
114	01/06/2024	10B	TM309949 4890	A shaded pond within woodland, with surface duckweed. Frogs were observed during the survey.	Below average	Negative eDNA result
115	13/06/2025	10B	TM303129 4947	A pond within woodland, influenced by one artificial pipe.	Not surveyed	Dry
116	N/A	10D	TM307649 5701	No access available during 2025 and 2026 survey.	Not surveyed	Positive (Precautionary)
117	30/06/2026	10C	TM300209 5853	Small shaded pond surrounded by trees adjacent to road.	Average	Negative eDNA result
118	01/07/2025	10C	TM303209 5535	Pond within small section of woodland with grassland boundaries; existing access track runs directly adjacent to the pond.	Good	Positive eDNA Result
119	01/07/2025	10D	TM307579 5763	Located in a narrow woodland strip within or adjacent to industrial land.	Average	Negative eDNA result
120	N/A	9	TM278349 7779	No access available during 2025 and 2026 survey.	Not surveyed	Positive (Precautionary)
121	N/A	9	TM275009 8260	No access available during 2025 and 2026 survey.	Not surveyed	Positive (Precautionary)
122	N/A	3	TM237539 1557	No access available during 2025 and 2026 survey.	Not surveyed	Positive (Precautionary)
123	01/07/2025	3	TM238459 1586	Pond within hedgerow of arable field. Adjacent grassland field margin.	Average	Positive eDNA Result
124	26/06/2025	3	TM240029 1453	Field pond within arable field, surrounded by grassland field margin	Good	Positive eDNA Result

Pond number	Survey Date	Closest Land Parcel	Grid Reference	Description	HSI Result	Survey Result
125	26/06/2025	3	TM242139 1299	Field pond in hedgerow, bordering arable field.	Average	Positive eDNA Result
126	19/06/2026	3	TM241269 1948	This is a field pond surrounded by scrub.	Average	Positive eDNA Result
127	26/06/2025	3	TM242739 1896	A relatively large waterbody situated within arable farmland.	Average	Positive eDNA Result
128	07/05/2026	3	TM245589 2398	A pond set within garden of tradition cottage	Average	Positive eDNA Result
129	12/06/2025 and 07/05/2026	3	TM241989 2083	A field edge basin surrounded by scrub and trees; dry in both 2025 and 2026.	Not assessed	Dry
130	N/A	3	TM244549 2402	No access available during 2025 and 2026 survey.	Not surveyed	Positive (Precautionary)
131	07/05/2026	3	TM249949 2437	No access available during 2025 and 2026 survey. A field edge basin surrounded by scrub and trees.	Not assessed	Dry
132	28/06/26	3	TM250029 2434	A dry field edge basin surrounded by scrub and trees.	Not assessed	Dry
133	12/06/2025	CRC6	TM243659 2208	Currently dry ditch in woodland. Not suitable for breeding GCN.	Not surveyed	Dry
134	01/07/2025	CRC6	TM243609 2164	Ditch located at the edge of an arable field; the ditch was dry during the time of survey. Not suitable for breeding GCN.	Not surveyed	Dry
135	N/A	3	TM251879 1842	No access available during 2025 and 2026 survey.	Not surveyed	Positive (Precautionary)
136	N/A	3	TM251769 1639	No access available during 2025 survey. Pond no longer within the 250m survey buffer zone.	Not surveyed	Positive (Precautionary)

Pond number	Survey Date	Closest Land Parcel	Grid Reference	Description	HSI Result	Survey Result
137	28/06/26	3	TM250029 2447	A dry field edge basin surrounded by scrub and trees.	Not assessed	Dry
138	20/06/2026	4A	TM199359 4330	A corner pond with surrounding trees.	Below average	Positive eDNA Result
139	23/06/2025 and 27/06/2026	4A	TM195999 4345	The pond is located along the edge of a field and was dry at the time of the surveys in both 2025 and 2026.	Not assessed	Dry
140	23/06/2025	4A	TM198919 4234	Pond within arable field hedgerow, surrounding trees.	Average	Positive eDNA Result
141	NA	4B	TM204859 3591	No access available during 2025 and 2026 survey.	Not surveyed	Positive (Precautionary)
142	28/06/2026	4B	TM202609 3760	This pond is located in open pasture subject to grazing pressure, with sheep present at the time of survey. 2019/2020 desk study records confirm GCN presence within this pond.	Below average	Positive eDNA Result
143	28/06/2026	4B	TM203109 3706	A shallow drainage feature associated with the Long Stratton bypass, with a terrestrial sward; likely to be wet only on a temporary basis after rain.	Not assessed	Dry
144	N/A	6	TM251929 3493	No access available during 2025 and 2026 survey.	Not surveyed	Positive (Precautionary)
145	N/A	6	TM250319 3804	No access available during 2025 and 2026 survey.	Not surveyed	Positive (Precautionary)
146	28/06/26	6	TM255299 3395	No access available during 2025 and 2026 survey. A pond located in a garden and only viewed from the adjacent road.	Average	Positive (Precautionary)

Pond number	Survey Date	Closest Land Parcel	Grid Reference	Description	HSI Result	Survey Result
147	N/A	6	TM252169 3464	No access available during 2025 and 2026 survey.	Not surveyed	Positive (Precautionary)
148	26/06/2025	6	TM251879 3711	A man-made pond within a farm complex.	Poor	Inconclusive
149	25/06/2025	5B	TM230229 3180	An isolated pond situated in the middle of an arable field, surrounded by a ring of mature trees. Moderate shading and leaf litter present. Desk study records from 2019 confirm negative eDNA records within this pond.	Below average	Negative eDNA result
150	17/06/2026	5B	TM229889 3228	Pond within border of arable field and residential garden.	Excellent	Positive eDNA Result
151	19/06/2025	5A	TM220069 3756	Field margin dry pond surrounded by trees. Pond recorded as being negative for GCN eDNA in 2019.	Not surveyed	Confirmed negative by desk study
152	23/06/2025 and 19/06/2026	5A	TM225069 4236	Dry pond within grassland area adjacent arable field. Dry in both 2025 and 2026.	Not assessed	Dry
153	N/A	5A	TM227659 4262	No access available during 2025 and 2026 survey.	Not surveyed	Positive (Precautionary)
154	N/A	5A	TM228859 4081	No access available during 2025 and 2026 survey.	Not surveyed	Positive (Precautionary)
155	N/A	5A	TM226489 4225	No access available during 2025 and 2026 survey.	Not surveyed	Positive (Precautionary)
158	19/06/2026	4A	TM201669 5312	Pond within arable field hedgerow, and surrounding trees, and within the valley bottom; densely vegetated with emergent sedges. The adjacent Pond 159 was found to be negative for GCN eDNA when tested in 2025. eDNA test of this pond in 2026 returned a negative result.	Average	Negative eDNA result

Pond number	Survey Date	Closest Land Parcel	Grid Reference	Description	HSI Result	Survey Result
159	30/06/2025	4A	TM201649 5382	Pond within woodland section in arable field. Close to A140.	Below average	Negative eDNA result
161	N/A	CRC4	TM194478 9588	No access available during 2025 and 2026 survey.	Not surveyed	Positive (Precautionary)
162	19/05/2025 and 19/06/2026	CRC4	TM204239 1600	In 2025 the pond was dry at the time of survey, but aquatic vegetation was still present. In 2026, wetland vegetation was absent and only terrestrial grasses and herbs were present; this is a shallow basin with an apparent drainage function and only likely to be wet on a temporary basis after rain.	Not assessed	Dry
163	19/05/2025	CRC4	TM204639 1597	A pond shown on mapping data could not be found in the field. The area appears to have been incorporated into arable land and ploughed over.	Not surveyed	Non existent
164	N/A	CRC4	TM212319 1652	No access available during 2025 survey. Now located over 250m from CRC4.	Not surveyed	Positive (Precautionary)
165	21/05/2026	CRC7	TM229509 4707	A pond within a valley bottom woodland, densely vegetated with both native and invasive species. Himalayan balsam <i>Impatiens glandulifera</i> was recorded on the margins.	Good	Negative eDNA result
166	01/07/2025	3	TM239049 2168	Pond used by waterfowl, with murky water and turbid conditions. Moderate disturbance. Pond now located outside of the 250m survey buffer zone.	Poor	Positive eDNA Result
168	- 20/06/2026	7F	TM245079 5354	This pond is within a field boundary hedgerow and shaded	Good	Negative eDNA result
169	17/06/2025	3	TM251349 1819	Pond located at end of ditch system. The water was low in ditch at the time of survey.	Average	Inconclusive

Pond number	Survey Date	Closest Land Parcel	Grid Reference	Description	HSI Result	Survey Result
170	19/06/2026	CRC9	TM284799 5553	This is a raised lagoon used for agricultural purposes.	Good	Negative eDNA result
171	28/06/2026	CRC9	TM285149 5627	A small woodland pond.	Below average	Negative eDNA result
172	28/06/2026	CRC9	TM285209 5236	A large woodland pond with only sparse overtopping tree cover and abundant aquatic and marginal vegetation.	Excellent	Negative eDNA result
173	28/06/2026	CRC9	TM291329 5423	Data received from NBIS confirmed the pond as positive for GCN in 2023. This is a field edge pond with nearby woodland and partially enclosed by scrub.	Average	Positive eDNA Result
174	30/06/2025	1C	TM161308 8295	A large pond located along the edge of an arable field. Now located over 250m from 1C.	Below average	Negative eDNA result
175	N/A	1C	TM166308 8045	No access available during 2025 and 2026 survey.	Not surveyed	Positive (Precautionary)
176	16/06/2025 and 19/06/2026	BESS	TM169508 8494	A shallow basin set within woodland. Dry in both 2025 and 2026.	Not assessed	Dry
177	16/06/2025	BESS	TM169158 8373	Pond couldn't be located. Presumed to have been lost through succession.	Not surveyed	Non existent
178	N/A	2B	TM186668 9217	No access available during 2025 and 2026 survey.	Not surveyed	Positive (Precautionary)
179	30/06/2026	7B	TM225319 4576	Pooling of a small watercourse identified as a tributary of a nearby river. Although the channel is narrow and shallow, it had minimal flow and is likely to have permanent open water.	Average	Negative eDNA result
180	01/07/2025	7A	TM209299 5204	Access to the pond was restricted due to dense vegetation, however, a wet ditch connected to the pond was surveyed.	Below average	Inconclusive

Pond number	Survey Date	Closest Land Parcel	Grid Reference	Description	HSI Result	Survey Result
181	26/06/2025	CRC	TM250149 4458	Pond within hedgerow of arable field. Adjacent grassland field margin. Now located outside of the 250m survey buffer zone.	Good	Negative eDNA result
182	N/A	2A	TM179558 8070	No access available during 2025 and 2026 survey.	Not surveyed	Positive (Precautionary)
183	N/A	2A	TM180068 8102	No access available during 2025 and 2026 survey.	Not surveyed	Positive (Precautionary)
184	29/07/2025	CRC2	TM179998 8153	Long pond within small woodland section within grassland field.	Average	Positive eDNA Result
185	27/06/2026	CRC2	TM180758 8217	A small pond within the garden of a traditional cottage.	Poor	Negative eDNA result
186	29/07/2025	CRC2	TM180578 8246	Pond within small woodland section within grassland field.	Good	Inconclusive
187	30/06/2025	CRC4	TM197219 0090	A pond located within a working farm complex next to arable field. Data received from NBIS confirmed the pond as negative for GCN eDNA in 2023. Inconclusive eDNA laboratory results.	Below average	Confirmed negative by desk study
188	19/06/2025	CRC4	TM204809 1528	This pond is within a farm complex and bordered by trees. 2020 desk study records confirm GCN presence within this pond.	Good	Positive eDNA Result
189	N/A	CRC4	TM200699 1499	No access available during 2025 survey. Desk study records (most recent from 2020 confirm GCN presence within this pond.	Not surveyed	Confirmed positive by desk study
190	19/06/2025	CRC4	TM202919 1928	Pond within arable field border, surrounding trees. Desk study records (most recent from 2020) confirm GCN presence within this pond. Inconclusive eDNA laboratory results.	Good	Confirmed positive by desk study

Pond number	Survey Date	Closest Land Parcel	Grid Reference	Description	HSI Result	Survey Result
192	N/A	3	TM248849 2593	No access available during 2025 and 2026 survey.	Not surveyed	Positive (Precautionary)
193	17/06/2026	3	TM245109 2518	A large garden pond with a liner, with an abundance of aquatic and marginal vegetation and no fish. The eDNA test result was negative, but the owner provided a photograph of a GCN apparently taken within the garden, adopting a precautionary approach, this has been accepted as evidence of GCN presence.	Good	Confirmed positive by desk study
194	N/A	3	TM239259 2043	No access available during 2025 and 2026 survey.	Not surveyed	Positive (Precautionary)
195	N/A	5A	TM222689 4356	No access available during 2025 and 2026 survey.	Not surveyed	Positive (Precautionary)
196	26/06/2025	5A	TM218439 4285	Pond within hedgerow grassland field. Adjacent to B-road.	Below average	Negative eDNA result
197	26/06/2025	5A	TM218389 4235	Dry pond, recorded as negative for GCN eDNA in 2019.	Not surveyed	Confirmed negative by desk study
198	25/06/2025	CRC6	TM236129 3977	Arable field pond with wide grassland buffer.	Average	Positive eDNA Result
199	N/A	4B	TM201469 3955	No access available during 2025 and 2026 survey. 2020 desk study records confirm GCN presence within this pond.	Not surveyed	Confirmed positive by desk study
200	N/A	8B	TM255789 7529	No access available during 2025 and 2026 survey.	Not surveyed	Positive (Precautionary)
201	N/A	8B	TM256199 7457	No access available during 2025 and 2026 survey.	Not surveyed	Positive (Precautionary)

Pond number	Survey Date	Closest Land Parcel	Grid Reference	Description	HSI Result	Survey Result
202	21/05/2026	10E	TM315119 6337	A field edge dry basin surrounded by scrub.	Not assessed	Dry
203	25/06/2025	BESS	TM173718 8117	Pond in middle of arable field. Surrounding trees.	Below average	Negative eDNA result
204	N/A	CRC4	TM200258 9322	No access available during 2025 and 2026 survey.	Not surveyed	Positive (Precautionary)
205	N/A	7C	TM221879 6029	No access available during 2025 and 2026 survey.	Not surveyed	Positive (Precautionary)
206	N/A	7C	TM223419 6127	No access available during 2025 and 2026 survey.	Not surveyed	Positive (Precautionary)
207	01/07/2025	10E	TM309299 6724	Woodland pond, heavily shaded.	Not surveyed	Dry
208	24/06/2025	2A	TM184728 8161	Large woodland pond.	Average	Negative eDNA result
209	30/06/2025	2C	TM187848 9553	Heavily vegetated dry woodland pond.	Not surveyed	Dry
210	29/07/2025	2C	TM187928 9504	Pond within large area of woodland. Heavily shaded.	Average	Negative eDNA result
213	20/05/2025	2B	TM190288 9081	A pond situated along a field margin, connected to a ditch system and bordered by a hedgerow. Partial shading present.	Good	Negative eDNA result
214	01/07/2025	3	TM253549 2142	Landowner advised that this pond was no longer present, and aerial images indicate that it has been removed, and the land has been developed for business use.	Not surveyed	Non existent
215	21/05/2026	CRC6	TM236759 3282	A shallow basin located in woodland, dry at the time of survey.	Not assessed	Dry

Pond number	Survey Date	Closest Land Parcel	Grid Reference	Description	HSI Result	Survey Result
216	N/A	CRC6	TM239139 2481	No access available during 2025 and 2026 survey.	Not surveyed	Positive (Precautionary)
217	30/06/2026	CRC3	TM1814188 82	Pond recorded during Autumn 2025 habitat surveys, no access available during 2026 survey. A roadside pond surrounded by trees and scrub. Inspected from the roadside only, to generate a Habitat Suitability Index.	Poor	Positive (Precautionary)
218	11/08/2025	CRC6	TM2420492 28	Large pond surrounded by willow scrub and pond surface covered in extensive aquatic plant cover. Pond recorded during Autumn 2025 habitat surveys, no access available during 2026 survey.	Above average.	Positive (Precautionary)
219	11/08/2025	CRC6	TM2428392 72	Dry pond with lots of dead wood present within basin. Pond recorded during Autumn 2025 habitat surveys.	Not surveyed	Dry
220	30/06/2026	CRC4	TM2153593 47	Pond located next to hedgerow, with some aquatic vegetation present. Pond recorded during Autumn 2025 habitat surveys. This appears to be a relatively modern feature with a drainage function, but is closed at both ends.	Poor	Negative eDNA result
221	28/06/2026	CRC8	TM2658194 82	Pond located on the edge of arable field, with lots of aquatic vegetation present. Pond recorded during Autumn 2025 habitat surveys. This was dry in 2026.	Poor	Dry
222	29/08/2025	CRC8	TM2707294 98	. Pond located within the centre of an arable field surrounded by a narrow layer of scrub. Recorded during Autumn 2025 habitat surveys. No access available during 2026 survey.	Not surveyed	Positive (Precautionary)
223	08/10/2025	CRC9	TM2906295 90	Field margin pond, adjacent to woodland/scrub. Pond recorded during Autumn 2025 habitat surveys. No access available during 2026 survey.	Not surveyed	Positive (Precautionary)

Pond number	Survey Date	Closest Land Parcel	Grid Reference	Description	HSI Result	Survey Result
224	30/06/2026	CRC14	TM2681697 50	Field pond with lots of dead foliage and brambles within the pond itself and surrounding it. Pond recorded during Autumn 2025 habitat surveys.	Below average	Negative eDNA result
225	02/09/2025 and 28/06/2026	CRC14	TM2649597 55	Dry pond with lots of dead foliage and brambles within the pond itself and surrounding it. Pond recorded during Autumn 2025 habitat surveys. The basin was dry in both 2025 and 2026.	Not assessed	Dry
226	28/06/2026	CRC14	TM2666397 57	Pond recorded during Autumn 2025 habitat surveys. Hedgerow pond, heavily shaded by scrub and trees.	Below average	Negative eDNA result
227	28/06/2026	CRC14	TM2688197 59	Pond recorded during Autumn 2025 habitat surveys. Hedgerow pond, heavily shaded by scrub and trees.	Poor	Negative eDNA result
228	N/A	CRC4	TM1993791 489	Pond no longer present	Not surveyed	Non existent
229	N/A	LSR1	TM1727387 3	Pond originally located outside of the 250m survey area during the 2024 and 2025 survey season. No access available during 2026 survey.	Not surveyed	Positive (Precautionary)
230	N/A	LSR2	TM1730987 34	Pond originally located outside of the 250m survey area during the 2024 and 2025 survey season. No access available during 2026 survey.	Not surveyed	Positive (Precautionary)
231	N/A	LSR4	TM1831486 74	Pond originally located outside of the 250m survey area during the 2024 and 2025 survey season. No access available during 2026 survey.	Not surveyed	Positive (Precautionary)
232	N/A	LHL	TM1996792 25	Pond originally located outside of the 250m survey area during the 2024 and 2025 survey season. No access available during 2026 survey.	Not surveyed	Positive (Precautionary)

Pond number	Survey Date	Closest Land Parcel	Grid Reference	Description	HSI Result	Survey Result
233	N/A	LHL	TM199789223	Pond originally located outside of the 250m survey area during the 2024 and 2025 survey season. No access available during 2026 survey.	Not surveyed	Positive (Precautionary)
234	N/A	LCH	TM229879724	Pond originally located outside of the 250m survey area during the 2024 and 2025 survey season. No access available during 2026 survey.	Not surveyed	Positive (Precautionary)
235	N/A	LCH	TM229919733	Pond originally located outside of the 250m survey area during the 2024 and 2025 survey season. No access available during 2026 survey.	Not surveyed	Positive (Precautionary)
236	N/A	LCH	TM229979726	Pond originally located outside of the 250m survey area during the 2024 and 2025 survey season. No access available during 2026 survey.	Not surveyed	Positive (Precautionary)
237	N/A	LCH	TM232079707	Pond originally located outside of the 250m survey area during the 2024 and 2025 survey season. No access available during 2026 survey.	Not surveyed	Positive (Precautionary)
238	N/A	LCH	TM232339707	Pond originally located outside of the 250m survey area during the 2024 and 2025 survey season. No access available during 2026 survey.	Not surveyed	Positive (Precautionary)
239	N/A	LSR3	TM180788691	Pond originally located outside of the 250m survey area during the 2024 and 2025 survey season. No access available during 2026 survey.	Not surveyed	Positive (Precautionary)
240	N/A	LSR2	TM174828754	Pond originally located outside of the 250m survey area during the 2024 and 2025 survey season. No access available during 2026 survey.	Not surveyed	Positive (Precautionary)
241	N/A	LSR1	TM171058762	Pond originally located outside of the 250m survey area during the 2024 and 2025 survey season. No access available during 2026 survey.	Not surveyed	Positive (Precautionary)

Pond number	Survey Date	Closest Land Parcel	Grid Reference	Description	HSI Result	Survey Result
242	N/A	LSR1	TM1734687 61	Pond originally located outside of the 250m survey area during the 2024 and 2025 survey season. No access available during 2026 survey.	Not surveyed	Positive (Precautionary)
243	19/06/2026	2A	TM1769287 54	Pond originally located outside of the 250m survey area during the 2024 and 2025 survey season. Surveyed directly in 2026. Understood to have been excavated in the 1980s, this is a large pond with an abundance of aquatic and marginal vegetation; fish were evident.	Good	Negative eDNA result
244	N/A	CRC2	TM1790188 15	Pond originally located outside of the 250m survey area during the 2024 and 2025 survey season. No access available during 2026 survey.	Not surveyed	Positive (Precautionary)
245	N/A	1B	TM1759789 37	Pond originally located outside of the 250m survey area during the 2024 and 2025 survey season. No access available during 2026 survey.	Not surveyed	Positive (Precautionary)
246	30/06/2026	CRC6	TM2383992 37	Pond located outside of the 250m survey area during the 2024 and 2025 survey season. A large pond with likely permanent water; extensive marginal vegetation, but submerged aquatic vegetation was not visible.	Excellent	Positive eDNA result
247	N/A	CRC6	TM2383992 37	Pond originally located outside of the 250m survey area during the 2024 and 2025 survey season. No access available during 2026 survey.	Not surveyed	Positive (Precautionary)
248	N/A	CRC6	TM2485294 12	Pond originally located outside of the 250m survey area during the 2024 and 2025 survey season. No access available during 2026 survey.	Not surveyed	Positive (Precautionary)
249	07/05/2026	LSR1	TM1746987 66	Pond located outside of the 250m survey area during the 2024 and 2025 survey season. A large pond with likely permanent water; extensive marginal vegetation but	Poor	Negative eDNA result

