

Hornsea 4



Hornsea Project Four: Preliminary Environmental Information Report (PEIR)

Volume 6, Annex 3.4: Great Crested Newt Environmental DNA (eDNA) Survey Report

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Glossary

Term	Definition
Development Consent Order (DCO)	An order made under the Planning Act 2008 granting development consent for one or more Nationally Significant Infrastructure Projects (NSIP).
Export cable route (ECC) corridor	The specific corridor of seabed (seaward of Mean High Water Springs (MHWS)) and land (landward of MHWS) from the Hornsea Project Four array area to the Creyke Beck National Grid substation, within which the export cables will be located. The final ECC corridor will be located within the ECC corridor search area and will be defined via a site selection process considering technical, physical and environmental constraints.
Hornsea Project Four	The proposed Hornsea Four offshore wind farm project; the term covers all elements within the Development Consent Order (i.e. both the offshore and onshore components).

Acronyms

Acronym	Definition
DCO	Development Consent Order
ECC	Export Cable Corridor
eDNA	Environmental DNA
EcIA	Ecological Impact Assessment
EIA	Environmental Impact Assessment
EP1HS	Extended Phase 1 Habitat Survey
EPS	European Protected Species
GCN	Great Crested Newt
HSI	Habitat Suitability Index
OS	Ordnance Survey
PEIR	Preliminary Environmental Information Report

1 Introduction

1.1 Project Background

1.1.1.1 Ørsted Hornsea Project Four Limited (hereafter the 'Applicant') is proposing to develop Hornsea Project Four offshore wind farm (hereafter Hornsea Four). Hornsea Four will be located approximately 65 km offshore the East Riding of Yorkshire in the Southern North Sea and will be the fourth project to be developed in the former Hornsea Zone. Hornsea Four will include both offshore and onshore infrastructure including an offshore generating station (wind farm), export cables to landfall, and connection to the electricity transmission network. The location of Hornsea Four is illustrated on [Figure 1](#).

1.1.1.2 Royal HaskoningDHV was commissioned to undertake a Great crested newt (GCN) *Triturus cristatus* survey within and around Hornsea Four.

1.2 Aims and objectives

1.2.1.1 The aims of the GCN survey was to:

- Complete a Habitat Suitability Index (HSI) assessment of all identified ponds within and up to 250 m from the Hornsea Four PEIR boundary; and
- Collect an environmental DNA (eDNA) survey sample of all identified ponds where landowner access had been granted to determine the likely presence/absence of GCN.

1.2.1.2 The purpose of this report is to present the findings of the 2019 Hornsea Four GCN survey and to provide an initial understanding of the presence or likely absence of GCN in ponds within Hornsea Four GCN study area. It should be noted that an accompanying assessment has not been undertaken as the baseline data collection was incomplete at the point of preparing this technical report. This was agreed in consultation with Natural England (NE), The Yorkshire Wildlife Trust (YWT), the Royal Society for the Protection of Birds (RSPB) and the Environment Agency (EA) during the third Hornsea Four Ecology and Nature Conservation Evidence Plan meeting on 8th April 2019. Therefore, this technical report will instead support the development of the ES which will contain all assessments required to inform the Development Consent Order (DCO) application in [Volume 3, Chapter 3: Ecology and Nature Conservation](#).

1.2.1.3 This report has been prepared following the guidelines as set out in the Chartered Institute of Ecology and Environmental Management's (CIEEM) Guidelines on Ecological Report Writing (CIEEM, 2017).

2 Legislation

2.1.1.1 **Table 1** summarises the relevant policy and legislation relating to the legal protection afforded to GCN. It should be noted that this is for information only and is not intended to be comprehensive or to replace specialised legal advice.

Table 1: Summary of key legislation and policy relevant to GCN.

Legislation	Relevance
European Union (EU) Directive 92/43/EEC (The Habitats Directive) (Habitats Directive, 1992)	This Directive provides protection for specific habitats listed in Annex I and species listed in Annex II of the Directive. The Directive sets out decision making procedures for the protection of Special Areas of Conservation (SAC) and Special Protection Areas (SPA) and these are implemented in the UK through The Conservation of Habitats and Species Regulations 2017. GCN are listed on Annex II of this directive.
The Conservation of Habitats and Species Regulations 2017 (as amended) (Conservation of Habitats and Species Regulations, 2017)	Codifies the EU Directive 92/43/EEC (The Habitats Directive) into UK law; provides legal protection for European Protected Species (EPS). GCN are an EPS.
Wildlife and Countryside Act 1981 (as amended) (WCA, 1981)	This Act makes it an offence to intentionally kill, injure or take any animal listed in Schedule 5 of the Act and protects occupied and unoccupied places used for shelter or protection. GCN are listed on Schedule 5 of this Act
Natural Environment and Rural Communities Act 2006 (NERC, 2006)	Section 41 of the Act requires the Secretary of State to compile a list of habitats and species of principal importance for the conservation of biodiversity in England. Decision makers of public bodies, in the execution of their duties, must have regard to the conservation of biodiversity in England, and the list is intended to guide them. Natural England have compiled a list of species of Principal Importance and GCN are on this list.
Policy	Relevance
UK Post-2010 Biodiversity Framework (JNCC, 2012)	Supersedes the UK Biodiversity Action Plan (UK BAP), which fulfilled a legal obligation under the Convention on Biological Diversity to identify and produce action plans for priority habitats and species.