Pond number	Survey Date	Closest Land Parcel	Grid Reference	Description	HSI Result	Survey Result
				submerged aquatic vegetation was not visible. Set within the grounds of a traditional farmhouse.		
250	27/06/2026	1B	TM167768 9718	Landowner provided surveyors with the location of this additional field edge pond with partial shade from trees and scrub and without aquatic vegetation.	Average	Positive eDNA result
251	N/A	1B	TM168628 9902	Landowner provided a plan which indicated the presence of this pond on adjacent land. No access available during 2026 survey.	Not assessed	Positive (Precautionary)
252	N/A	1B	TM168778 9894	Landowner provided a plan which indicated the presence of this pond on adjacent land. No access available during 2026 survey.	Not assessed	Positive (Precautionary)
253	N/A	1B	TM168918 9915	Landowner provided a plan which indicated the presence of this pond on adjacent land. No access available during 2026 survey.	Not assessed	Positive (Precautionary)
254	30/06/2026	CRC4	TM200749 1705	A shallow drainage feature associated with the Long Stratton bypass (understood to have been recently created in 2025), with a terrestrial sward; likely to be wet only on a temporary basis after rain. Inspected from accessible land in 2026.	Not assessed	Dry
255	30/06/2026	CRC4	TM200239 1470	A A shallow drainage feature associated with the Long Stratton bypass (understood to have been recently created in 2025), with a terrestrial sward; likely to be wet only on temporary basis after rain. Inspected from accessible land in 2026.	Not assessed	Dry
256	30/06/2026	CRC4	TM200239 1470	A shallow drainage feature associated with the Long Stratton bypass (understood to have been recently created in 2025) , with a terrestrial sward; likely to be wet	Not assessed	Dry

Pond number	Survey Date	Closest Land Parcel	Grid Reference	Description	HSI Result	Survey Result
				only on temporary basis after rain. Inspected from accessible land in 2026.		
257	30/06/2026	CRC4	TM200239 1470	A shallow drainage feature associated with the Long Stratton bypass (understood to have been recently created in 2025), with a terrestrial sward; likely to be wet only on temporary basis after rain. Inspected from accessible land in 2026.	Not assessed	Dry
258	17/06/2026	7D	TM234859 6698	Landowner provided surveyors with the location of this additional small ornamental pond within a residential garden.	Poor	Positive eDNA result
259	30/06/2026	4B	TM203449 3585	A shallow drainage feature associated with the Long Stratton bypass (understood to have been recently created in 2025), with a terrestrial sward; likely to be wet only on temporary basis after rain. Inspected from accessible land in 2026.	Not assessed	Dry
260	30/06/2026	4B	TM203449 3585	A shallow drainage feature associated with the Long Stratton bypass (understood to have been recently created in 2025), with a terrestrial sward; likely to be wet only on temporary basis after rain. Inspected from accessible land in 2026.	Not assessed	Dry
261	30/06/2026	4B	TM203449 3585	A shallow drainage feature associated with the Long Stratton bypass (understood to have been recently created in 2025), with a terrestrial sward; likely to be wet only on temporary basis after rain. Inspected from accessible land in 2026.	Not assessed	Dry
262	30/06/2026	4B	TM203449 3585	A shallow drainage feature associated with the Long Stratton bypass (understood to have been recently created in 2025), with a terrestrial sward; likely to be wet	Not assessed	Dry

Pond number	Survey Date	Closest Land Parcel	Grid Reference	Description	HSI Result	Survey Result
				only on temporary basis after rain. Inspected from accessible land in 2026.		
263	30/06/2026	BESS	TM169528 8543	Dry basin beneath dense tree canopy discovered by surveyors during the 2026 surveys.	Not surveyed	Dry
264	N/A	10C	TM292939 5402	Field margin pond surrounded by scrub. Location provided by landowner following the completion of the 2026 surveys.	Not assessed	Positive (Precautionary)
265	N/A	CRC9	TM283509 5654	Field margin pond adjacent to woodland habitats. Location provided by landowner following the completion of the 2026 surveys.	Not assessed	Positive (Precautionary)

4 Evaluation and Impacts

4.1 Summary

- 4.1.1 GCNs have suffered major declines in Britain over the last century, through habitat loss (both aquatic for breeding, and terrestrial for foraging, commuting, and hibernating) and habitat fragmentation. The rate of population loss is difficult to calculate; however, England is thought to support a significant number of breeding sites on a European scale (English Nature, 2001). This species is also thought to be widespread throughout Norfolk (Norfolk & Norwich Naturalists' Society, 1985-1991).
- 4.1.2 GCNs receive full protection under The Conservation of Habitats and Species Regulations 2017 (as amended) through their inclusion on Schedule 2. They are also protected under the Wildlife and Countryside Act 1981 (as amended), are nationally a Species of Principal Importance (SPI)⁴ and are a Local Biodiversity Action Plan (LBAP) Species in Norfolk (Norfolk Biodiversity, 2002).
- 4.1.3 At the end of the 2024 and 2025 survey season, of the 249 ponds identified within the Order Limits or within 250m of the Order Limits boundaries, the following results were received/recorded:
- 20 returned positive eDNA results (including five within the Site);
 - 36 returned negative eDNA results (including fifteen within the Site);
 - 17 returned inconclusive eDNA results (including six within the Site);
 - 47 ponds were not surveyed as they were dry (including eight within the Site);
 - 5 ponds are no longer present (including one within the Site);
 - 109 ponds were considered positive for GCN on a precautionary basis;
 - 7 were confirmed positive for GCN via desk study; and
 - 8 were confirmed negative for GCN via desk study.
- 4.1.4 The 20 surveyed ponds which returned positive results for GCN, included five within the Order Limits (Ponds 21, 43, 56, 57 and 62) and 15 were outside the Site boundary but within 250m (Ponds 11, 41, 70, 80, 118, 123, 124, 125, 127, 140, 142, 166, 184, 188, and 198) at the time of survey, following Order Limits amendments Pond 166 is now located beyond 250m from the Order Limits boundaries.
- 4.1.5 The seven ponds confirmed as positive via desk study are all located within the 250m survey boundary (Ponds 20, 33, 107, 173, 189, 190, and 199).

⁴ In the UK, species of principal importance are defined under the Natural Environment and Rural Communities (NERC) Act 2006. This legislation requires public authorities to consider these species in their decision-making processes to help conserve biodiversity. [Habitats and species of principal importance in England - GOV.UK](https://www.gov.uk/government/publications/habitats-and-species-of-principal-importance-in-england)

- 4.1.6 Sub-sites containing positive ponds within the Order Limits are restricted to Sub-Sites 3, 4B, 8B, and 10C, with a notable cluster of six ponds (Ponds 62, 123, 124, 125, 127, 166) present within and around Site 3 and likely represents a metapopulation dispersing between the various ponds in that vicinity.
- 4.1.7 Additional ponds and basins were identified during the 2026 surveys, including drainage features for the Long Stratton bypass, with a total of 265 ponds or other features identified. The surveys in 2026 covered mostly ponds not previously inspected but some were re-surveyed / inspected, and are summarised as follows:
- 86 ponds were surveyed to some extent in 2026. Thirteen were surveyed from adjacent land with public access, for the purposes of calculating the HSI; eight of these ponds are drainage features for the Long Stratton bypass.
 - A total of 73 ponds were therefore inspected directly in 2026, and eDNA sampling was undertaken of all ponds with open water. A total of 49 were sampled for eDNA testing, of which 19 were found to be positive and 30 negative via laboratory analysis.
 - Of the 86 ponds inspected in 2026, either directly or from adjacent land 34 were dry and considered unsuitable for supporting successful breeding by great crested newts on this basis.
- 4.1.8 The ponds with positive records from 2026 are Ponds 5, 18, 37, 38, 40, 76, 82, 100, 105, 113, 126, 128, 138, 142, 150, 173, 246, 250, and 258. Pond 193 has photographic evidence to demonstrate presence. The general locations of the positive records are summarised as follows:
- Saxlingham Nethergate (Ponds 5, 18, 105 and 258)
 - East of the B1332 (Pond 113)
 - West of and B1332 (Pond 173)
 - South-west of Hempnall (Pond 150)
 - North-west of Hempnall (Pond 100)
 - North-east of Hempnall (Ponds 76 and 82)
 - Great Moulton (Ponds 38, 37, 40 and 250)
 - Lundy Green, central and neighbouring farmland (Ponds 246, 126, 128, and 193 [photographic evidence])
 - West of the A140 north of Long Stratton (Pond 138)
 - East of the A140 north of Long Stratton (Pond 142).
- 4.1.9 **Table 3.7** (below) provides a summary of the results gathered from the 2024, 2025 and 2026 surveys:

Table 3.7: 2024 – 2026 Survey Results Summary

Survey Result	Count	Percentage ⁵
Positive eDNA result	38	14.3%
Negative eDNA result	65	24.5%
Inconclusive eDNA result	11	4.2%
Positive (precautionary approach)	78	29.4%
Confirmed positive via desk-study	7	2.6%
Confirmed negative via desk study	8	3.0%
Dry	52	19.6%
Non-existent	6	2.3%
Total	265	

4.2 Geographic Evaluation

- 4.2.1 There are 78 ponds that have not been surveyed, due to a variety of reasons summarised above, or desk study data is unavailable for these ponds, there are also 11 ponds which resulted in inconclusive laboratory results. Therefore, the presence or likely absence of GCNs cannot be determined at these locations. Consequently, a precautionary approach has been adopted and (whilst it is considered unlikely to be the case) all un-surveyed or inconclusive ponds (unless confirmed to be negative via desk study) are considered to be GCN positive. Given the extent and distribution of ponds that were successfully surveyed, the available data provides a good spatial coverage across the zone of influence for GCN (Order Limits and wider 250m buffer zone). The results indicate that GCN are uncommon, yet widely distributed within and surrounding the Order Limits, it is therefore reasonable to assume the un-surveyed ponds are likely to follow a similar pattern of presence/absence, with a comparable proportion of positive, negative and dry ponds.
- 4.2.2 Based on the assumptions above, a similar number of the un-surveyed ponds are likely to support GCN (based on these assumptions, approximately 15 of the 89 ponds discussed above would likely be found to be positive for GCN), however, these are largely expected to be associated with existing populations already confirmed through the surveys, rather than representing isolated populations. Therefore, the inclusion of the un-surveyed ponds does not negatively alter the overall evaluation or introduce any new areas requiring mitigation.
- 4.2.3 Numerous ponds are present within the Order Limits and wider survey area, however, suitable terrestrial habitat connectivity is limited and largely represented by linear habitats such as hedgerows, ditches, field margins and

⁵ Percentages are rounded to one decimal place, so the displayed figures total 99.9% due to rounding.

occasional areas of woodland/scrub. The busy A140 road (and newly built Long Stratton bypass) that runs through the western part of the Order Limits is likely to form a near-complete barrier to dispersal of GCNs from one site to the other, but the majority of the Order Limits lies to the east of the road with only the Substation Site, BESS, Subsites 1, 2 and 4A, CRC 1, CRC 2, CRC 3, and a section of CRC4 are present to the west. A variety of other A and B roads, watercourses, urban settlements are also present within and surrounding the Order Limits which also limit landscape-wide connectivity. It is considered likely that the survey area supports multiple separate populations (such as the population within and around Great Moulton), which form part of a wider meta-population within the south Norfolk area. Based on the presence of widely distributed positive ponds (and assumption of GCN presence in un-surveyed ponds), the availability (albeit limited within the Order Limits) of suitable terrestrial habitat, and taking into account the wider distribution of the species in Norfolk, the Site is considered to be of **local level importance** for GCN.

4.3 Potential Impact Pathways

- 4.3.1 The Scheme layout primarily impacts arable land (and occasional heavily grazed pastoral farmland), which are considered suboptimal for GCN. The ponds with positive GCN eDNA are located within field margins and boundary woodlands. Therefore, the suitable habitats immediately surrounding the ponds, such as grassland margins, hedgerows and woodland, have the potential to support GCN in their terrestrial phase. Therefore, it is considered likely that GCNs will be at least occasionally present within these suitable on-Site terrestrial habitats.
- 4.3.2 The Scheme avoids impacts upon suitable aquatic habitats, and additionally avoids permanent terrestrial habitat impacts within 50m of positive GCN ponds (or those considered to be positive on a precautionary basis) within Solar PV Module Sites. Suitable terrestrial habitats such as woodland, hedgerows, lowland fen, scrub are largely avoided and incorporated within the Scheme design. With only potential minor direct impacts upon terrestrial habitats suitable for GCN during the construction (and potentially decommissioning phases) such as the removal of short hedgerow sections (maximum of 10m) to accommodate internal haul routes/access tracks and the temporary and localised CRC works (construction phase). See **Design Principles, Parameters and Commitments [EN0110014/APP/7.18]**.
- 4.3.3 The Scheme will include the provision (and long-term management) of extensive areas of suitable terrestrial grassland mosaics/woodland/scrub habitats suitable for use by terrestrial GCN, creation of seven ponds (identified as Norfolk ghost ponds) and the enhancement (and long-term management) of existing pond habitats within the Solar PV Sites for the benefit of GCN, thereby providing much increased opportunities for the species (and amphibians in general), ensuring that the favourable conservation status of GCN within the Site is maintained. Further information regarding habitat delivery is provided within the **Outline Landscape and Ecological Management Plan (oLEMP) [EN0110014/APP/7.4]**.

4.4 Mitigation and Avoidance Measures

- 4.4.1 No ponds will be lost to the Scheme; however, GCN are potentially present within suitable terrestrial habitats within the Order Limits. In the absence of avoidance measures the Schemes construction and decommissioning phases may potentially result in direct impact to GCN through injury, mortality and temporary loss of terrestrial habitat. Therefore, embedded mitigation measures will be adopted through sensitive design, and species-specific precautionary working measures. See the **outline Protected Species Mitigation Strategy (oPSMS): ES, Vol III, Appendix 8.10** for further information.
- 4.4.2 Habitat enhancement measures will be informed by the **oLEMP [EN0110014/APP/7.4]**, and will include native shrub, hedgerow, and woodland planting, and new grassland to be managed to provide suitable habitat for amphibians (including GCN), creating structurally diverse grassland and hedgerow and woodland/scrub areas to provide shelter and foraging and opportunities and connectivity around the Order Limits. Additional proposed pond enhancements and creation will enhance opportunities for breeding GCN within the Order Limits.
- 4.4.3 An assessment of potential impacts of the Scheme on GCN has been provided within **Chapter 8 Ecology and Biodiversity [EN0110014/APP/6.1.8]** and an outline mitigation strategy is provided within the **outline Protected Species Mitigation Strategy (oPSMS): ES, Vol III, Appendix 8.10**, which details appropriate precautionary working measures required to avoid impacts upon individual GCN.
- 4.4.4 Based on the habitat avoidance commitments and habitat enhancement information provided within the **Design Principles, Parameters and Commitments [EN0110014/APP/7.18]** and the **Outline Landscape and Ecological Management Plan (oLEMP) [EN0110014/APP/7.4]** and in adherence to the **oPSMS** temporary impacts upon GCN will be reasonably avoided through sensitive design, and species-specific precautionary working measures.
- 4.4.5 In the unlikely event that following from the provision of the final LEMP and CEMP (secured as a DCO commitment) the risk of likely GCN impacts increases, GCN specific consultation will be undertaken with Natural England to determine the most appropriate derogation licensing route for the Scheme.
- 4.4.6 If a derogation licence is required prior to the proposed works commencing, one of the following three options can be pursued:
- GCN EPSML issued by Natural England prior to any works commencing to suitable terrestrial habitats with 250m of confirmed (or assumed) GCN ponds, unless considerable barriers are present that prevent GCN from accessing the land (such as the A140 corridor);

- Focused/localised works undertaken in adherence with the CL33 Great Crested Newt Low Impact Class Licence⁶;
 - Enrolment within the Natural England (Norfolk) GCN DLL scheme⁷; or
 - Contributing to the emerging Nature Restoration Fund⁸; which would be used by Natural England to implement the Environmental Delivery Plan that Natural England delivers in partnership with a range of local organisations and bodies for the benefit of wider biodiversity (including GCN).
- 4.4.7 If required, enrolment within one of the above options will ensure that the favourable conservation status of the species will be maintained. Otherwise, reasonable avoidance measures (RAMs) for amphibians will be sufficient to minimise any potential impacts on individual GCN.

4.5 Additional Survey Requirements

- 4.5.1 Given the large scale of the Order Limits and in adherence with Natural England's EPSML Policy 4⁹ (*Appropriate and relevant surveys where the impacts of development can be confidently predicted*), in the unlikely event that a GCN EPSML is required, it is not considered appropriate to undertake traditional population surveys as:
- The costs or delays associated with carrying out standard survey requirements would be disproportionate to the additional certainty that it would bring;
 - The ecological impacts of development upon GCN can be predicted with sufficient certainty; and
 - Mitigation will ensure that the licensed activity does not detrimentally affect the conservation status of the local population of GCN.
- 4.5.2 Following the 2026 season, a total of 78 ponds located within 250m of the Order Limits have not been surveyed for GCN for reasons discussed in the sections above or desk study data is unavailable for these ponds and 11 ponds returned inconclusive eDNA results. However, the combined data is considered to provide a strong spatial coverage of the Order Limits and surrounding environment. It is therefore considered that further survey data is not required to inform this process.

⁶ <https://www.gov.uk/guidance/great-crested-newts-apply-for-a-mitigation-class-licence-for-low-impact-work-cl33>

⁷ <https://www.gov.uk/government/publications/great-crested-newts-district-level-licensing-schemes-for-developers>

⁸ <https://naturalengland.blog.gov.uk/2025/09/11/accelerating-development-and-nature-recovery-a-natural-england-perspective-on-the-nature-restoration-fund/>

⁹ <https://www.gov.uk/government/consultations/wildlife-licensing-comment-on-new-policies-for-european-protected-species-licences>

5 References

ARG UK. (2010). *ARG UK Advice Note 5: GCN Habitat Suitability Index*. Amphibian and Reptile Groups of the United Kingdom.

Biggs, J., Ewald, N., Valentini, A., Gaboriaud, C., Griffiths, R.A., Foster J., Wilkinson, J., Arnett, A., Williams P. and Dun, F. (2014). *Analytical and Methodological Development for Improved Surveillance of the GCN. Appendix 5. Technical Advice Note for Field and Laboratory Sampling of GCN (Triturus cristatus) Environmental DNA*. Freshwater Habitats Trust, Oxford.

British Standards. (2013). *42020:2013 Biodiversity - Code of Practice for Biodiversity and Development*. London

CIEEM. (2017). *Guidelines for Ecological Report Writing, 2nd edition*. Chartered Institute of Ecology and Environmental Management, Winchester.

CIEEM. (2018). *Guidelines for Ecological Impact Assessment in the UK and Ireland: Terrestrial, Freshwater, Coastal and Marine version 1.2*. Chartered Institute of Ecology and Environmental Management, Winchester.

Ministry for Housing, Communities and Local Government (2025). *National Planning Policy Framework*. [Online] Available at: <https://www.gov.uk/government/publications/national-planning-policy-framework--2> [accessed December 2025].

English Nature (2001) *Great Crested Newt Mitigation Guidelines*. Natural England, Peterborough.

Natural England (2015) *Great Crested Newt: Surveys and Mitigation for Development Projects*. Natural England, Peterborough.

Natural England. (2016). *Wildlife Licensing: Comment on New Policies for European protected species licences*. [Online] Available at: <https://www.gov.uk/government/consultations/wildlife-licensing-comment-on-new-policies-for-european-protected-species-licences> [accessed December 2025].

Natural England. (2017). *Method Statement Template for Great Crested Newt Mitigation Licence*. [Online] Available at: <https://www.gov.uk/government/publications/great-crested-newts-apply-for-a-mitigation-licence> [accessed December 2025].

Natural England. (2025). *Great Crested Newts: District Level Licensing Schemes for Developers, Ecologists and Landowners*. [Online] Available at: <https://www.gov.uk/government/publications/great-crested-newts-district-level-licensing-schemes-for-developers> [accessed December 2025].

Norfolk Biodiversity (2002) *Norfolk Biodiversity Action Plan Great Crested Newt (Triturus cristatus)*. [Online] available at

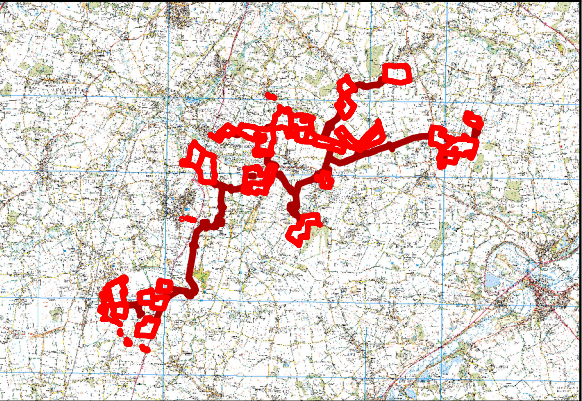
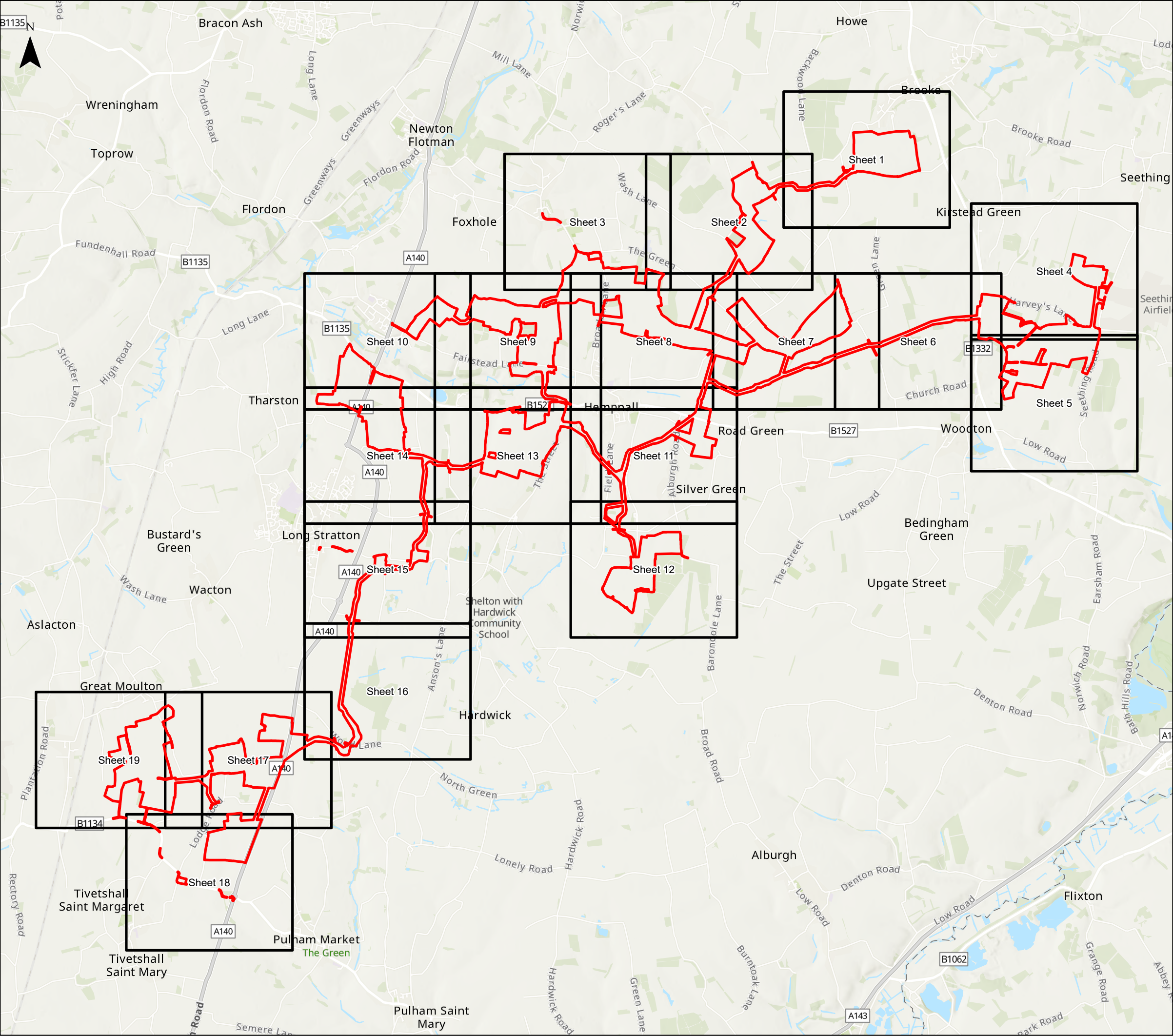
<https://www.norfolkbiodiversity.org/habitats-and-species/amphibians/>
[accessed December 2025].

Norfolk & Norwich Naturalists' Society. (1985-1991). *Transactions of Norfolk & Norwich Naturalists' Society* (vols 27-29).

Oldham R.S., Keeble J., Swan M.J.S. & Jeffcote M. (2000). Evaluating the suitability of habitat for the GCN (*Triturus cristatus*). *Herpetological Journal* 10 (4), 143-155.

6 Figures

Figure 1: Pond Locations and eDNA Survey Results Plans



- Legend**
- Order Limits
 - Sheet Index

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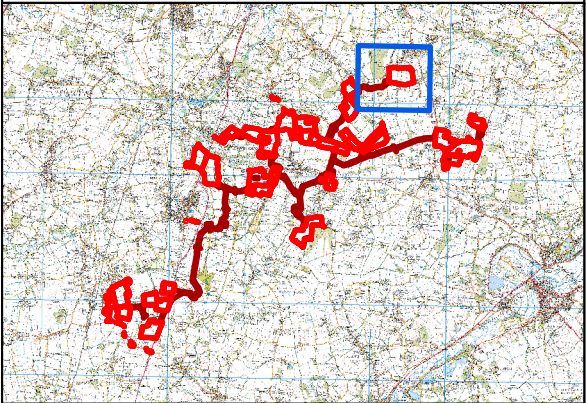
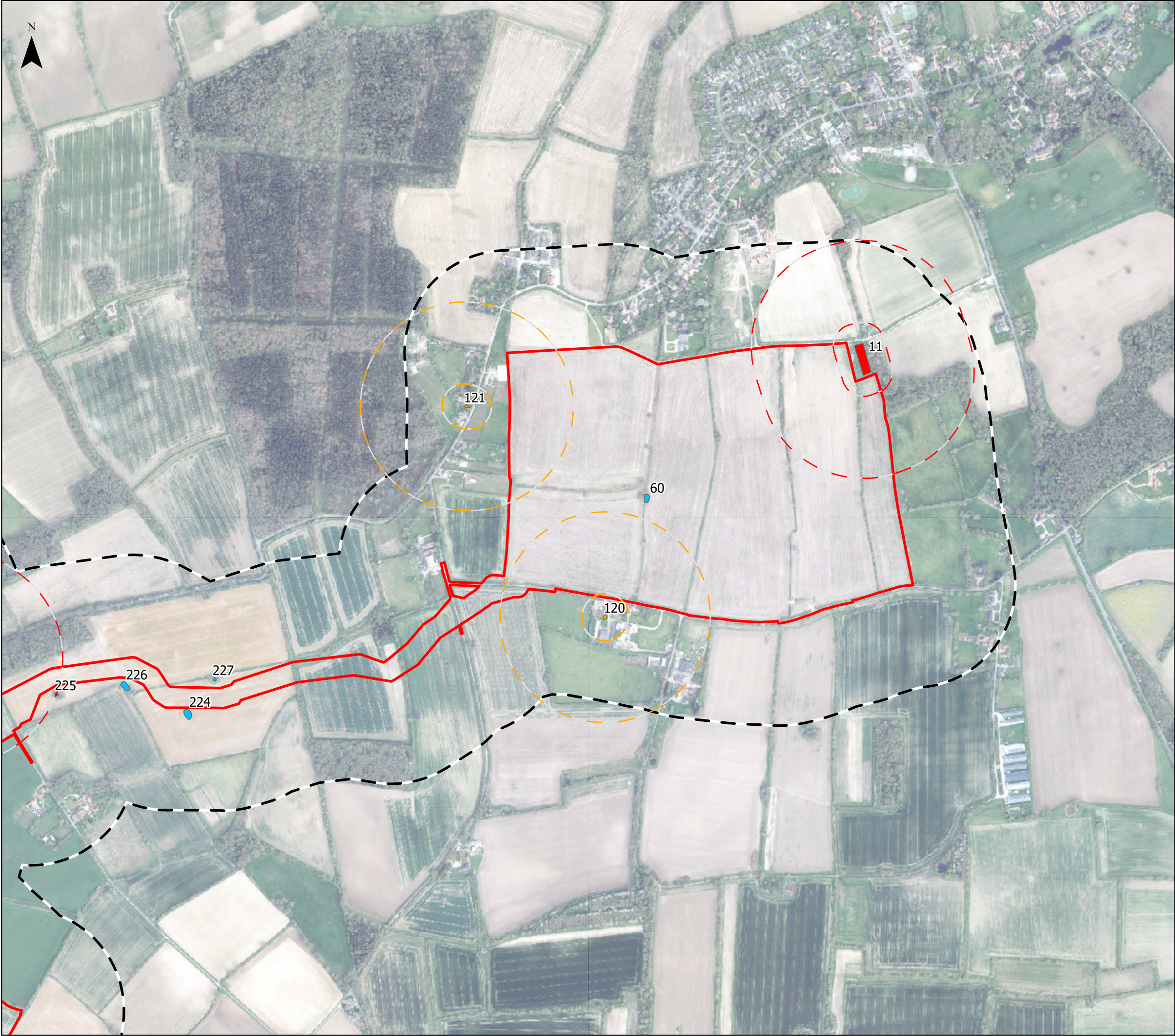
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Figure 1: Pond Locations and eDNA Survey Results Plans Overview

Revision 2





- Legend**
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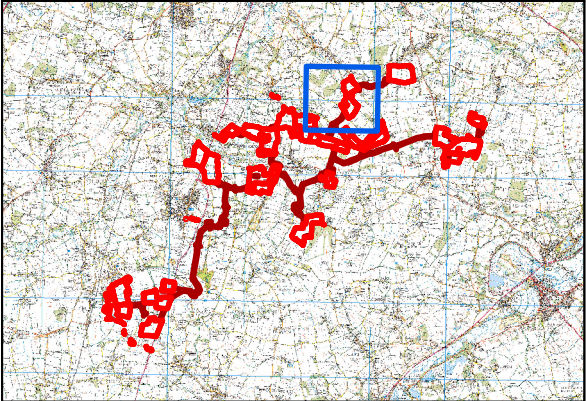
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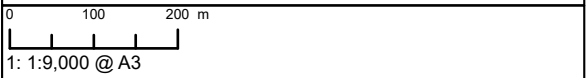
Figure 1: Pond Locations and eDNA Survey Results Plans

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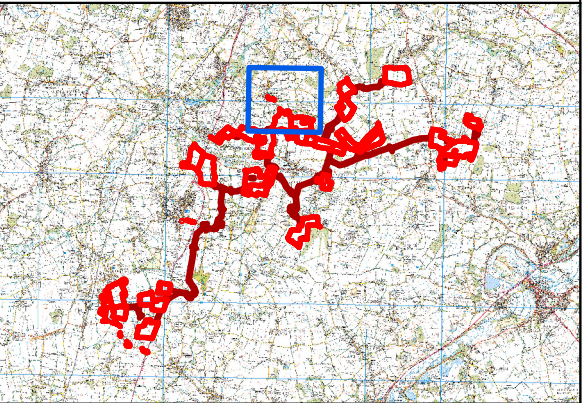
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Figure 1: Pond Locations and eDNA Survey Results Plans

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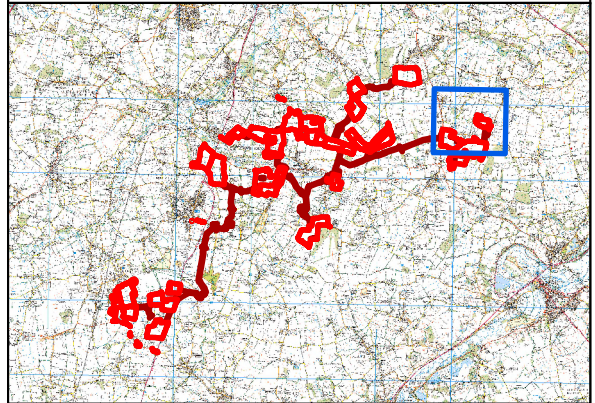
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Figure 1: Pond Locations and eDNA Survey Results Plans

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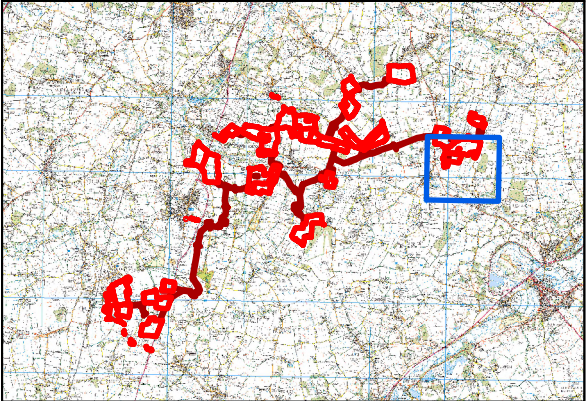
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Figure 1: Pond Locations and eDNA Survey Results Plans

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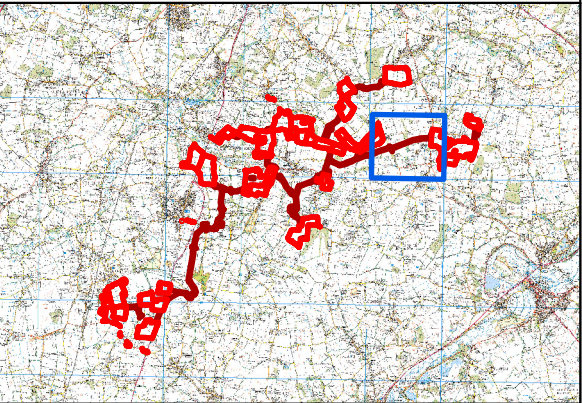
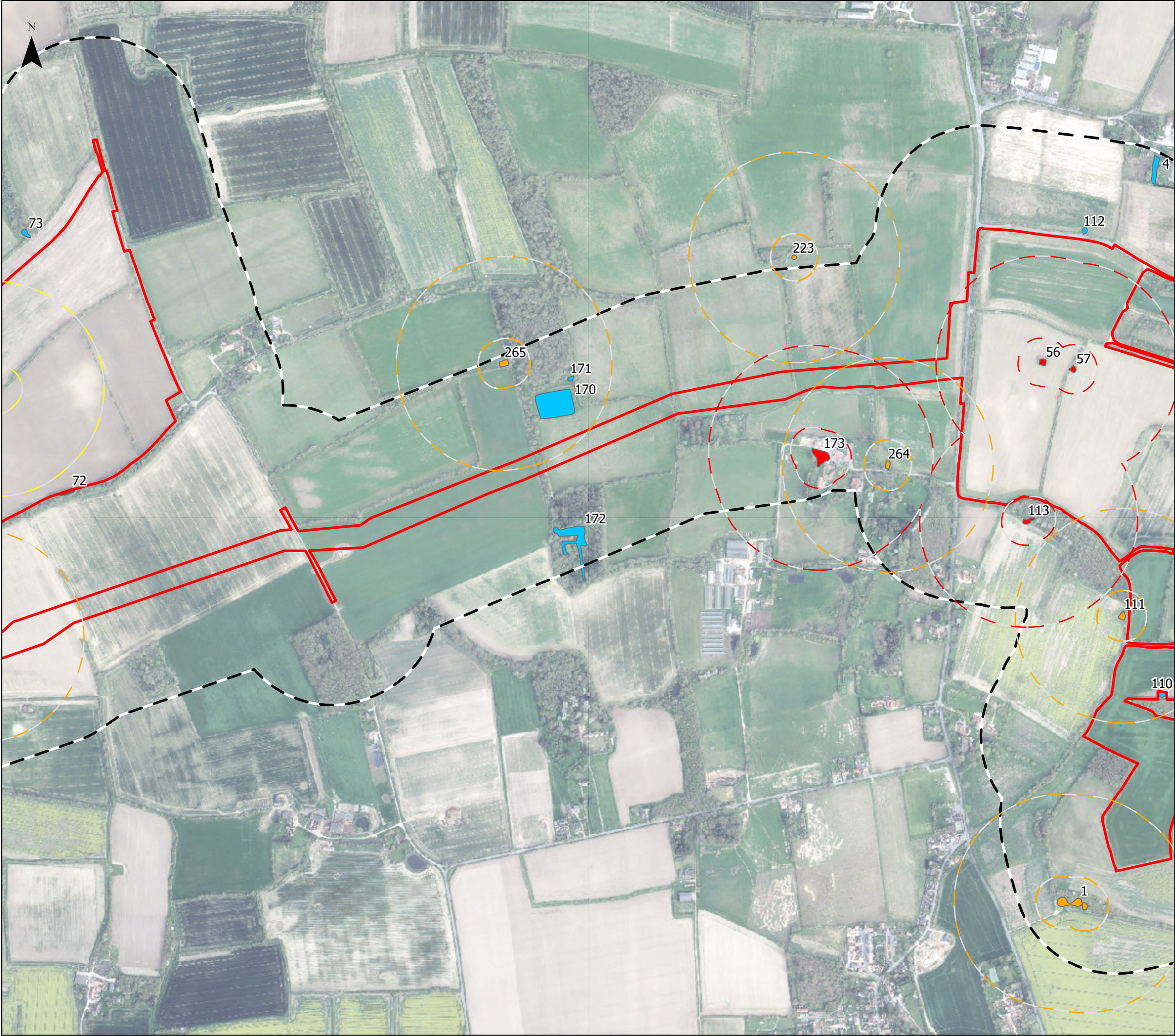
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Figure 1: Pond Locations and eDNA Survey Results Plans

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Legend

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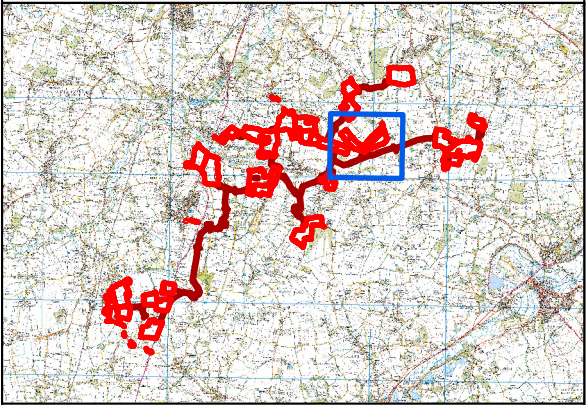
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Figure 1: Pond Locations and eDNA Survey Results Plans

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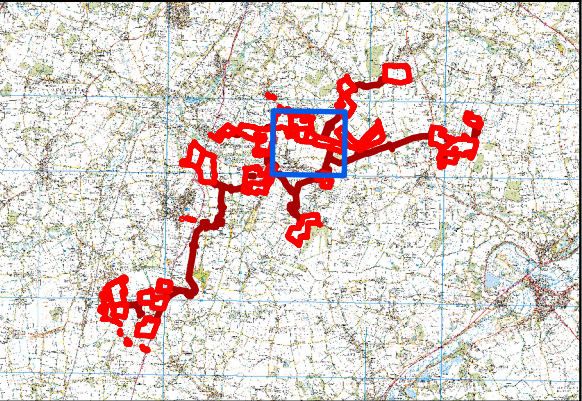
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Figure 1: Pond Locations and eDNA Survey Results Plans

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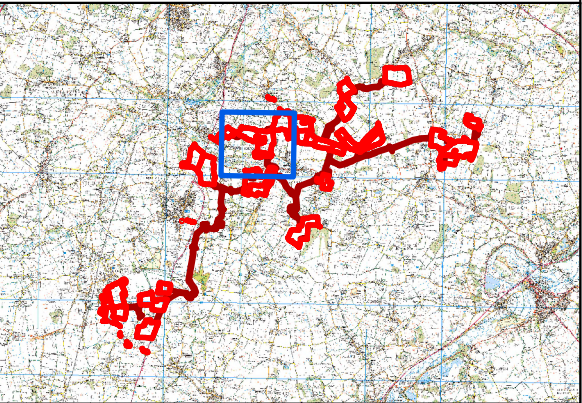
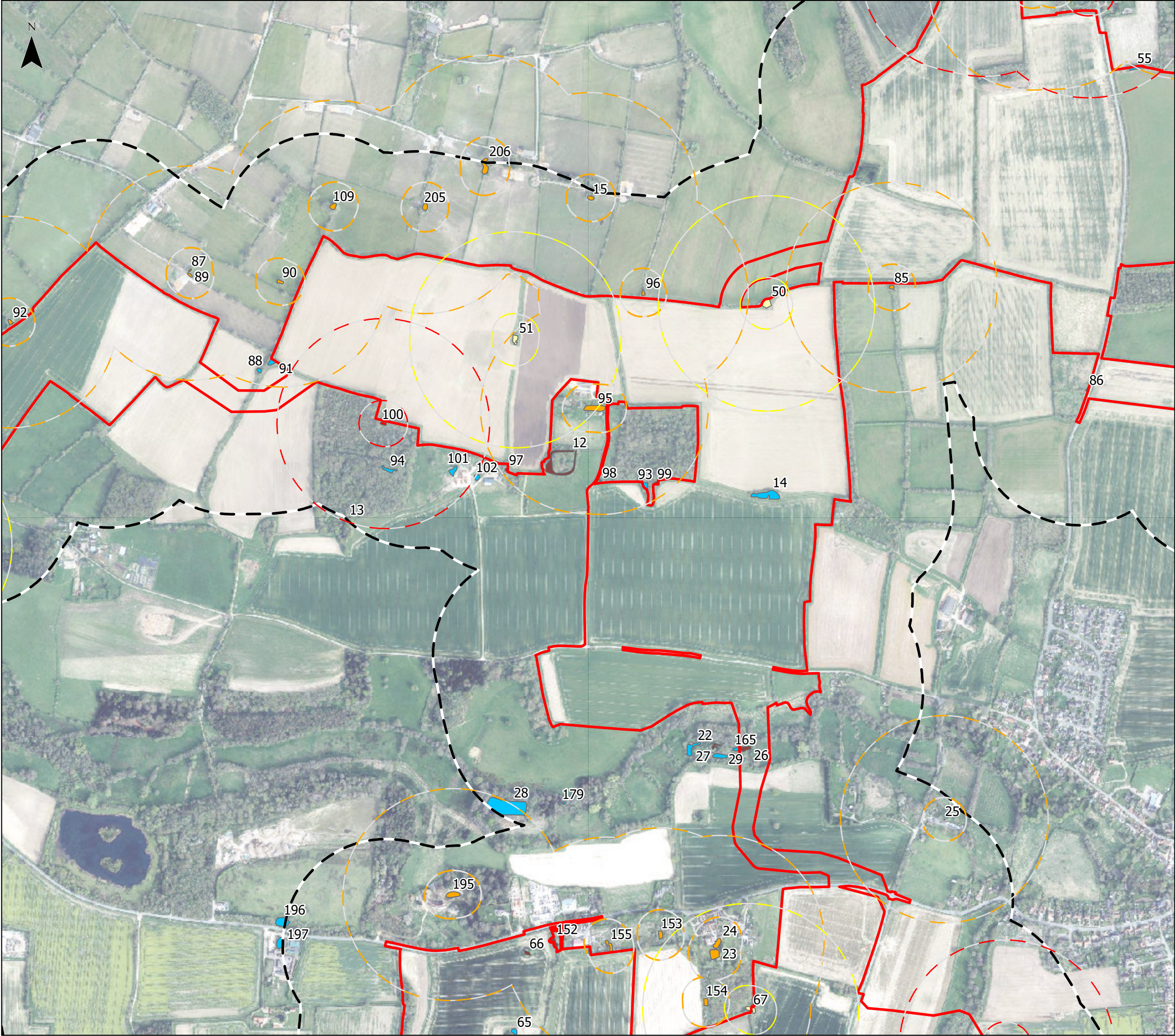
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Figure 1: Pond Locations and eDNA Survey Results Plans

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Legend

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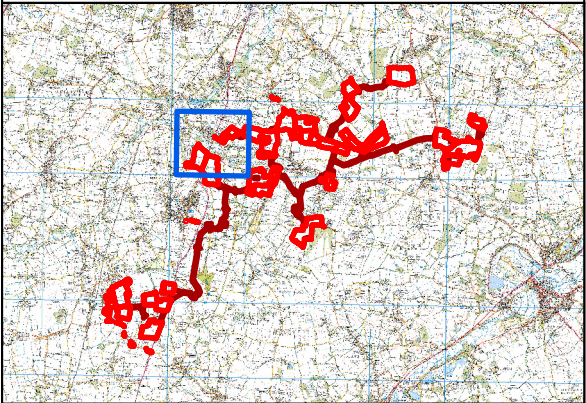
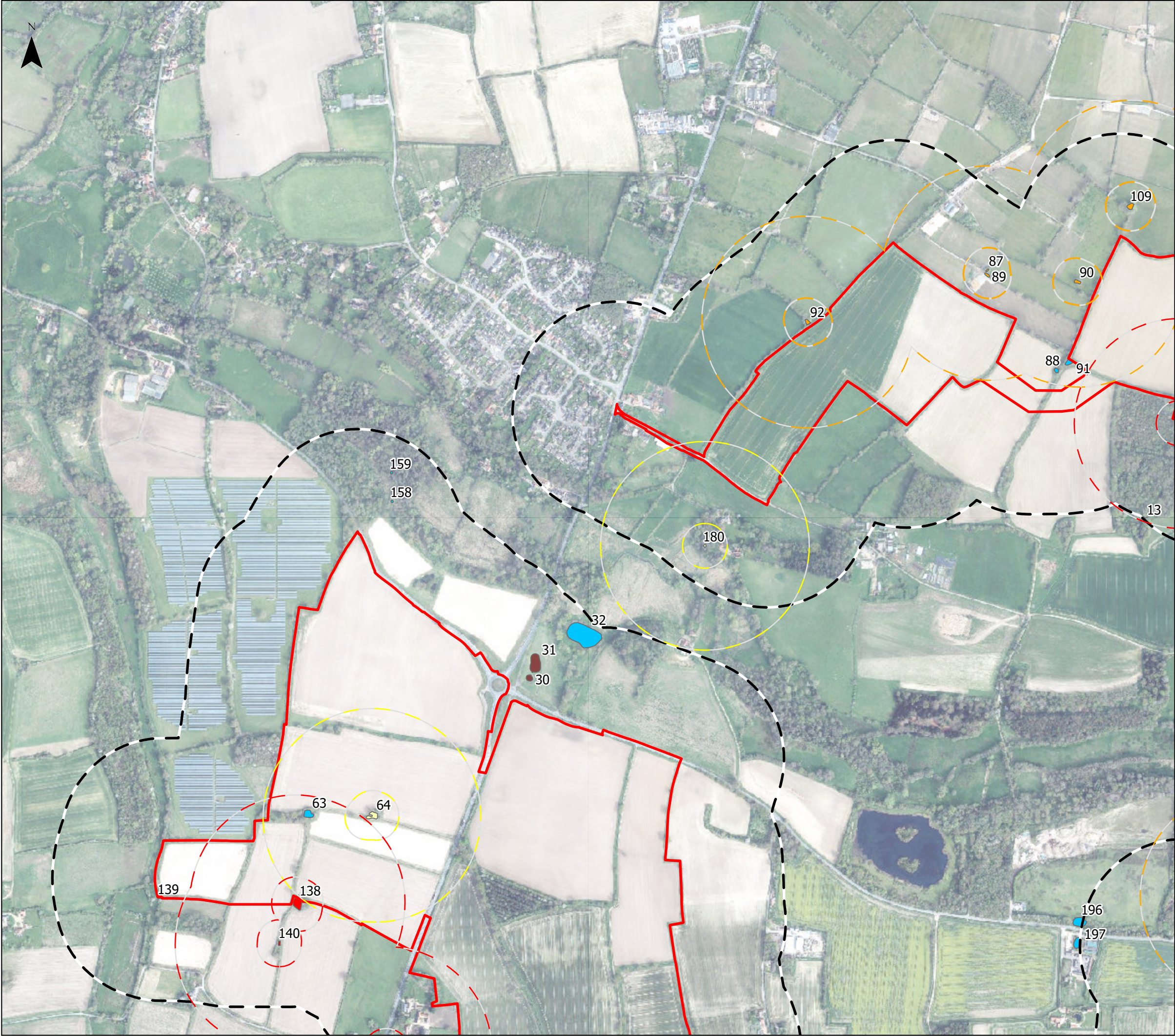
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Figure 1: Pond Locations and eDNA Survey Results Plans

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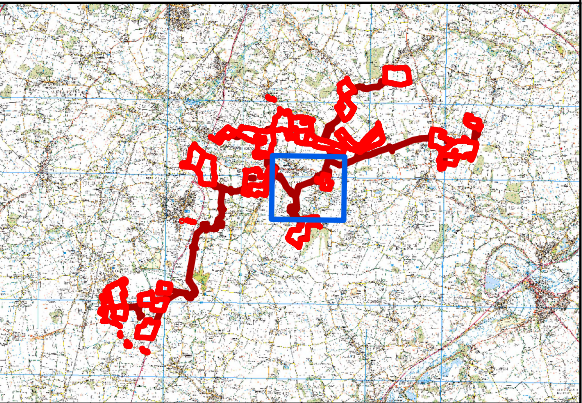
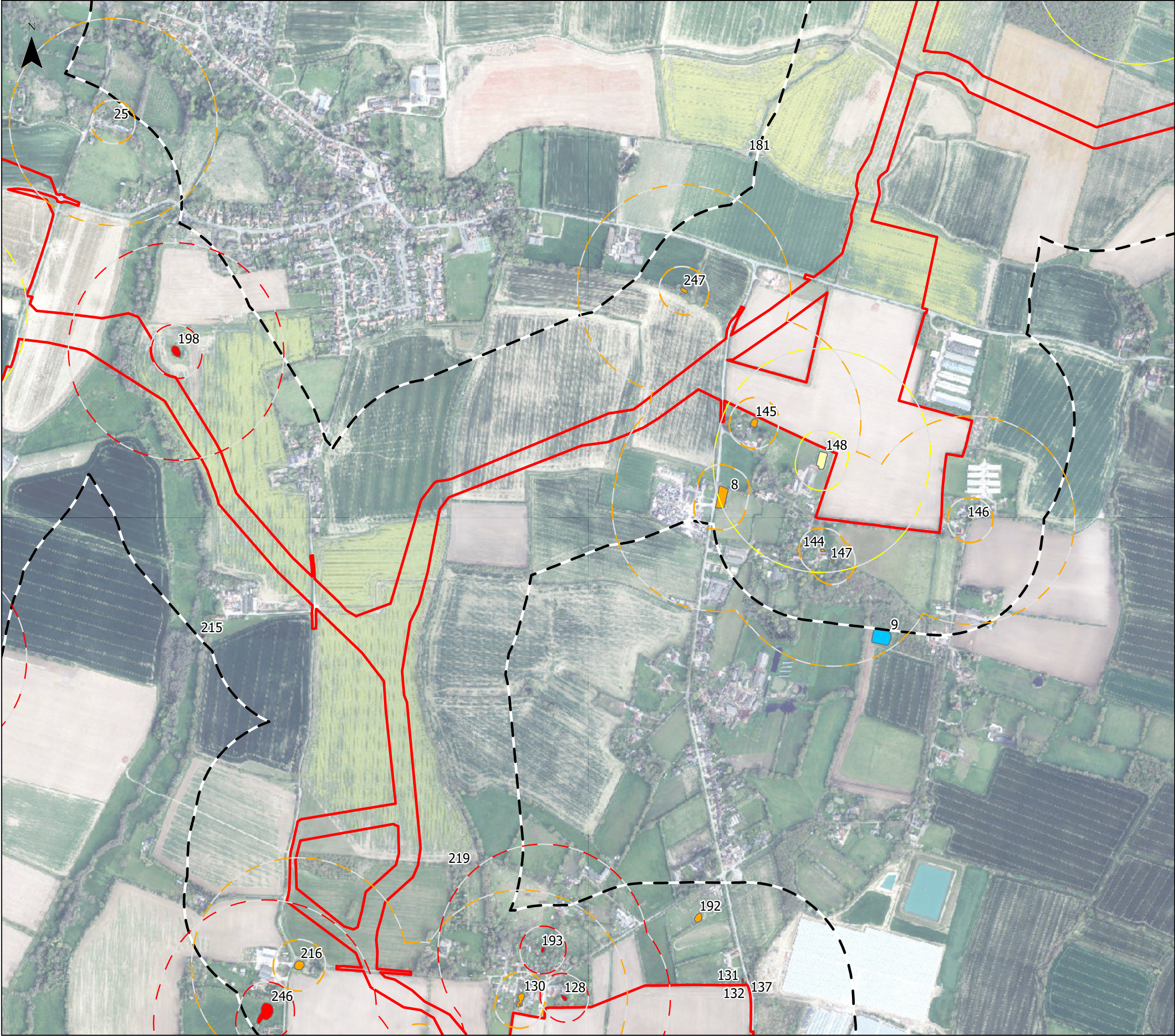
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Figure 1: Pond Locations and eDNA Survey Results Plans

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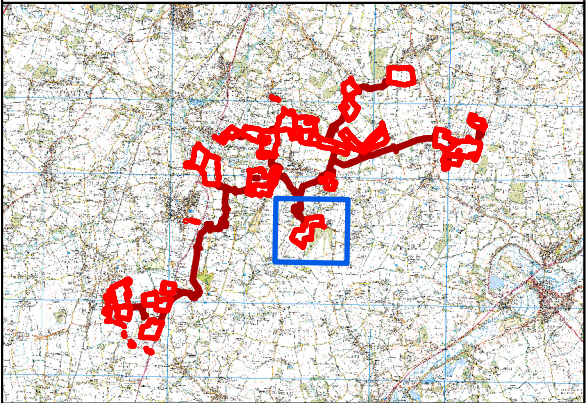
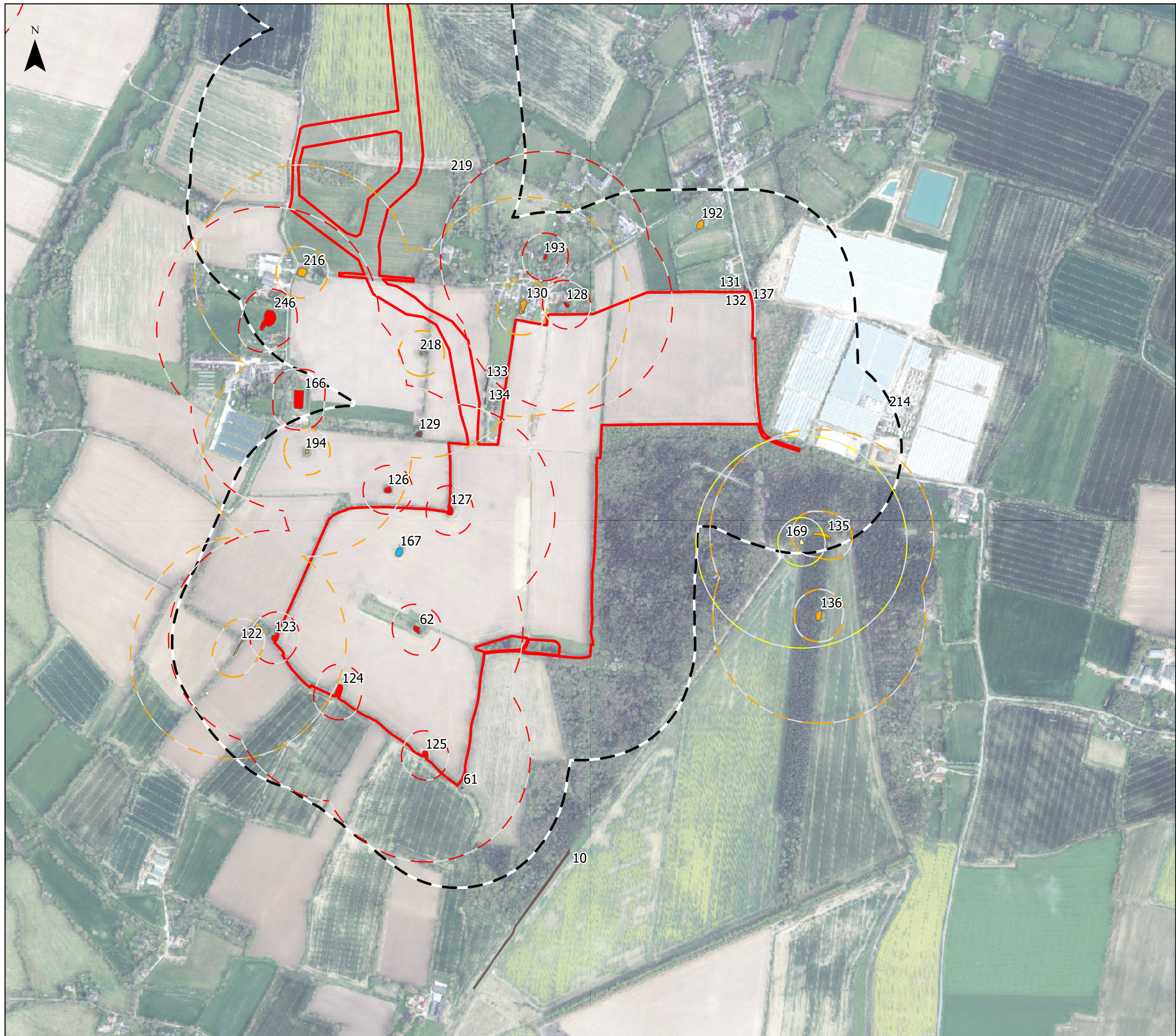
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Figure 1: Pond Locations and eDNA Survey Results Plans

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Legend

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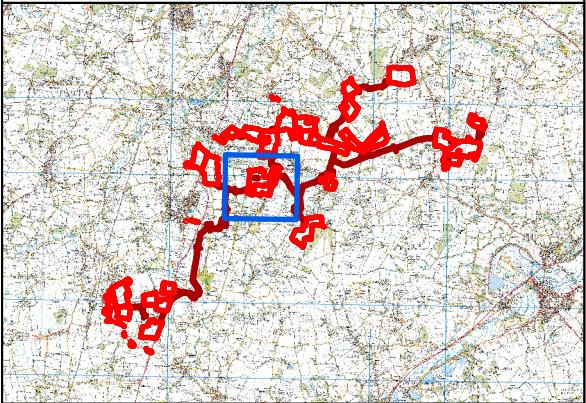
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Figure 1: Pond Locations and eDNA Survey Results Plans

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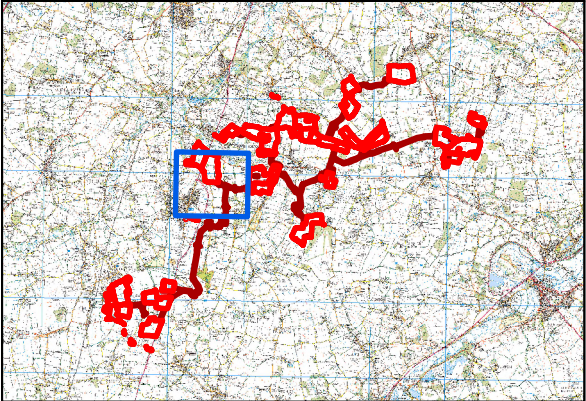
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Figure 1: Pond Locations and eDNA Survey Results Plans

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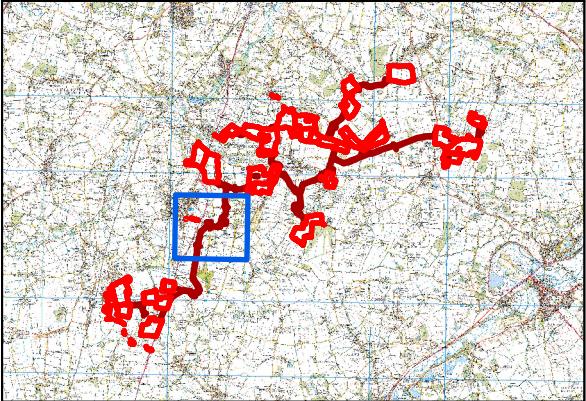
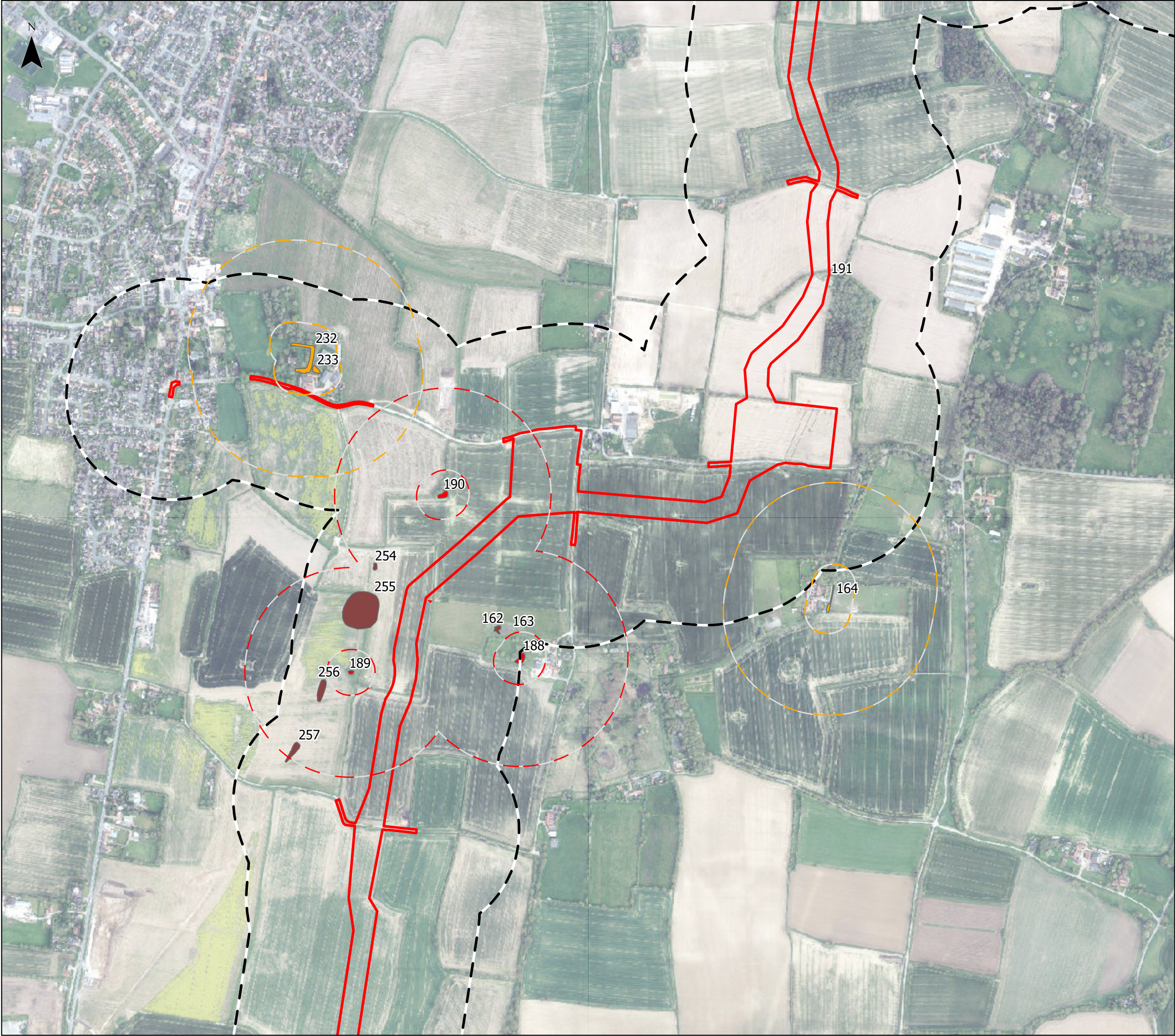
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APFP Regulation: 5(2)(a)	Application Doc No. APP/6.3.8.3
Ref: Appendix 8.3	Date: 8/17/2026
Drawn: AD	Checked: DF

Figure 1: Pond Locations and eDNA Survey Results Plans

Sheet 14 of 19
Revision 2



- Legend**
- Order Limits
 - 250m Buffer from Order Limits
 - 50m and 250m Buffer from Positive GCN Pond
 - 50m and 250m Buffer from Unsurveyed GCN Pond
 - 50m and 250m Buffer from Inconclusive GCN Pond
 - Dry Ponds
 - Inconclusive GCN results pond (precautionary positive approach)
 - Negative GCN results pond
 - Un-surveyed ponds (precautionary positive approach)
 - Positive GCN results pond

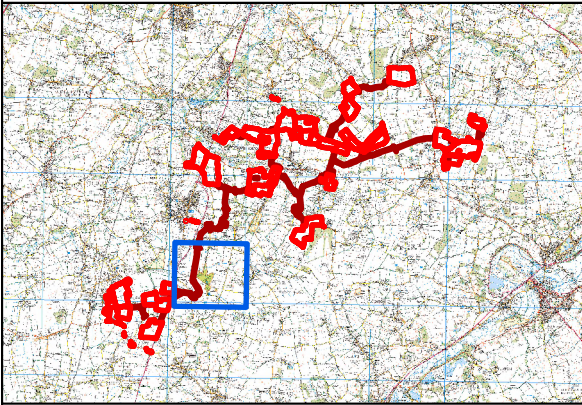
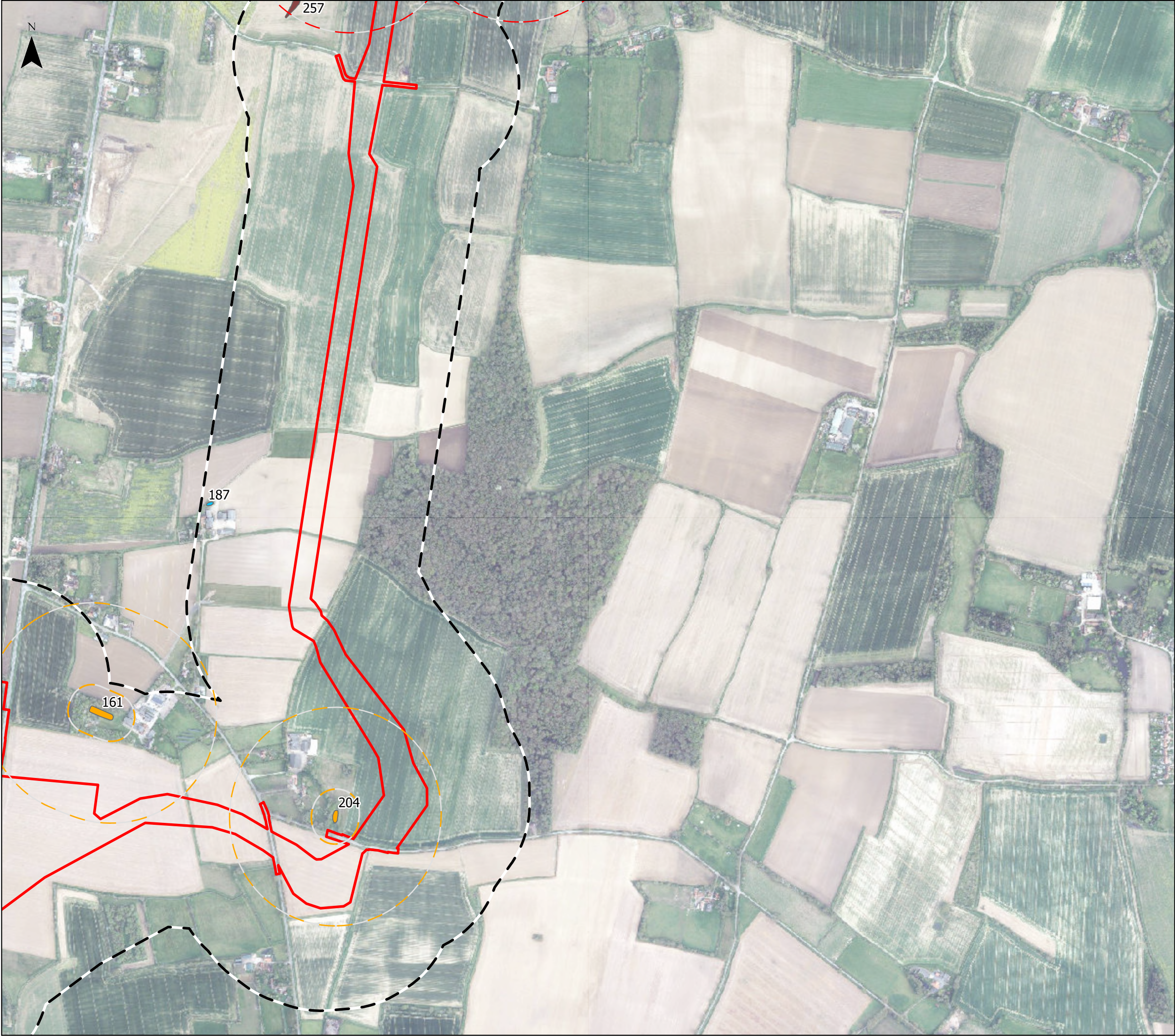
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Figure 1: Pond Locations and eDNA Survey Results Plans

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Revision 2



Legend

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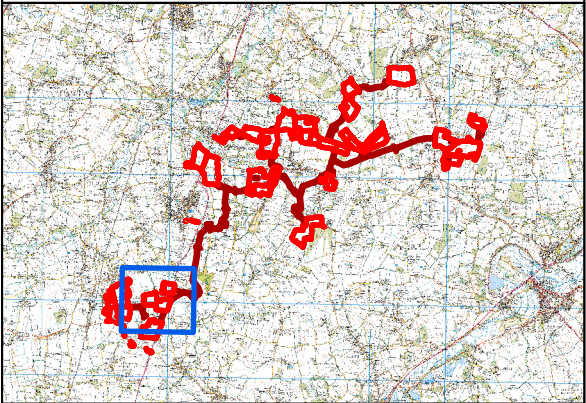
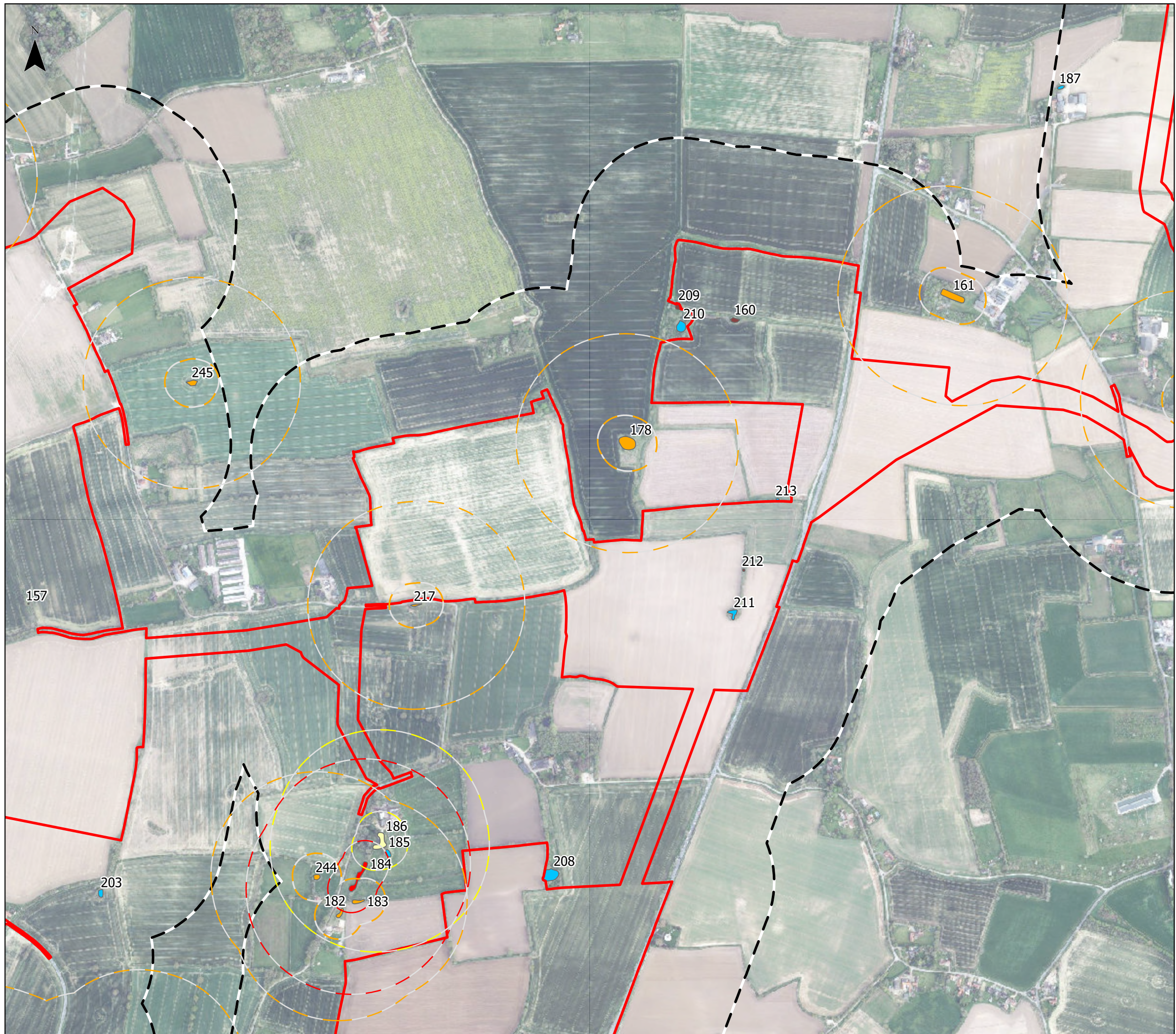
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Figure 1: Pond Locations and eDNA Survey Results Plans

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Revision 2



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 - Positive GCN results pond

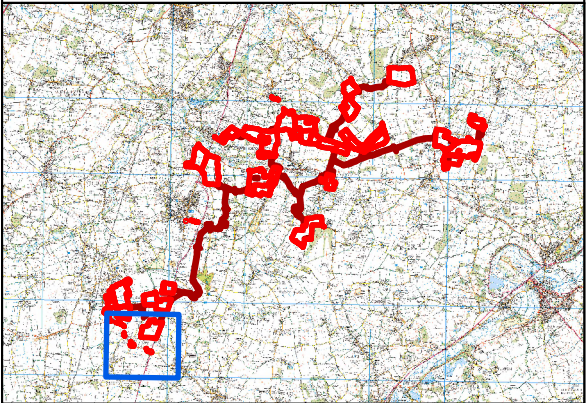
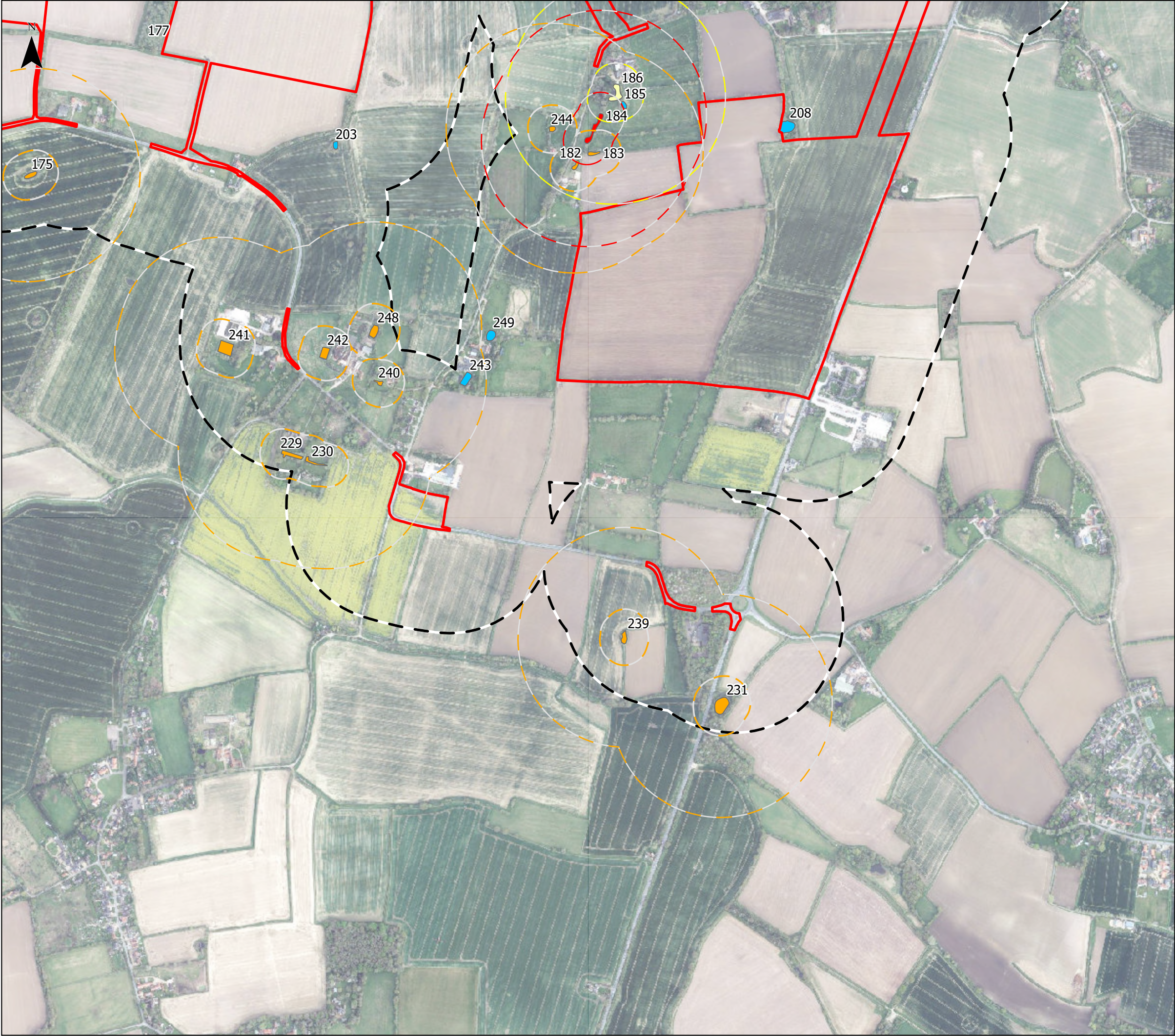
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Drawn: AD	Checked: DF

Figure 1: Pond Locations and eDNA Survey Results Plans

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Revision 2



Legend

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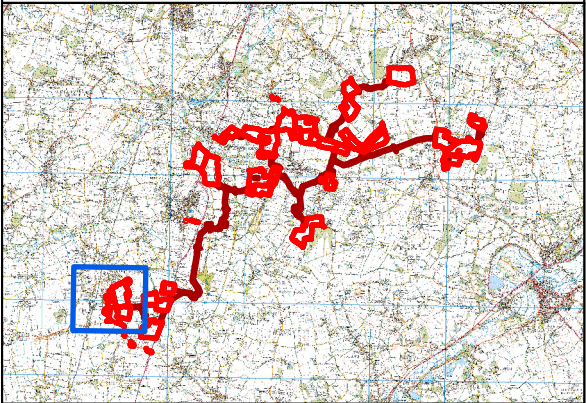
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Drawn: AD	Checked: DF

Figure 1: Pond Locations and eDNA Survey Results Plans

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Revision 2



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Drawn: AD	Checked: DF

Figure 1: Pond Locations and eDNA Survey Results Plans

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Revision 2

Annex A: Photographs

Photograph 1

Pond 140. eDNA survey results show that this pond supports great crested newts. The pond returned an Average HSI score and is located within 250m buffer of 4A.



Photograph 2

Pond 56. eDNA survey revealed it to support great crested newts. The HSI result was Below Average. The pond is located within parcel 10C.



Photograph 3

Pond 118. eDNA survey revealed this pond to support great crested newts, and it received a Good HSI result. It is located within the 250m buffer from the Site.



Photograph 4

Pond 123. eDNA survey revealed this pond to support great crested newts and it scored an Average HSI result. The pond is in the 250m buffer from the Site and has good connectivity to the habitats on-Site.



Photograph 5

Pond 124. eDNA survey found this pond to support great crested newts. It is located within the 250m buffer from the Site.



Photograph 6

Pond 184. eDNA survey revealed it to support great crested newts, the HSI results rating was Average.



Photograph 7

An example of a dry basin (Pond 176).



Photograph 8

An example of attenuation basin on the Long Stratton bypass (Pond 256).



Photograph 9

An example of pond positive by eDNA (Pond 138), and rated as Below Average using the Habitat Suitability Index.



Photograph 10

An example of pond positive by eDNA (Pond 76), and rated as Below Average using the Habitat Suitability Index.



Annex B: Habitat Suitability Index Results

Pond number	Geographic location	Pond area m ²	Permanence	Water quality	Shade	Waterfowl	Fish	Pond count	Terrestrial habitat	Macro-phytes	Resulting HSI Score	Pond suitability
003	Zone A	<50	Rarely dries	Poor	70	Minor	Absent	11	Moderate	15	0.55	Below average
004	Zone A	500	Sometimes dries	Poor	0	Minor	Possible	13+	Moderate	5	0.67	Average
005	Zone A	150	Sometimes	Good	30	Absent	Absent	24	Good	80	0.81	Excellent
008	Zone A	200	Never	Poor	10	Absent	Possible	25	Poor	5	0.63	Average
009	Zone A	375	Never	Moderate	10	Minor	Minor	40	Moderate	5	0.68	Average
022	Zone A	400	Sometimes dries	Moderate	90	Absent	Absent	11	Good	0	0.57	Below average
027	Zone A	150	Rarely dries	Good	95	Minor	Absent	13+	Good	55	0.74	Good
028	Zone A	3007	Never dries	Bad	85	Minor	Absent	13+	Good	5	0.49	Poor
029	Zone A	300	Rarely dries	Bad	100	Absent	Absent	12	Good	5	0.46	Poor
032	Zone A	>2000	Never dries	Poor	100	Major	Major	13+	Good	10	0.27	Poor
034	Zone A	50	Rarely	Good	100	Absent	Absent	16	Poor	10	0.55	Below Average
037	Zone A	50	Sometimes	Moderate	10	Absent	Absent	25	Moderate	10	0.62	Average
038	Zone A	50	Sometimes	Good	10	Absent	Absent	25	Moderate	10	0.65	Average
039	Zone A	400	Never	Good	10	Absent	Major	22	Moderate	20	0.55	Below Average
040	Zone A	12	Annually	Moderate	0	Absent	Absent	25	Moderate	70	0.54	Below Average
045	Zone A	150	Rarely dries	Poor	95	Minor	Absent	9	Moderate	0	0.53	Below average
054	Zone A	50	Sometimes dries	Poor	95	Absent	Absent	3	Moderate	20	0.47	Poor
055	Zone A	<50	Sometimes dries	Poor	100	Absent	Absent	11	Poor	0	0.39	Poor
056	Zone A	150	Rarely dries	Poor	100	Absent	Absent	8	Moderate	0	0.53	Below average
060	Zone A	150	Rarely dries	Poor	80	Major	Possible	3	Moderate	0	0.35	Poor
067	Zone A	100	Rarely dries	Good	0	Absent	Possible	8	Good	55	0.79	Good
073	Zone A	80	Sometimes	Good	100	Absent	Absent	32	Good	0	0.58	Below Average
074	Zone A	<50	Dries annually	Bad	100	Absent	Absent	4	Moderate	0	0.24	Poor
076	Zone A	30	Sometimes	Good	100	Absent	Absent	17	Moderate	0	0.52	Below Average
077	Zone A	30	Sometimes	Good	100	Absent	Absent	18	Moderate	0	0.52	Below Average
078	Zone A	1000	Never dries	Poor	90	Minor	Minor	11	Poor	0	0.55	Below average
079	Zone A	80	Sometimes	Good	100	Absent	Absent	29	Good	0	0.58	Below Average
080	Zone A	250	Rarely dries	Poor	95	Minor	Absent	12	Poor	0	0.43	Poor
081	Zone A	80	Sometimes	Good	100	Absent	Absent	23	Good	0	0.58	Below Average
082	Zone A	250	Never dries	Poor	80	Minor	Possible	9	Poor	0	0.57	Below average
083	Zone A	350	Rarely dries	Poor	100	Minor	Possible	13+	Moderate	0	0.58	Below average
084	Zone A	500	Rarely dries	Poor	100	Minor	Possible	12	Poor	0	0.56	Below average
093	Zone A	150	Never dries	Moderate	90	Minor	Possible	12	Moderate	50	0.62	Average
094	Zone A	50	Sometimes	Good	95	Absent	Absent	27	Good	0	0.58	Below Average
098	Zone A	40	Annually	Moderate	25	Absent	Absent	34	Good	0	0.54	Below Average
100	Zone A	60	Annually	Moderate	90	Absent	Absent	24	Good	0	0.49	Poor
105	Zone A	80	Rarely	Good	80	Absent	Absent	20	Poor	5	0.63	Average
112	Zone A	30	Annually	Good	100	Absent	Absent	21	Good	0	0.46	Poor
113	Zone A	120	Sometimes	Moderate	90	Absent	Absent	36	Moderate	50	0.65	Average
117	Zone A	50	Annually	Moderate	100	Absent	Absent	16	Poor	0	0.41	Poor
118	Zone A	200	Never dries	Moderate	0	Minor	Possible	13+	Moderate	30	0.73	Good

Pond number	Geographic location	Pond area m ²	Permanence	Water quality	Shade	Waterfowl	Fish	Pond count	Terrestrial habitat	Macro-phytes	Resulting HSI Score	Pond suitability
119	Zone A	400	Never dries	Moderate	80	Minor	Minor	13+	Moderate	20	0.67	Average
123	Zone A	<50	Never dries	Good	70	Absent	Absent	13+	Moderate	5	0.62	Average
124	Zone A	50	Never dries	Good	0	Absent	Absent	13+	Moderate	30	0.72	Good
125	Zone A	<50	Never dries	Moderate	0	Absent	Absent	13+	Moderate	60	0.67	Average
126	Zone A	120	Sometimes	Good	90	Absent	Absent	29	Good	0	0.64	Average
127	Zone A	150	Never dries	Moderate	0	Minor	Possible	4	Moderate	10	0.66	Average
128	Zone A	80	Never	Moderate	30	Absent	Absent	40	Poor	5	0.64	Average
138	Zone A	100	Sometimes	Good	100	Absent	Absent	25	Moderate	5	0.58	Below Average
142	Zone A	200	Rarely dries	Poor	100	Absent	Absent	6	Moderate	0	0.58	Below average
146	Zone A	16	Sometimes	Moderate	50	Absent	Absent	24	Moderate	80	0.64	Average
149	Zone A	100	Sometimes dries	Moderate	90	Absent	Absent	1	Moderate	0	0.55	Below average
150	Zone A	220	Never	Good	30	Absent	Absent	27	Poor	50	0.80	Excellent
156	Zone A	<50	Sometimes dries	Poor	90	Absent	Absent	3	Poor	0	0.43	Poor
158	Zone A	120	Annually	Moderate	10	Absent	Absent	16	Good	80	0.6496964	Average
159	Zone A	500	Sometimes dries	Poor	100	Minor	Possible	13+	Good	0	0.58	Below average
163	Zone A	<50	Dries annually	Bad	0	Absent	Absent	5	Good	10	0.33	Poor
165	Zone A	250	Annually	Good	70	Absent	Absent	22	Good	40	0.71	Good
166	Zone A	800	Never dries	Bad	0	Major	Possible	13+	Moderate	0	0.32	Poor
167	Zone A	250	Never dries	Good	0	Minor	Possible	12	Good	5	0.71	Good
168	Zone A	30	Sometimes	Moderate	100	Absent	Absent	7	Moderate	0	0.49	Poor
169	Zone A	100	Rarely dries	Poor	0	Absent	Absent	2	Good	20	0.62	Average
170	Zone A	5500	Never	Moderate	0	Minor	Possible	36	Good	0	0.76	Good
171	Zone A	30	Sometimes	Moderate	100	Absent	Absent	36	Good	5	0.53	Below Average
172	Zone A	4000	Never	Good	10	Absent	Possible	25	Good	20	0.87	Excellent
173	Zone A	50	Sometimes	Moderate	60	Absent	Absent	34	Good	5	0.64	Average
174	Zone A	1600	Never dries	Good	0	Major	Possible	13+	Moderate	5	0.51	Below average
179	Zone A	20	Sometimes	Moderate	80	Absent	Absent	32	Good	40	0.61	Average
180	Zone A	50	Rarely dries	Moderate	100	Absent	Possible	5	Moderate	40	0.53	Below average
181	Zone A	400	Sometimes dries	Moderate	0	Minor	Absent	13+	Poor	20	0.7	Good
182	Zone A	16	Annually	Moderate	100	Absent	Absent	31	Good	0	0.4268936	Poor
184	Zone A	350	Sometimes dries	Poor	80	Minor	Absent	13+	Moderate	0	0.63	Average
185	Zone A	15	Never	Poor	0	Absent	Minor	33	Poor	5	0.47	Poor
186	Zone A	500	Rarely dries	Moderate	0	Minor	Possible	13+	Moderate	10	0.78	Good
187	Zone A	100	Never dries	Poor	0	Minor	Possible	13+	Poor	5	0.56	Below average
188	Zone A	350	Never dries	Good	0	Minor	Possible	5	Good	0	0.76	Good
190	Zone A	300	Never dries	Moderate	0	Major	Possible	5	Good	30	0.51	Below average
193	Zone A	150	Rarely	Good	0	Absent	Absent	44	Moderate	30	0.79	Good
196	Zone A	500	Never dries	Moderate	0	Minor	Major	13+	Moderate	10	0.51	Below average
198	Zone A	300	Never dries	Moderate	0	Minor	Possible	3	Moderate	0	0.68	Average
203	Zone A	250	Rarely dries	Poor	90	Minor	Possible	13+	Poor	0	0.56	Below average
210	Zone A	400	Never dries	Poor	80	Minor	Possible	13+	Moderate	0	0.65	Average
211	Zone A	100	Rarely dries	Poor	100	Absent	Absent	1	Poor	0	0.47	Poor
213	Zone A	<50	Sometimes dries	Good	75	Absent	Absent	7	Moderate	30	0.79	Good
217	Zone A	50	Sometimes	Moderate	100	Absent	Absent	23	Poor	0	0.48	Poor

Pond number	Geographic location	Pond area m ²	Permanence	Water quality	Shade	Waterfowl	Fish	Pond count	Terrestrial habitat	Macro-phytes	Resulting HSI Score	Pond suitability
220	Zone A	10	Annually	Moderate	0	Absent	Absent	33	Moderate	10	0.50	Poor
224	Zone A	80	Annually	Poor	0	Absent	Absent	23	Poor	40	0.51	Below Average
226	Zone A	80	Sometimes	Moderate	100	Absent	Absent	23	Poor	0	0.50	Below Average
227	Zone A	50	Sometimes	Moderate	100	Absent	Absent	24	Poor	0	0.48	Poor
243	Zone A	650	Never	Good	75	Minor	Absent	32	Poor	10	0.75	Good
246	Zone A	650	Never	Good	5	Minor	Possible	28	Moderate	10	0.80	Excellent
249	Zone A	400	Rarely	Poor	5	Absent	Major	34	Moderate	5	0.48	Poor
250	Zone A	75	Sometimes	Moderate	80	Absent	Absent	20	Moderate	5	0.61	Average
258	Zone A	15	Never	Good	60	Absent	Major	20	Moderate	20	0.41	Poor

Annex C: Nature Metrics EDNA Reports



NATURE
METRICS
DNA-BASED MONITORING

Environmental DNA Report

Great Crested Newt



Order number	SO04187
Prepared for	The Temple Group - Norwich
Project	T10372 South Norfolk Solar DCO
Number of samples	19
Sampling dates	2024/06/26 - 2024/06/28
Report ID	GCN-CNQ823
Date of report	2024/07/12



Thank you for choosing NatureMetrics

Welcome to your report. We are the leading provider of powerful, scalable biodiversity data delivered using environmental DNA.

Your sample(s) have been processed in accordance with the protocol set out in Appendix 5 of Biggs et al. (2014). Results are based on the samples as supplied by the client to the laboratory. Incorrect sampling methodology may affect the results. Note that a negative result does not preclude the presence of GCN at a level below the [limits of detection](#).

A results interpretation guide and a glossary of terms highlighted throughout this report can be found at the end of the report.

GCN Detection Results

Pond ID	Inhibition	Degradation	GCN Score	Result
14	No	No	0	Negative
42	No	No	0	Negative
48	No	No	0	Negative
52	No	No	0	Negative
65	No	No	0	Negative
Pond 2	No	No	0	Negative
Pond 11	No	No	3	Positive
Pond 21	No	No	4	Positive
Pond 41	No	No	11	Positive
Pond 43	No	No	9	Positive
Pond 46	No	No	0	Negative
Pond 47	No	No	0	Negative
Pond 53	No	No	0	Negative
Pond 57	No	No	10	Positive
Pond 62	No	No	12	Positive
Pond 63	No	No	0	Negative
Pond 70	No	No	12	Positive



Pond 110	No	No	0	Negative
Pond 114	No	No	0	Negative

Sample Information

Pond ID	Kit ID	Sampling Date	Received Date
14	GCN-24-03552	2024/06/26	2024/07/02
42	GCN-24-03537	2024/06/28	2024/07/02
48	GCN-24-03538	2024/06/27	2024/07/02
52	GCN-24-03549	2024/06/26	2024/07/02
65	GCN-24-03553	2024/06/26	2024/07/02
Pond 2	GCN-24-03535	2024/06/27	2024/07/02
Pond 11	GCN-24-03542	2024/06/28	2024/07/02
Pond 21	GCN-24-03548	2024/06/26	2024/07/02
Pond 41	GCN-24-03545	2024/06/26	2024/07/02
Pond 43	GCN-24-03536	2024/06/27	2024/07/02
Pond 46	GCN-24-03550	2024/06/27	2024/07/02
Pond 47	GCN-24-03547	2024/06/27	2024/07/02
Pond 53	GCN-24-03546	2024/06/27	2024/07/02
Pond 57	GCN-24-03544	2024/06/28	2024/07/02
Pond 62	GCN-24-03551	2024/06/27	2024/07/02
Pond 63	GCN-24-03554	2024/06/26	2024/07/02
Pond 70	GCN-24-03540	2024/06/27	2024/07/02
Pond 110	GCN-24-03543	2024/06/28	2024/07/02
Pond 114	GCN-24-03539	2024/06/28	2024/07/02

Methods

eDNA was precipitated via centrifugation at 14,000 x g and then extracted using Qiagen DNeasy Blood and Tissue extraction kits. **qPCR** amplification was carried out in 12 replicates per sample, using GCN specific **primers** and **probe** (developed by Thomsen et al. (2012) and adopted by Biggs et al. (2014)), in

the presence of **extraction negative controls**, **qPCR positive controls**, and **qPCR negative controls**. A score is given for the number of positive replicates out of 12.

The **qPCR** method follows the recommendations set out by NatureMetrics for Natural England in the qPCR validation project and helps improve the reliability of the interpretation of the data.

Results from the GCN assay are considered to have a **high** confidence rating according to our **Validation Scale** (Harper et al. 2021).

The quality control methods exceed the requirements outlined in Appendix 5 of Biggs et al. (2014). These consist of the use of **kit blanks**, additional **extraction negative controls**, **qPCR negative controls**, and **qPCR positive controls**. Using these controls ensures assay performance is as expected and increases confidence in any weak or late amplifications.

The extraction and qPCR negative controls analysed alongside your samples showed no target amplification and the triplicate positive controls performed as expected.

END OF REPORT

Contact: **team@naturemetrics.co.uk**



Result Interpretation Guide

Positive

Target DNA has been detected in this sample, meaning that at least 1 of the 12 qPCR replicates has amplified. This is not a quantitative test, so you should not interpret a high eDNA score (e.g. 12/12) as necessarily indicating a larger population of GCN than a low eDNA score (e.g. 1/12).

Negative

No target DNA has been detected in this sample, but the internal and external controls worked as expected. This tells us that if there had been GCN DNA in the sample, we would have detected it, so we can be confident in its absence from the sample provided.

Inconclusive

No GCN DNA was detected in the sample, but the internal controls failed to amplify as expected. This means that any GCN DNA in the sample might also have failed to amplify properly, so we cannot have confidence in this negative result. Inconclusive results can be caused by the degradation of the DNA (as indicated by delayed or no amplification of the DNA marker contained in the ethanol in the kits) or by inhibition of the reaction (as indicated by delayed or no amplification of the DNA marker added in the lab) caused by certain chemicals or organic compounds that may be present in the water sample.

Validation Scale

We have developed our own confidence assessment tool for qPCR eDNA assays that builds upon the Thalinger et al. (2021) validation scale and helps end-users to interpret the qPCR outputs but also contextualise these with the level of validation that the assay itself has gone through. Briefly, the level of confidence that can be assigned to results coming from an assay is derived from several validation steps:

- Basic analysis - can the assay work in principle on the computer?
- PCR protocol - has the protocol been optimised in the lab?
- Specificity analysis - has the assay been tested in the lab against other co-inhabiting and/or closely related species?
- How extensively has the assay been tested with natural samples?
- Have the theoretical limits of detection been established?
- Have detection probabilities been estimated with extensive site occupancy modelling?
- Have external factors affecting detectability been extensively tested (e.g. seasonality, spatial heterogeneity)?

- Low

Results from these assays are difficult to interpret with confidence. It is impossible to conclusively tell if the target species is present or absent because of the limited amounts of *in silico*, *in vitro*, and *in vivo* testing.

- Medium

Assays with this rating have been tested *in silico*, have optimised lab protocols, specificity and sensitivity tested in and out of the lab, but with no estimates of detection probabilities or extensive testing of external factors that may affect the detectability of the target. Positive results can be interpreted as meaning the target species' DNA is present (assuming the correct sampling conditions), but negative results could mean that the target is absent or that external



factors such as ecology, seasonality, and spatial scales are influencing the detections.

- High

High rating assays have everything that a Medium assay has, in addition to site occupancy modelling and extensive testing of external effects such as ecological, temporal and spatial factors. Positive results can be conclusively interpreted, and negative results can be interpreted as meaning the target species' DNA is absent (assuming the correct sampling conditions). In some instances, a probability of target species presence at a site and in a sample can be given.

Glossary

Controls

Controls are used to monitor both the performance of the assays but also any contamination. Controls are treated in the same way as a normal sample. This is particularly important given the sensitivity of eDNA qPCR methods. Our full complement of controls enables us to fully monitor the whole GCN eDNA process from kits to data.

Kit blank

Used to determine if the kits are contaminated but also to monitor the early stages of the pipelines, e.g. sample reception. These samples also act as uninhibited samples that can be used as a baseline to compare against. This is an additional control not specifically mentioned in the Biggs et al. (2014) protocol.

Extraction negative

Extraction blank. Used to monitor potential contamination during the DNA extraction process.

qPCR negative

Template negative control. Used to monitor potential contamination during the qPCR setup process. For every qPCR reaction, we include more qPCR negative controls than are prescribed in the Biggs et al. (2014) protocol.

qPCR positive

Used to determine whether the qPCR run performed as expected. In addition to the 4 standard dilutions prescribed by the Biggs et al. (2014) protocol, we include an additional standard dilution and amplify all standards in triplicate. The increased number of standard dilutions and replicates allow us to generate standard curves to assess run performance and assay sensitivity.

Limit of detection

The lowest standard positive control concentration at which 95 % of technical replicates amplify. Target amplification below the LOD cannot automatically be considered as negative but should be further investigated as spurious amplifications are more prevalent at these low concentrations.

eDNA

Short for 'environmental DNA'. Refers to DNA deposited in the environment through excretions and secretions, such as mucus, skin cells, saliva, faeces, urine etc. This can be collected in environmental samples (e.g. water, sediment) and used to identify the organisms that it originated from. eDNA in water is broken down by environmental processes over a period of days to weeks. It can travel some distance from the point at which it was released from

the organism, particularly in running water. eDNA is sampled in low concentrations and can be degraded (i.e. broken into short fragments), which limits the analysis options.

Inhibitors

Chemicals/compounds that reduce or prevent DNA amplification, potentially resulting in false-negative results. Common inhibitors include tannins, humic acids and other organic compounds. Inhibitors can be overcome by either diluting the DNA (and the inhibitors), but dilution carries the risk of reducing the DNA concentration below the limits of detection.

qPCR

Stands for 'quantitative PCR'. A PCR reaction incorporating a coloured dye that fluoresces during DNA amplification, allowing a machine to track the progress of the reaction. Often used with species-specific primers and probe where amplification is used to infer the presence of the target species' DNA in the sample. If the species is not present in the sample, no fluorescence will be detected.

Primers

Short sections of synthesised DNA that bind to either end of the DNA segment to be amplified by qPCR.

Probe

A short section of synthesised DNA that binds to a specific section of the target species' DNA within the section flanked by the primers. The probe is designed to be totally specific to that species. The probe is labelled such that it fluoresces during amplification, which is used to infer the presence of the target species' DNA in the sample.

References

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

Environmental DNA Report

Great crested newt (*Triturus cristatus*) (for UK licensing)



Project code	PROJ07467
Prepared for	The Temple Group - Norwich
Project	East Pye - T10372
Number of samples	3
Sampling dates	12-Jun-2025 to 01-Jul-2025
Report ID	GCN-R3Q3FSC
Date of report	16-Jul-2025



Thank you for choosing NatureMetrics

Welcome to your report. We are the leading provider of powerful, scalable biodiversity data delivered using environmental DNA.

Your sample(s) have been processed in accordance with the protocol set out in Appendix 5 of Biggs et al. (2014). Results are based on the samples as supplied by the client to the laboratory. Incorrect sampling methodology may affect the results. Note that a negative result does not preclude the presence of GCN at a level below the [limits of detection](#).

A results interpretation guide and a glossary of terms highlighted throughout this report can be found at the end of the report.

GCN Detection Results

Pond ID	Inhibition	Degradation	GCN Score	Result
180 - ditch adj	Pass	Fail	0	Inconclusive
G4(4A)	Pass	Fail	0	Inconclusive
S1(7C)	Pass	Fail	0	Inconclusive

Sample Information

Pond ID	Kit ID	Sampling Date	Received Date
G4(4A)	GCN-25-01890	12-Jun-2025	07-Jul-2025
S1(7C)	GCN-25-01892	12-Jun-2025	07-Jul-2025
180 - ditch adj	GCN-25-02096	01-Jul-2025	07-Jul-2025

Methods

eDNA was precipitated via centrifugation at 14,000 x g and then extracted using Qiagen DNeasy Blood and Tissue extraction kits. **qPCR** amplification was carried out in 12 replicates per sample, using GCN specific **primers** and **probe** (developed by Thomsen et al. (2012) and adopted by Biggs et al. (2014)), in the presence of **extraction negative controls**, **qPCR positive controls**, and **qPCR negative controls**. A score is given for the number of positive replicates out of 12.

The **qPCR** method follows the recommendations set out by NatureMetrics for Natural England in the qPCR validation project and helps improve the reliability of the interpretation of the data.

Results from the GCN assay are considered to have a **high** confidence rating according to our **Validation Scale** (Harper et al. 2021).



The quality control methods exceed the requirements outlined in Appendix 5 of Biggs et al. (2014). These consist of the use of **kit blanks**, additional **extraction negative controls**, **qPCR negative controls**, and **qPCR positive controls**. Using these controls ensures assay performance is as expected and increases confidence in any weak or late amplifications.

The extraction and qPCR negative controls analysed alongside your samples showed no target amplification and the triplicate positive controls performed as expected.

END OF REPORT

Contact: [Customer Support Helpdesk](https://www.naturemetrics.com/contact)
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Result Interpretation Guide

Positive

Target DNA has been detected in this sample, meaning that at least 1 of the 12 qPCR replicates has amplified. This is not a quantitative test, so you should not interpret a high eDNA score (e.g. 12/12) as necessarily indicating a larger population of GCN than a low eDNA score (e.g. 1/12).

Negative

No target DNA has been detected in this sample, but the internal and external controls worked as expected. This tells us that if there had been GCN DNA in the sample, we would have detected it, so we can be confident in its absence from the sample provided.

Inconclusive

No GCN DNA was detected in the sample, but the internal controls failed to amplify as expected. This means that any GCN DNA in the sample might also have failed to amplify properly, so we cannot have confidence in this negative result. Inconclusive results can be caused by the degradation of the DNA (as indicated by delayed or no amplification of the DNA marker contained in the ethanol in the kits) or by inhibition of the reaction (as indicated by delayed or no amplification of the DNA marker added in the lab) caused by certain chemicals or organic compounds that may be present in the water sample.

Validation Scale

We have developed our own confidence assessment tool for qPCR eDNA assays that builds upon the Thalinger et al. (2021) validation scale and helps end-users to interpret the qPCR outputs but also contextualise these with the level of validation that the assay itself has gone through. Briefly, the level of confidence that can be assigned to results coming from an assay is derived from several validation steps:

- Basic analysis - can the assay work in principle on the computer?
- PCR protocol - has the protocol been optimised in the lab?
- Specificity analysis - has the assay been tested in the lab against other co-inhabiting and/or closely related species?
- How extensively has the assay been tested with natural samples?
- Have the theoretical limits of detection been established?
- Have detection probabilities been estimated with extensive site occupancy modelling?
- Have external factors affecting detectability been extensively tested (e.g. seasonality, spatial heterogeneity)?

- Low

Results from these assays are difficult to interpret with confidence. It is impossible to conclusively tell if the target species is present or absent because of the limited amounts of *in silico*, *in vitro*, and *in vivo* testing.

- Medium

Assays with this rating have been tested *in silico*, have optimised lab protocols, specificity and sensitivity tested in and out of the lab, but with no estimates of detection probabilities or extensive testing of external factors that may affect the detectability of the target. Positive results can be interpreted as meaning the target species' DNA is present (assuming the correct sampling conditions), but negative results could mean that the target is absent or that external



factors such as ecology, seasonality, and spatial scales are influencing the detections.

- High

High rating assays have everything that a Medium assay has, in addition to site occupancy modelling and extensive testing of external effects such as ecological, temporal and spatial factors. Positive results can be conclusively interpreted, and negative results can be interpreted as meaning the target species' DNA is absent (assuming the correct sampling conditions). In some instances, a probability of target species presence at a site and in a sample can be given.

Glossary

Controls

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Limit of detection

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the organism, particularly in running water. eDNA is sampled in low concentrations and can be degraded (i.e. broken into short fragments), which limits the analysis options.

Inhibitors

Chemicals/compounds that reduce or prevent DNA amplification, potentially resulting in false-negative results. Common inhibitors include tannins, humic acids and other organic compounds. Inhibitors can be overcome by either diluting the DNA (and the inhibitors), but dilution carries the risk of reducing the DNA concentration below the limits of detection.

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Environmental DNA Report

Great crested newt (*Triturus cristatus*) (for UK licensing)



Project code	PROJ07467
Prepared for	The Temple Group - Norwich
Project	East Pye - T10372
Number of samples	11
Sampling dates	16-Jun-2025 to 18-Jun-2025
Report ID	GCN-5GD6JZK
Date of report	02-Jul-2025



Thank you for choosing NatureMetrics

Welcome to your report. We are the leading provider of powerful, scalable biodiversity data delivered using environmental DNA.

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A results interpretation guide and a glossary of terms highlighted throughout this report can be found at the end of the report.

GCN Detection Results

Pond ID	Inhibition	Degradation	GCN Score	Result
10c_P56	Pass	Fail	3	Positive
7E_P54	Pass	Fail	0	Inconclusive
7K_P45	Pass	Fail	0	Inconclusive
JA_P169	Pass	Fail	0	Inconclusive
10375-1A-156	Pass	Pass	0	Negative
7F-P55	Pass	Pass	0	Negative
7R_P3	Pass	Pass	0	Negative
9_P60	Pass	Pass	0	Negative
JB_P167	Pass	Pass	0	Negative
SS_P35	Pass	Pass	0	Negative
ZB_P159	Pass	Pass	0	Negative

Sample Information

Pond ID	Kit ID	Sampling Date	Received Date
7F-P55	GCN-25-01889	17-Jun-2025	20-Jun-2025
JA_P169	GCN-25-01891	17-Jun-2025	20-Jun-2025
10375-1A-156	GCN-25-01893	16-Jun-2025	20-Jun-2025



10c_P56	GCN-25-01896	18-Jun-2025	20-Jun-2025
ZB_P159	GCN-25-01897	16-Jun-2025	20-Jun-2025
7R_P3	GCN-25-01898	18-Jun-2025	20-Jun-2025
9_P60	GCN-25-01899	18-Jun-2025	20-Jun-2025
SS_P35	GCN-25-01900	16-Jun-2025	20-Jun-2025
7E_P54	GCN-25-01901	18-Jun-2025	20-Jun-2025
7K_P45	GCN-25-01903	18-Jun-2025	20-Jun-2025
JB_P167	GCN-25-01904	17-Jun-2025	20-Jun-2025

Methods

eDNA was precipitated via centrifugation at 14,000 x g and then extracted using Qiagen DNeasy Blood and Tissue extraction kits. **qPCR** amplification was carried out in 12 replicates per sample, using GCN specific **primers** and **probe** (developed by Thomsen et al. (2012) and adopted by Biggs et al. (2014)), in the presence of **extraction negative controls**, **qPCR positive controls**, and **qPCR negative controls**. A score is given for the number of positive replicates out of 12.

The **qPCR** method follows the recommendations set out by NatureMetrics for Natural England in the qPCR validation project and helps improve the reliability of the interpretation of the data.

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The extraction and qPCR negative controls analysed alongside your samples showed no target amplification and the triplicate positive controls performed as expected.

END OF REPORT

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Result Interpretation Guide

Positive

Target DNA has been detected in this sample, meaning that at least 1 of the 12 qPCR replicates has amplified. This is not a quantitative test, so you should not interpret a high eDNA score (e.g. 12/12) as necessarily indicating a larger population of GCN than a low eDNA score (e.g. 1/12).

Negative

No target DNA has been detected in this sample, but the internal and external controls worked as expected. This tells us that if there had been GCN DNA in the sample, we would have detected it, so we can be confident in its absence from the sample provided.

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Template negative control. Used to monitor potential contamination during the qPCR setup process. For every qPCR reaction, we include more qPCR negative controls than are prescribed in the Biggs et al. (2014) protocol.

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Inhibitors

Chemicals/compounds that reduce or prevent DNA amplification, potentially resulting in false-negative results. Common inhibitors include tannins, humic acids and other organic compounds. Inhibitors can be overcome by either diluting the DNA (and the inhibitors), but dilution carries the risk of reducing the DNA concentration below the limits of detection.

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References

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Environmental DNA Report

Great crested newt (*Triturus cristatus*) (for UK licensing)



Project code	PROJ07467
Prepared for	The Temple Group - Norwich
Project	East Pye - T10372
Number of samples	20
Sampling dates	26-Jun-2025 to 30-Jun-2025
Report ID	GCN-6TMKLQL
Date of report	14-Jul-2025



Thank you for choosing NatureMetrics

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GCN Detection Results

Pond ID	Inhibition	Degradation	GCN Score	Result
127	Pass	Fail	12	Positive
124	Pass	Fail	9	Positive
184	Pass	Fail	5	Positive
123	Pass	Fail	3	Positive
125	Pass	Fail	3	Positive
80	Pass	Pass	1	Positive
186	Pass	Fail	0	Inconclusive
187	Pass	Fail	0	Inconclusive
6_p148	Pass	Fail	0	Inconclusive
75	Pass	Fail	0	Inconclusive
82	Pass	Fail	0	Inconclusive
83	Pass	Fail	0	Inconclusive
159	Pass	Pass	0	Negative
174	Pass	Pass	0	Negative
181	Pass	Pass	0	Negative
210	Pass	Pass	0	Negative
32	Pass	Pass	0	Negative



78	Pass	Pass	0	Negative
84	Pass	Pass	0	Negative
East Pye 196	Pass	Pass	0	Negative

Sample Information

Pond ID	Kit ID	Sampling Date	Received Date
181	GCN-25-02048	26-Jun-2025	02-Jul-2025
184	GCN-25-02050	27-Jun-2025	02-Jul-2025
78	GCN-25-02051	29-Jun-2025	02-Jul-2025
210	GCN-25-02052	27-Jun-2025	02-Jul-2025
East Pye 196	GCN-25-02054	26-Jun-2025	02-Jul-2025
32	GCN-25-02067	30-Jun-2025	02-Jul-2025
159	GCN-25-02068	30-Jun-2025	02-Jul-2025
83	GCN-25-02070	29-Jun-2025	02-Jul-2025
187	GCN-25-02072	30-Jun-2025	02-Jul-2025
174	GCN-25-02073	30-Jun-2025	02-Jul-2025
127	GCN-25-02076	26-Jun-2025	02-Jul-2025
84	GCN-25-02078	29-Jun-2025	02-Jul-2025
125	GCN-25-02079	26-Jun-2025	02-Jul-2025
80	GCN-25-02080	29-Jun-2025	02-Jul-2025
82	GCN-25-02081	29-Jun-2025	02-Jul-2025
186	GCN-25-02082	27-Jun-2025	02-Jul-2025
75	GCN-25-02093	26-Jun-2025	02-Jul-2025
123	GCN-25-02097	26-Jun-2025	02-Jul-2025
124	GCN-25-02098	26-Jun-2025	02-Jul-2025
6_p148	GCN-25-02099	26-Jun-2025	02-Jul-2025

Methods

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- How extensively has the assay been tested with natural samples?
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- Have detection probabilities been estimated with extensive site occupancy modelling?
- Have external factors affecting detectability been extensively tested (e.g. seasonality, spatial heterogeneity)?

- Low

Results from these assays are difficult to interpret with confidence. It is impossible to conclusively tell if the target species is present or absent because of the limited amounts of *in silico*, *in vitro*, and *in vivo* testing.

- Medium

Assays with this rating have been tested *in silico*, have optimised lab protocols, specificity and sensitivity tested in and out of the lab, but with no estimates of detection probabilities or extensive testing of external factors that may affect the detectability of the target. Positive results can be interpreted as meaning the target species' DNA is present (assuming the correct sampling conditions), but negative results could mean that the target is absent or that external



factors such as ecology, seasonality, and spatial scales are influencing the detections.

- High

High rating assays have everything that a Medium assay has, in addition to site occupancy modelling and extensive testing of external effects such as ecological, temporal and spatial factors. Positive results can be conclusively interpreted, and negative results can be interpreted as meaning the target species' DNA is absent (assuming the correct sampling conditions). In some instances, a probability of target species presence at a site and in a sample can be given.

Glossary

Controls

Controls are used to monitor both the performance of the assays but also any contamination. Controls are treated in the same way as a normal sample. This is particularly important given the sensitivity of eDNA qPCR methods. Our full complement of controls enables us to fully monitor the whole GCN eDNA process from kits to data.

Kit blank

Used to determine if the kits are contaminated but also to monitor the early stages of the pipelines, e.g. sample reception. These samples also act as uninhibited samples that can be used as a baseline to compare against. This is an additional control not specifically mentioned in the Biggs et al. (2014) protocol.

Extraction negative

Extraction blank. Used to monitor potential contamination during the DNA extraction process.

qPCR negative

Template negative control. Used to monitor potential contamination during the qPCR setup process. For every qPCR reaction, we include more qPCR negative controls than are prescribed in the Biggs et al. (2014) protocol.

qPCR positive

Used to determine whether the qPCR run performed as expected. In addition to the 4 standard dilutions prescribed by the Biggs et al. (2014) protocol, we include an additional standard dilution and amplify all standards in triplicate. The increased number of standard dilutions and replicates allow us to generate standard curves to assess run performance and assay sensitivity.

Limit of detection

The lowest standard positive control concentration at which 95 % of technical replicates amplify. Target amplification below the LOD cannot automatically be considered as negative but should be further investigated as spurious amplifications are more prevalent at these low concentrations.

eDNA

Short for 'environmental DNA'. Refers to DNA deposited in the environment through excretions and secretions, such as mucus, skin cells, saliva, faeces, urine etc. This can be collected in environmental samples (e.g. water, sediment) and used to identify the organisms that it originated from. eDNA in water is broken down by environmental processes over a period of days to weeks. It can travel some distance from the point at which it was released from

the organism, particularly in running water. eDNA is sampled in low concentrations and can be degraded (i.e. broken into short fragments), which limits the analysis options.

Inhibitors

Chemicals/compounds that reduce or prevent DNA amplification, potentially resulting in false-negative results. Common inhibitors include tannins, humic acids and other organic compounds. Inhibitors can be overcome by either diluting the DNA (and the inhibitors), but dilution carries the risk of reducing the DNA concentration below the limits of detection.

qPCR

Stands for 'quantitative PCR'. A PCR reaction incorporating a coloured dye that fluoresces during DNA amplification, allowing a machine to track the progress of the reaction. Often used with species-specific primers and probe where amplification is used to infer the presence of the target species' DNA in the sample. If the species is not present in the sample, no fluorescence will be detected.

Primers

Short sections of synthesised DNA that bind to either end of the DNA segment to be amplified by qPCR.

Probe

A short section of synthesised DNA that binds to a specific section of the target species' DNA within the section flanked by the primers. The probe is designed to be totally specific to that species. The probe is labelled such that it fluoresces during amplification, which is used to infer the presence of the target species' DNA in the sample.

References

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NATURE
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DNA-BASED MONITORING

Environmental DNA Report

Great crested newt (*Triturus cristatus*) (for UK licensing)



Project code	PROJ07467
Prepared for	The Temple Group - Norwich
Project	East Pye - T10372
Number of samples	8
Sampling dates	19-Jun-2025 to 20-Jun-2025
Report ID	GCN-90X7GKV
Date of report	02-Jul-2025



Thank you for choosing NatureMetrics

Welcome to your report. We are the leading provider of powerful, scalable biodiversity data delivered using environmental DNA.

Your sample(s) have been processed in accordance with the protocol set out in Appendix 5 of Biggs et al. (2014). Results are based on the samples as supplied by the client to the laboratory. Incorrect sampling methodology may affect the results. Note that a negative result does not preclude the presence of GCN at a level below the [limits of detection](#).

A results interpretation guide and a glossary of terms highlighted throughout this report can be found at the end of the report.

GCN Detection Results

Pond ID	Inhibition	Degradation	GCN Score	Result
CRC_P188	Pass	Pass	1	Positive
7B-P22	Pass	Fail	0	Inconclusive
7B-P27	Pass	Fail	0	Inconclusive
7B-P28	Pass	Fail	0	Inconclusive
7C-P93	Pass	Fail	0	Inconclusive
CRC_190	Pass	Fail	0	Inconclusive
2B-213	Pass	Pass	0	Negative
7B-P29	Pass	Pass	0	Negative

Sample Information

Pond ID	Kit ID	Sampling Date	Received Date
CRC_P188	GCN-25-01894	19-Jun-2025	24-Jun-2025
CRC_190	GCN-25-01895	19-Jun-2025	24-Jun-2025
7C-P93	GCN-25-01902	19-Jun-2025	24-Jun-2025
7B-P22	GCN-25-02045	19-Jun-2025	24-Jun-2025
7B-P27	GCN-25-02046	19-Jun-2025	24-Jun-2025
7B-P29	GCN-25-02047	19-Jun-2025	24-Jun-2025



2B-213	GCN-25-02049	20-Jun-2025	24-Jun-2025
7B-P28	GCN-25-02060	19-Jun-2025	24-Jun-2025

Methods

eDNA was precipitated via centrifugation at 14,000 x g and then extracted using Qiagen DNeasy Blood and Tissue extraction kits. **qPCR** amplification was carried out in 12 replicates per sample, using GCN specific **primers** and **probe** (developed by Thomsen et al. (2012) and adopted by Biggs et al. (2014)), in the presence of **extraction negative controls**, **qPCR positive controls**, and **qPCR negative controls**. A score is given for the number of positive replicates out of 12.

The **qPCR** method follows the recommendations set out by NatureMetrics for Natural England in the qPCR validation project and helps improve the reliability of the interpretation of the data.

Results from the GCN assay are considered to have a **high** confidence rating according to our **Validation Scale** (Harper et al. 2021).

The quality control methods exceed the requirements outlined in Appendix 5 of Biggs et al. (2014). These consist of the use of **kit blanks**, additional **extraction negative controls**, **qPCR negative controls**, and **qPCR positive controls**. Using these controls ensures assay performance is as expected and increases confidence in any weak or late amplifications.

The extraction and qPCR negative controls analysed alongside your samples showed no target amplification and the triplicate positive controls performed as expected.

END OF REPORT

Contact: [Customer Support Helpdesk](https://www.naturemetrics.com/contact)
www.naturemetrics.com/contact



Result Interpretation Guide

Positive

Target DNA has been detected in this sample, meaning that at least 1 of the 12 qPCR replicates has amplified. This is not a quantitative test, so you should not interpret a high eDNA score (e.g. 12/12) as necessarily indicating a larger population of GCN than a low eDNA score (e.g. 1/12).

Negative

No target DNA has been detected in this sample, but the internal and external controls worked as expected. This tells us that if there had been GCN DNA in the sample, we would have detected it, so we can be confident in its absence from the sample provided.

Inconclusive

No GCN DNA was detected in the sample, but the internal controls failed to amplify as expected. This means that any GCN DNA in the sample might also have failed to amplify properly, so we cannot have confidence in this negative result. Inconclusive results can be caused by the degradation of the DNA (as indicated by delayed or no amplification of the DNA marker contained in the ethanol in the kits) or by inhibition of the reaction (as indicated by delayed or no amplification of the DNA marker added in the lab) caused by certain chemicals or organic compounds that may be present in the water sample.

Validation Scale

We have developed our own confidence assessment tool for qPCR eDNA assays that builds upon the Thalinger et al. (2021) validation scale and helps end-users to interpret the qPCR outputs but also contextualise these with the level of validation that the assay itself has gone through. Briefly, the level of confidence that can be assigned to results coming from an assay is derived from several validation steps:

- Basic analysis - can the assay work in principle on the computer?
- PCR protocol - has the protocol been optimised in the lab?
- Specificity analysis - has the assay been tested in the lab against other co-inhabiting and/or closely related species?
- How extensively has the assay been tested with natural samples?
- Have the theoretical limits of detection been established?
- Have detection probabilities been estimated with extensive site occupancy modelling?
- Have external factors affecting detectability been extensively tested (e.g. seasonality, spatial heterogeneity)?

- Low

Results from these assays are difficult to interpret with confidence. It is impossible to conclusively tell if the target species is present or absent because of the limited amounts of *in silico*, *in vitro*, and *in vivo* testing.

- Medium

Assays with this rating have been tested *in silico*, have optimised lab protocols, specificity and sensitivity tested in and out of the lab, but with no estimates of detection probabilities or extensive testing of external factors that may affect the detectability of the target. Positive results can be interpreted as meaning the target species' DNA is present (assuming the correct sampling conditions), but negative results could mean that the target is absent or that external



factors such as ecology, seasonality, and spatial scales are influencing the detections.

- High

High rating assays have everything that a Medium assay has, in addition to site occupancy modelling and extensive testing of external effects such as ecological, temporal and spatial factors. Positive results can be conclusively interpreted, and negative results can be interpreted as meaning the target species' DNA is absent (assuming the correct sampling conditions). In some instances, a probability of target species presence at a site and in a sample can be given.

Glossary

Controls

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Kit blank

Used to determine if the kits are contaminated but also to monitor the early stages of the pipelines, e.g. sample reception. These samples also act as uninhibited samples that can be used as a baseline to compare against. This is an additional control not specifically mentioned in the Biggs et al. (2014) protocol.

Extraction negative

Extraction blank. Used to monitor potential contamination during the DNA extraction process.

qPCR negative

Template negative control. Used to monitor potential contamination during the qPCR setup process. For every qPCR reaction, we include more qPCR negative controls than are prescribed in the Biggs et al. (2014) protocol.

qPCR positive

Used to determine whether the qPCR run performed as expected. In addition to the 4 standard dilutions prescribed by the Biggs et al. (2014) protocol, we include an additional standard dilution and amplify all standards in triplicate. The increased number of standard dilutions and replicates allow us to generate standard curves to assess run performance and assay sensitivity.

Limit of detection

The lowest standard positive control concentration at which 95 % of technical replicates amplify. Target amplification below the LOD cannot automatically be considered as negative but should be further investigated as spurious amplifications are more prevalent at these low concentrations.

eDNA

Short for 'environmental DNA'. Refers to DNA deposited in the environment through excretions and secretions, such as mucus, skin cells, saliva, faeces, urine etc. This can be collected in environmental samples (e.g. water, sediment) and used to identify the organisms that it originated from. eDNA in water is broken down by environmental processes over a period of days to weeks. It can travel some distance from the point at which it was released from

the organism, particularly in running water. eDNA is sampled in low concentrations and can be degraded (i.e. broken into short fragments), which limits the analysis options.

Inhibitors

Chemicals/compounds that reduce or prevent DNA amplification, potentially resulting in false-negative results. Common inhibitors include tannins, humic acids and other organic compounds. Inhibitors can be overcome by either diluting the DNA (and the inhibitors), but dilution carries the risk of reducing the DNA concentration below the limits of detection.

qPCR

Stands for 'quantitative PCR'. A PCR reaction incorporating a coloured dye that fluoresces during DNA amplification, allowing a machine to track the progress of the reaction. Often used with species-specific primers and probe where amplification is used to infer the presence of the target species' DNA in the sample. If the species is not present in the sample, no fluorescence will be detected.

Primers

Short sections of synthesised DNA that bind to either end of the DNA segment to be amplified by qPCR.

Probe

A short section of synthesised DNA that binds to a specific section of the target species' DNA within the section flanked by the primers. The probe is designed to be totally specific to that species. The probe is labelled such that it fluoresces during amplification, which is used to infer the presence of the target species' DNA in the sample.

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Environmental DNA Report

Great crested newt (*Triturus cristatus*) (for UK licensing)



Project code	PROJ07467
Prepared for	The Temple Group - Norwich
Project	East Pye - T10372
Number of samples	5
Sampling dates	01-Jul-2025
Report ID	GCN-AKJFJ9W
Date of report	22-Jul-2025



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A results interpretation guide and a glossary of terms highlighted throughout this report can be found at the end of the report.

GCN Detection Results

Pond ID	Inhibition	Degradation	GCN Score	Result
123	Pass	Pass	12	Positive
118	Pass	Fail	8	Positive
168	Pass	Fail	1	Positive
119	Pass	Pass	0	Negative
4	Pass	Pass	0	Negative

Sample Information

Pond ID	Kit ID	Sampling Date	Received Date
123	GCN-25-02064	01-Jul-2025	11-Jul-2025
168	GCN-25-02065	01-Jul-2025	11-Jul-2025
119	GCN-25-02071	01-Jul-2025	11-Jul-2025
4	GCN-25-02074	01-Jul-2025	11-Jul-2025
118	GCN-25-02075	01-Jul-2025	11-Jul-2025

Methods

eDNA was precipitated via centrifugation at 14,000 x g and then extracted using Qiagen DNeasy Blood and Tissue extraction kits. **qPCR** amplification was carried out in 12 replicates per sample, using GCN specific **primers** and **probe** (developed by Thomsen et al. (2012) and adopted by Biggs et al. (2014)), in



the presence of **extraction negative controls**, **qPCR positive controls**, and **qPCR negative controls**. A score is given for the number of positive replicates out of 12.

The **qPCR** method follows the recommendations set out by NatureMetrics for Natural England in the qPCR validation project and helps improve the reliability of the interpretation of the data.

Results from the GCN assay are considered to have a **high** confidence rating according to our **Validation Scale** (Harper et al. 2021).

The quality control methods exceed the requirements outlined in Appendix 5 of Biggs et al. (2014). These consist of the use of **kit blanks**, additional **extraction negative controls**, **qPCR negative controls**, and **qPCR positive controls**. Using these controls ensures assay performance is as expected and increases confidence in any weak or late amplifications.

The extraction and qPCR negative controls analysed alongside your samples showed no target amplification and the triplicate positive controls performed as expected.

END OF REPORT

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Result Interpretation Guide

Positive

Target DNA has been detected in this sample, meaning that at least 1 of the 12 qPCR replicates has amplified. This is not a quantitative test, so you should not interpret a high eDNA score (e.g. 12/12) as necessarily indicating a larger population of GCN than a low eDNA score (e.g. 1/12).

Negative

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- Low

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Environmental DNA Report

Great crested newt (*Triturus cristatus*) (for UK licensing)



Project code	PROJ07467
Prepared for	The Temple Group - Norwich
Project	East Pye - T10372
Number of samples	10
Sampling dates	23-Jun-2025 to 25-Jun-2025
Report ID	GCN-OFULAJJ
Date of report	09-Jul-2025



Thank you for choosing NatureMetrics

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GCN Detection Results

Pond ID	Inhibition	Degradation	GCN Score	Result
140	Pass	Pass	1	Positive
142	Pass	Pass	1	Positive
Pond 198	Pass	Pass	1	Positive
50	Pass	Fail	0	Inconclusive
67	Pass	Fail	0	Inconclusive
149	Pass	Pass	0	Negative
203 Buffer	Pass	Pass	0	Negative
208	Pass	Pass	0	Negative
88	Pass	Pass	0	Negative
91	Pass	Pass	0	Negative

Sample Information

Pond ID	Kit ID	Sampling Date	Received Date
142	GCN-25-02053	23-Jun-2025	27-Jun-2025
Pond 198	GCN-25-02055	25-Jun-2025	27-Jun-2025
203 Buffer	GCN-25-02056	25-Jun-2025	27-Jun-2025
50	GCN-25-02057	24-Jun-2025	27-Jun-2025



140	GCN-25-02058	23-Jun-2025	27-Jun-2025
208	GCN-25-02059	24-Jun-2025	27-Jun-2025
91	GCN-25-02066	24-Jun-2025	27-Jun-2025
88	GCN-25-02069	24-Jun-2025	27-Jun-2025
67	GCN-25-02077	25-Jun-2025	27-Jun-2025
149	GCN-25-02083	25-Jun-2025	27-Jun-2025

Methods

eDNA was precipitated via centrifugation at 14,000 x g and then extracted using Qiagen DNeasy Blood and Tissue extraction kits. qPCR amplification was carried out in 12 replicates per sample, using GCN specific primers and probe (developed by Thomsen et al. (2012) and adopted by Biggs et al. (2014)), in the presence of extraction negative controls, qPCR positive controls, and qPCR negative controls. A score is given for the number of positive replicates out of 12.

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The extraction and qPCR negative controls analysed alongside your samples showed no target amplification and the triplicate positive controls performed as expected.

END OF REPORT

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Result Interpretation Guide

Positive

Target DNA has been detected in this sample, meaning that at least 1 of the 12 qPCR replicates has amplified. This is not a quantitative test, so you should not interpret a high eDNA score (e.g. 12/12) as necessarily indicating a larger population of GCN than a low eDNA score (e.g. 1/12).

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Results from these assays are difficult to interpret with confidence. It is impossible to conclusively tell if the target species is present or absent because of the limited amounts of *in silico*, *in vitro*, and *in vivo* testing.

- Medium

Assays with this rating have been tested *in silico*, have optimised lab protocols, specificity and sensitivity tested in and out of the lab, but with no estimates of detection probabilities or extensive testing of external factors that may affect the detectability of the target. Positive results can be interpreted as meaning the target species' DNA is present (assuming the correct sampling conditions), but negative results could mean that the target is absent or that external



factors such as ecology, seasonality, and spatial scales are influencing the detections.

- High

High rating assays have everything that a Medium assay has, in addition to site occupancy modelling and extensive testing of external effects such as ecological, temporal and spatial factors. Positive results can be conclusively interpreted, and negative results can be interpreted as meaning the target species' DNA is absent (assuming the correct sampling conditions). In some instances, a probability of target species presence at a site and in a sample can be given.

Glossary

Controls

Controls are used to monitor both the performance of the assays but also any contamination. Controls are treated in the same way as a normal sample. This is particularly important given the sensitivity of eDNA qPCR methods. Our full complement of controls enables us to fully monitor the whole GCN eDNA process from kits to data.

Kit blank

Used to determine if the kits are contaminated but also to monitor the early stages of the pipelines, e.g. sample reception. These samples also act as uninhibited samples that can be used as a baseline to compare against. This is an additional control not specifically mentioned in the Biggs et al. (2014) protocol.

Extraction negative

Extraction blank. Used to monitor potential contamination during the DNA extraction process.

qPCR negative

Template negative control. Used to monitor potential contamination during the qPCR setup process. For every qPCR reaction, we include more qPCR negative controls than are prescribed in the Biggs et al. (2014) protocol.

qPCR positive

Used to determine whether the qPCR run performed as expected. In addition to the 4 standard dilutions prescribed by the Biggs et al. (2014) protocol, we include an additional standard dilution and amplify all standards in triplicate. The increased number of standard dilutions and replicates allow us to generate standard curves to assess run performance and assay sensitivity.

Limit of detection

The lowest standard positive control concentration at which 95 % of technical replicates amplify. Target amplification below the LOD cannot automatically be considered as negative but should be further investigated as spurious amplifications are more prevalent at these low concentrations.

eDNA

Short for 'environmental DNA'. Refers to DNA deposited in the environment through excretions and secretions, such as mucus, skin cells, saliva, faeces, urine etc. This can be collected in environmental samples (e.g. water, sediment) and used to identify the organisms that it originated from. eDNA in water is broken down by environmental processes over a period of days to weeks. It can travel some distance from the point at which it was released from



the organism, particularly in running water. eDNA is sampled in low concentrations and can be degraded (i.e. broken into short fragments), which limits the analysis options.

Inhibitors

Chemicals/compounds that reduce or prevent DNA amplification, potentially resulting in false-negative results. Common inhibitors include tannins, humic acids and other organic compounds. Inhibitors can be overcome by either diluting the DNA (and the inhibitors), but dilution carries the risk of reducing the DNA concentration below the limits of detection.

qPCR

Stands for 'quantitative PCR'. A PCR reaction incorporating a coloured dye that fluoresces during DNA amplification, allowing a machine to track the progress of the reaction. Often used with species-specific primers and probe where amplification is used to infer the presence of the target species' DNA in the sample. If the species is not present in the sample, no fluorescence will be detected.

Primers

Short sections of synthesised DNA that bind to either end of the DNA segment to be amplified by qPCR.

Probe

A short section of synthesised DNA that binds to a specific section of the target species' DNA within the section flanked by the primers. The probe is designed to be totally specific to that species. The probe is labelled such that it fluoresces during amplification, which is used to infer the presence of the target species' DNA in the sample.

References

- Biggs J, Ewald N, Valentini A, Gaboriaud C, Griffiths RA, Foster J, Wilkinson J, Arnett A, Williams P and Dunn F (2014). Analytical and methodological development for improved surveillance of the Great Crested Newt. Appendix 5. Technical advice note for field and laboratory sampling of great crested newt (*Triturus cristatus*) environmental DNA. Freshwater Habitats Trust, Oxford.
- Harper KJ, Tang CQ, Bruce K, Ross-Gillespie A, Ross-Gillespie V, and Egeter B (2021). A framework for assessing confidence in environmental DNA qPCR assays and results. Natural England Report.
- Thalinger B, Deiner K, Harper LR, Rees HC, Blackman RC, Sint D, Traugott M, Goldberg CS and Bruce K (2021). A validation scale to determine the readiness of environmental DNA assays for routine species monitoring. *Environmental DNA*, 3, 823–836.
- Thomsen PF, Kielgast JOS, Iversen LL, Wiuf C, Rasmussen M, Gilbert MTP, Orlando L and Willerslev E (2012). Monitoring endangered freshwater biodiversity using environmental DNA. *Molecular Ecology*, 21, 2565-2573.

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Received Date: 21.05.2026

GCN Report

Technical Report



SureScreen Scientifics

Folio No: 1824-2026
Purchase Order: E PYE
Contact: Hopkins Ecology
Issue Date: 05.06.2026
Received Date: 21.05.2026

GCN eDNA Analysis

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 analyzing water samples, we can detect these small traces of environmental DNA (eDNA) to confirm GCN habitation or establish GCN absence.

Results

Lab ID	Site Name	OS Reference	Degradation Check	Inhibition Check	Result	Positive Replicates
GCN26 05506	E PYE - 39	TM 16826 89576	Pass	Pass	Negative	0/12
GCN26 05508	E PYE - 113	TM 29595 95291	Pass	Pass	Positive	1/12
GCN26 05509	E PYE - 112	TM 29775 95980	Pass	Pass	Negative	0/12
GCN26 05511	E PYE - 128	TM 24543 92431	Pass	Pass	Positive	12/12
GCN26 05512	E PYE - 249	TM 17752 87653	Pass	Pass	Negative	0/12
GCN26 05513	E PYE - 105	TM 23542 96600	Pass	Pass	Positive	2/12

Matters affecting result: none

Reported by: Ruby Fielding

Approved by: Alice Cunningham

Methodology

The samples detailed above have been analyzed 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 tube which then undergoes DNA extraction. The extracted sample is then analyzed 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 attention to detail to prevent the 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 analytical security.

SureScreen Scientifics Ltd is ISO9001 accredited and participates in Natural England's proficiency testing scheme for GCN eDNA testing.

Interpretation of Results

Sample Integrity Check:

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. Any samples which fail this test are rejected and eliminated before analysis.

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.

Inhibition Check:

Pass/Fail. The presence of inhibitors within a sample is assessed using a DNA marker. If inhibition is detected, samples are purified and re-analyzed. 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 the WC1067 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.

Inconclusive: Controls indicate inhibition or degradation of the sample, resulting in the inability to provide conclusive evidence for GCN presence or absence.



Folio No: 2085-2026
Purchase Order: EPYE2
Contact: Hopkins Ecology
Issue Date: 12.06.2026
Received Date: 29.05.2026

GCN Report

Technical Report



SureScreen Scientifics

Folio No: 2085-2026
Purchase Order: EPHY2
Contact: Hopkins Ecology
Issue Date: 12.06.2026
Received Date: 29.05.2026

GCN eDNA Analysis

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 analyzing water samples, we can detect these small traces of environmental DNA (eDNA) to confirm GCN habitation or establish GCN absence.

Results

Lab ID	Site Name	OS Reference	Degradation Check	Inhibition Check	Result	Positive Replicates
GCN26 05507	EPYE 100	TM 22089 95509	Pass	Pass	Positive	3/12
GCN26 05510	EPYE 005	TM 24881 96415	Pass	Pass	Positive	5/12
GCN26 05522	EPYE 098	TM 22627 95368	Pass	Pass	Negative	0/12
GCN26 05524	EPYE 93	TM 22716 95373	Pass	Pass	Negative	0/12
GCN26 05525	EPYE 165	TM 22985 94764	Pass	Pass	Negative	0/12
GCN26 05526	EPYE 94	TM 22103 95403	Pass	Pass	Negative	0/12
GCN26 05527	EPYE 022	TM 22850 94717	Pass	Pass	Negative	0/12

Matters affecting result: none

Reported by: Chelsea Warner

Approved by: Alice Cunnington

Methodology

The samples detailed above have been analyzed 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 tube which then undergoes DNA extraction. The extracted sample is then analyzed 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 attention to detail to prevent the 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 analytical security.

SureScreen Scientifics Ltd is ISO9001 accredited and participates in Natural England's proficiency testing scheme for GCN eDNA testing.

Interpretation of Results

Sample Integrity Check:

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. Any samples which fail this test are rejected and eliminated before analysis.

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.

Inhibition Check:

Pass/Fail. The presence of inhibitors within a sample is assessed using a DNA marker. If inhibition is detected, samples are purified and re-analyzed. 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 the WC1067 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.

Inconclusive: Controls indicate inhibition or degradation of the sample, resulting in the inability to provide conclusive evidence for GCN presence or absence.



Folio No: 2765-2026
Purchase Order: E PYE 3
Contact: Hopkins Ecology
Issue Date: 03.07.2026
Received Date: 19.06.2026

GCN Report

Technical Report



SureScreen Scientifics

Folio No: 2765-2026
Purchase Order: E PYE 3
Contact: Hopkins Ecology
Issue Date: 03.07.2026
Received Date: 19.06.2026

GCN eDNA Analysis

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 analyzing water samples, we can detect these small traces of environmental DNA (eDNA) to confirm GCN habitation or establish GCN absence.

Results

Lab ID	Site Name	OS Reference	Degradation Check	Inhibition Check	Result	Positive Replicates
GCN26 05503	E PYE - 18A	TM 23485 96698	Pass	Pass	Positive	10/12
GCN26 05504	E PYE - 018	TM 23503 96708	Pass	Pass	Positive	12/12
GCN26 05505	E PYE - 150	TM 22922 93041	Pass	Pass	Positive	7/12
GCN26 05523	E PYE - 193	TM 24502 92500	Pass	Pass	Negative	0/12

Matters affecting result: none

Reported by: Ruby Fielding

Approved by: Jennifer Higginbottom

Methodology

The samples detailed above have been analyzed 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 tube which then undergoes DNA extraction. The extracted sample is then analyzed 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 attention to detail to prevent the 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 analytical security.

SureScreen Scientifics Ltd is ISO9001 accredited and participates in Natural England's proficiency testing scheme for GCN eDNA testing.

Interpretation of Results

Sample Integrity Check:

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. Any samples which fail this test are rejected and eliminated before analysis.

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.

Inhibition Check:

Pass/Fail. The presence of inhibitors within a sample is assessed using a DNA marker. If inhibition is detected, samples are purified and re-analyzed. 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 the WC1067 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.

Inconclusive: Controls indicate inhibition or degradation of the sample, resulting in the inability to provide conclusive evidence for GCN presence or absence.



Folio No: 2928-2026
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Contact: Hopkins Ecology
Issue Date: 07.07.2026
Received Date: 23.06.2026

GCN Report

Technical Report



SureScreen Scientifics

Folio No: 2928-2026
Purchase Order: EPYE 4
Contact: Hopkins Ecology
Issue Date: 07.07.2026
Received Date: 23.06.2026

GCN eDNA Analysis

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 analyzing water samples, we can detect these small traces of environmental DNA (eDNA) to confirm GCN habitation or establish GCN absence.

Results

Lab ID	Site Name	OS Reference	Degradation Check	Inhibition Check	Result	Positive Replicates
GCN26 05498	EPYE - 170	TM 28514 95571	Pass	Pass	Negative	0/12
GCN26 05499	EPYE - 126	TM 24087 92083	Pass	Pass	Positive	3/12
GCN26 05501	EPYE - 243	TM 17688 87545	Pass	Pass	Negative	0/12
GCN26 05514	EPYE - 34	TM 16402 88668	Pass	Pass	Negative	0/12
GCN26 05516	EPYE - 138	TM 19875 94560	Pass	Pass	Positive	3/12
GCN26 05517	EPYE - 28	TM 21694 94784	Pass	Pass	Negative	0/12
GCN26 05518	EPYE - 168	TM 24422 95398	Pass	Pass	Negative	0/12
GCN26 05521	EPYE - 09	TM 2530 93263	Pass	Pass	Negative	0/12

Matters affecting result: none

Reported by: Ruby Fielding

Approved by: Alice Cunningham

Methodology

The samples detailed above have been analyzed 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 tube which then undergoes DNA extraction. The extracted sample is then analyzed 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.

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SureScreen Scientifics Ltd is ISO9001 accredited and participates in Natural England's proficiency testing scheme for GCN eDNA testing.

Interpretation of Results

Sample Integrity Check:

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. Any samples which fail this test are rejected and eliminated before analysis.

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.

Inhibition Check:

Pass/Fail. The presence of inhibitors within a sample is assessed using a DNA marker. If inhibition is detected, samples are purified and re-analyzed. 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 the WC1067 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.

Inconclusive: Controls indicate inhibition or degradation of the sample, resulting in the inability to provide conclusive evidence for GCN presence or absence.



Folio No: 3492-2026
Purchase Order: EPYE 6
Contact: Hopkins Ecology
Issue Date: 16.07.2026
Received Date: 01.07.2026

GCN Report

Technical Report



SureScreen Scientifics

Folio No: 3492-2026
Purchase Order: EPYE 6
Contact: Hopkins Ecology
Issue Date: 16.07.2026
Received Date: 01.07.2026

GCN eDNA Analysis

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 analyzing water samples, we can detect these small traces of environmental DNA (eDNA) to confirm GCN habitation or establish GCN absence.

Results

Lab ID	Site Name	OS Reference	Degradation Check	Inhibition Check	Result	Positive Replicates
GCN26 07247	EPYE - 172	TM 28548 628548	Pass	Pass	Negative	0/12
GCN26 07253	EPYE - 173	TM 29283 629283	Pass	Pass	Positive	2/12
GCN26 07254	EPYE - 227	TM 26882 97604	Pass	Pass	Negative	0/12
GCN26 07255	EPYE - 79	TM 26764 95610	Pass	Pass	Negative	0/12
GCN26 07261	EPYE - 226	TM 26659 97579	Pass	Pass	Negative	0/12
GCN26 07263	EPYE - 81	TM 26542 95342	Pass	Pass	Negative	0/12
GCN26 07264	EPYE - 142	TM 20268 93774	Pass	Pass	Positive	12/12
GCN26 07265	EPYE - 83	TM 26816 95637	Pass	Pass	Negative	0/12

Matters affecting result: none

Reported by: Ruby Fielding

Approved by: Chelsea Warner

Methodology

The samples detailed above have been analyzed 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 tube which then undergoes DNA extraction. The extracted sample is then analyzed 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 attention to detail to prevent the 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 analytical security.

SureScreen Scientifics Ltd is ISO9001 accredited and participates in Natural England's proficiency testing scheme for GCN eDNA testing.

Interpretation of Results

Sample Integrity Check:

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. Any samples which fail this test are rejected and eliminated before analysis.

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.

Inhibition Check:

Pass/Fail. The presence of inhibitors within a sample is assessed using a DNA marker. If inhibition is detected, samples are purified and re-analyzed. 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.

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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.

Inconclusive: Controls indicate inhibition or degradation of the sample, resulting in the inability to provide conclusive evidence for GCN presence or absence.



Folio No: 3487-2026
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Contact: Hopkins Ecology
Issue Date: 16.07.2026
Received Date: 01.07.2026

GCN Report

Technical Report



SureScreen Scientifics

Folio No: 3487-2026
Purchase Order: EPYE5
Contact: Hopkins Ecology
Issue Date: 16.07.2026
Received Date: 01.07.2026

GCN eDNA Analysis

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 analyzing water samples, we can detect these small traces of environmental DNA (eDNA) to confirm GCN habitation or establish GCN absence.

Results

Lab ID	Site Name	OS Reference	Degradation Check	Inhibition Check	Result	Positive Replicates
GCN26 05502	E PYE - 250	TM 16801 89807	Pass	Pass	Positive	1/12
GCN26 05515	E PYE - 40	TM 16860 89900	Pass	Pass	Positive	10/12
GCN26 05519	E PYE - 37	TM 16802 89835	Pass	Pass	Positive	12/12
GCN26 05520	E PYE - 38	TM 16831 89833	Pass	Pass	Positive	1/12
GCN26 07252	E PYE - 185	TM 17955 88063	Pass	Pass	Negative	0/12
GCN26 07256	E PYE - 158	TM 20197 95447	Pass	Pass	Negative	0/12

Matters affecting result: none

Reported by: Ruby Fielding

Approved by: Chelsea Warner

Methodology

The samples detailed above have been analyzed 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 tube which then undergoes DNA extraction. The extracted sample is then analyzed 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.

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SureScreen Scientifics Ltd is ISO9001 accredited and participates in Natural England's proficiency testing scheme for GCN eDNA testing.

Interpretation of Results

Sample Integrity Check:

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. Any samples which fail this test are rejected and eliminated before analysis.

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.

Inhibition Check:

Pass/Fail. The presence of inhibitors within a sample is assessed using a DNA marker. If inhibition is detected, samples are purified and re-analyzed. 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.

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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.

Inconclusive: Controls indicate inhibition or degradation of the sample, resulting in the inability to provide conclusive evidence for GCN presence or absence.



Folio No: 3666-2026
Purchase Order: EPYE 7
Contact: Hopkins Ecology
Issue Date: 20.07.2026
Received Date: 03.07.2026

GCN Report

Technical Report



SureScreen Scientifics

Folio No: 3666-2026
Purchase Order: EPYE 7
Contact: Hopkins Ecology
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GCN eDNA Analysis

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 analyzing water samples, we can detect these small traces of environmental DNA (eDNA) to confirm GCN habitation or establish GCN absence.

Results

Lab ID	Site Name	OS Reference	Degradation Check	Inhibition Check	Result	Positive Replicates
GCN26 07185	EPYE - 179	TM 22486 94568	Pass	Pass	Negative	0/12
GCN26 07244	EPYE - 224	TM 26824 97506	Pass	Pass	Negative	0/12
GCN26 07246	EPYE - 117	TM 30034 95838	Pass	Pass	Negative	0/12
GCN26 07249	EPYE - 82	TM 22486 94568	Pass	Pass	Positive	1/12
GCN26 07250	EPYE - 73	TM 27180 95999	Pass	Pass	Negative	0/12
GCN26 07257	EPYE - 171	TM 28504 95633	Pass	Pass	Negative	0/12
GCN26 07258	EPYE - 220	TM 21538 93493	Pass	Pass	Negative	0/12
GCN26 07259	EPYE - 246	TM 23833 92377	Pass	Pass	Positive	10/12
GCN26 07260	EPYE - 76	TM 25784 95624	Pass	Pass	Positive	3/12
GCN26 07262	EPYE - 77	TM 25691 95422	Pass	Pass	Negative	0/12

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Matters affecting result: none

Reported by: Ruby Fielding

Approved by: Vanessa Hind

Methodology

The samples detailed above have been analyzed 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 tube which then undergoes DNA extraction. The extracted sample is then analyzed 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 attention to detail to prevent the 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 analytical security.

SureScreen Scientifics Ltd is ISO9001 accredited and participates in Natural England's proficiency testing scheme for GCN eDNA testing.

Interpretation of Results

Sample Integrity Check:

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. Any samples which fail this test are rejected and eliminated before analysis.

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.

Inhibition Check:

Pass/Fail. The presence of inhibitors within a sample is assessed using a DNA marker. If inhibition is detected, samples are purified and re-analyzed. 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 the WC1067 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.

Inconclusive: Controls indicate inhibition or degradation of the sample, resulting in the inability to provide conclusive evidence for GCN presence or absence.



Annex D: Legislation

Important Notice: This section contains details of legislation applicable in England and Wales only (i.e., not including Scotland, the Isle of Man, Northern Ireland, the Republic of Ireland or the Channel Islands) and is provided for general guidance only. While every effort has been made to represent the current (at the time of writing) situation with respect to the UK's position outside of the EU and to ensure accuracy throughout, this section should not be relied upon as a definitive statement of the law.

Over the past few years, three important bills have been published which are intended to shape how growing pressures on the environment post-Brexit (post-transition period) are tackled. Both the Agriculture Bill and Fisheries Bill gained Royal Assent in November 2020 and are now the Agriculture Act 2020 and Fisheries Act 2020 respectively; and, more recently, the Environment Bill was passed into law in November 2021, becoming the Environment Act 2021. *N.B. as environment policy is a devolved matter, most of this Act applies to England only.*

Legislation Afforded to Species

The objective of the EC Habitats Directive¹⁰ is to conserve the various species of plant and animal which are considered rare across Europe. The Directive is transposed into UK law by **The Conservation of Habitats and Species Regulations 2017 (as amended)** and **The 'Conservation of Offshore Marine Habitats and Species Regulations 2017 (as amended)**.

Various amendments to the 2017 Regulations in England and Wales have been made through the Conservation of Habitats and Species (Amendment) (EU Exit) Regulations 2019. These changes came into effect on the 1 January 2021 following the UK's departure from the EU and the end of the Transition Period. The changes are limited to 'operability changes' that will ensure the Regulations can continue to have the same working effect as before.

The Wildlife and Countryside Act 1981 (as amended) is a key piece of national legislation which implements the Convention on the Conservation of European Wildlife and Natural Habitats (Bern Convention) and implements the species protection obligations of Council Directive 2009/147/EC (formerly 79/409/EEC) on the Conservation of Wild Birds (EC Birds Directive) in Great Britain.

Since the passing of the Wildlife & Countryside Act 1981, various amendments have been made, details of which can be found on www.opsi.gov.uk. Key amendments have been made through the Countryside and Rights of Way (CRoW) Act (2000).

As well as delivering long-term targets to reduce waste and improve resource efficiency and improve air and water quality targets, the **Environment Act 2021** aims to halt the decline of nature by 2030, mandates Biodiversity Net Gain for developments in England and amends the Wildlife and Countryside Act 1981 (as amended) to introduce an additional purpose for granting a protected species license in relation to development which is 'for reasons of overriding public interest'. The Act also introduces the Office for Environmental Protection (OEP), which will be a new public body intended to hold government and public authorities to

¹⁰ Council Directive 92/43/EEC on the Conservation of Natural Habitats and of Wild Fauna and Flora

account, although the government will be able to issue guidance to the OEP on how it enforces policies and legislation.

Some of the key biodiversity elements in the Act that will have a bearing on species protection in the UK include:

- A strengthened biodiversity duty on Local Planning Authorities.
- Biodiversity net gain to ensure developments, including Nationally Significant Infrastructure Projects (NSIP), deliver at least 10% increase in biodiversity;
- Local Nature Recovery Strategies to support a Nature Recovery Network;
- Duty upon Local Authorities to consult on street tree felling;
- Strengthen woodland protection enforcement measures;
- Conservation Covenants;
- Protected Site Strategies and Species Conservation Strategies to support the design and delivery of strategic approaches to deliver better outcomes for nature;
- Introduces the power for the Habitats Regulations to be amended or 'refocused' to 'to deliver creative public policy thinking that delivers results.'

Other legislative Acts affording protection to wildlife and their habitats include:

- Salmon and Freshwater Fisheries Act 1975
- Deer Act 1991
- Protection of Badgers Act 1992
- Wild Mammals (Protection) Act 1996
- Countryside and Rights of Way (CROW) Act 2000
- Natural Environment & Rural Communities (NERC) Act 2006
- The Eels (England and Wales) Regulations 2009
- Environment (Wales) Act 2016

Species and species groups that are protected or otherwise regulated under the aforementioned legislation, and that are most likely to be affected by development activities, include herpetofauna (amphibians and reptiles), badger, bats, birds, dormouse, invasive species, otter, plants, red squirrel, water vole and white clawed crayfish.

Explanatory notes relating to species protected under The Conservation of Habitats and Species Regulations 2017 (as amended), which includes smooth snake, sand lizard, great crested newt, natterjack toad, all bat species, otter, dormouse and some plant, invertebrate and fish species, are given below. **These should be read in conjunction with the relevant species sections that follow.**

- In the Habitats Directive, the term 'deliberate' is interpreted as being somewhat wider than intentional and may be thought of as including an element of recklessness.
- The Conservation of Habitats and Species Regulations 2017 (as amended) does not define the act of 'migration' and therefore, as a precaution, it is recommended that short distance movement of animals for e.g. foraging, breeding or dispersal purposes are also considered where relevant.
- In order to obtain a mitigation licence for species protected under the Conservation of Habitats and Species Regulations 2017 (as amended), the application must demonstrate that it meets all of the following three 'tests': i) the action(s) are necessary for the purpose of preserving public health or safety or other imperative reasons of overriding public interest including those of a social or economic nature and beneficial consequence of primary importance for the environment; ii) that there is no satisfactory alternative and iii) that the action authorised will not be detrimental to the maintenance of the species concerned at a favourable conservation status in their natural range.

Herpetofauna (Amphibians)

The great crested newt *Triturus cristatus* receives full protection under The Conservation of Habitats and Species Regulations 2017 (as amended) through their inclusion on Schedule 2. Regulation 43 prohibits:

- Deliberate killing, injuring or capturing of species listed on Schedule 2
- Deliberate disturbance of any Schedule 2 species as:
 - a) to impair their ability:
 - (i) to survive, breed, or reproduce, or to rear or nurture young;
 - (ii) in the case of animals of a hibernating or migratory species, to hibernate or migrate
 - b) to significantly affect the local distribution or abundance of the species
- Deliberate taking or destroying of the eggs of a Schedule 2 species
- Damage or destruction of a breeding site or resting place
- Keeping, transporting, selling, exchanging, or offering for sale whether live or dead or of any part thereof.

The species is also currently listed on Schedule 5 of the Wildlife and Countryside Act 1981 (as amended). Under this Act, they are additionally protected from:

- Intentional or reckless disturbance (at any level)
- Intentional or reckless obstruction of access to any place of shelter or protection
- Selling, offering, or exposing for sale, possession or transporting for purpose of sale.