3 Methodology

3.1 Study Area

3.1.1.1 The Hornsea Four GCN survey study area consisted of the landfall, onshore export cable corridor (ECC) and onshore substation (OnSS), which together comprise the onshore Hornsea Four PEIR boundary. An additional 250 m around all of these elements was included as current accepted research indicates that although GCN can travel up to 500 m from a breeding pond they are unlikely to do so where barriers to their movement may be present and if there are limited hibernation and/or foraging opportunities within 250 m of a breeding pond (Cresswell and Whitworth, 2004).

3.1.1.2 The updated Extended Phase 1 Habitat Survey (EP1HS) undertaken in February 2019 confirmed that the predominant habitat surveyed consisted of arable fields, either in crop or ploughed, which act as a natural barrier to GCN movement. This was discussed and confirmed in consultation with Natural England (NE), The Yorkshire Wildlife Trust (YWT), the Royal Society for the Protection of Birds (RSPB) and the Environment Agency (EA) during the third Hornsea Four Ecology and Nature Conservation Evidence Plan meeting on 8th April 2019. For further details on the EP1HS see [Volume 6, Annex 3.1: Extended Phase 1 Habitat Survey Report](#).

3.1.1.3 The Hornsea Four GCN survey study area is shown on [Figure 1](#).

3.2 Survey Methodology

3.2.1 Desk study

3.2.1.1 Biological data received from the North and East Yorkshire Data Centre (NEYEDC) during the scoping stages of the project was reviewed for information on GCN presence within the Hornsea Four GCN survey study area.

3.2.1.2 Ordnance Survey (OS) mapping was then used identify all ponds within the Hornsea Four GCN survey study area. A total of 84 potential ponds were identified as shown on [Figure 2 - Figure 26](#). During the GCN eDNA survey undertaken in June 2019, an additional pond was recorded by surveyors, therefore giving a total of 85 ponds. These 85 ponds form the basis of the 2019 GCN eDNA survey.



Figure 1: GCN Survey Area (Not to Scale).

3.2.2 Field survey

- 3.2.2.1 The GCN survey was undertaken using the environmental DNA (eDNA) approach (Briggs et al. 2014). This is an approved and valid method for undertaking a GCN presence/absence survey and was discussed and agreed with The Yorkshire Wildlife Trust (YWT), the Royal Society for the Protection of Birds (RSPB) and the Environment Agency (EA) during the third Hornsea Four Ecology and Nature Conservation Evidence Plan meeting on 8th April 2019.
- 3.2.2.2 Surveys using the eDNA method have a benefit over more traditional surveys as they can be completed within a single visit to each water body from mid-May to the end of June.
- 3.2.2.3 The 2019 GCN field survey was conducted in two stages, to accommodate the available survey access at the time. The first GCN eDNA survey was undertaken in April 2019 and a subsequent survey visit was undertaken in June 2019.
- 3.2.2.4 Access was granted to a total of 74 ponds (out of the 85 ponds identified) in April and June 2019. Of those 74 accessible ponds, 15 ponds were dry, 14 ponds were no longer present, and one pond was inaccessible due to the presence of livestock. These ponds can be seen on [Figure 2 – Figure 26](#). Furthermore, two ponds were inaccessible due to electric fencing and locked gates. However, a visual assessment was undertaken which noted that these ponds were large fishing ponds with a high number of geese, swan and ducks as well as chickens foraging within the areas of grassland surrounding these ponds (see [Appendix B - 2019 GCN eDNA Survey Pond Descriptions and Photographs](#)). In light of these observations, both of these ponds were therefore assessed as being unsuitable for GCN and have not been considered or surveyed any further.
- 3.2.2.5 A total of 42 surveyed ponds were assessed for their potential to support GCN using the Habitat Suitability Index (HSI) (see [Section 3.2.3](#)) and were subsequently sample for eDNA (see [Section 3.2.4](#)).

3.2.3 Habitat Suitability Index Assessment

- 3.2.3.1 All ponds surveyed for their potential to support GCN using the Habitat Suitability Index (HSI) where surveyed in accordance with standard methodology (Oldham et al. 2000). These HSI assessments were undertaken in April and June 2019 by two GCN licensed ecologists with experience of undertaking HSI assessments:
- 3.2.3.2 Each HSI assessment considered the following ten standard habitat attributes that are considered to influence the suitability of a pond for breeding GCN:
- **Location** – within a UK-wide context reflecting the differences in national distribution of this species;
 - **Area** – water bodies between 100 and 300 m² in size are considered to represent the most suitable habitat for GCN;

- **Drying** – occasional drying kills fish which is beneficial for GCN, but the species predominantly favours ponds that do not dry out every year.
- **Water quality** – qualitative evidence-based assessment to infer good (diverse aquatic invertebrate assemblage), moderate (moderate invertebrate diversity), poor (low invertebrate diversity, few submerged plants) or bad (clearly polluted) water quality.
- **Shade** – percentage of pond perimeter shaded to at least 1 m from the shore. GCN favour lightly shaded water bodies;
- **Waterfowl** – qualitative evidence-based assessment of presence or absence and numbers is made. Large numbers of waterfowl can result in nutrient enrichment of the water and habitat damage, which is less favourable for GCN;
- **Fish** – qualitative evidence-based assessment of likely presence or absence is made. GCN favour breeding ponds that do not support fish because their open-water swimming larvae are vulnerable to fish predation;
- **Number of waterbodies within 1 km** – GCN populations are typically best developed where they have access to a network of ponds, and therefore the species is more likely to be found where there are several ponds within 1 km that are linked by suitable terrestrial habitat; and
- **Macrophyte cover** – percentage of pond surface area occupied by macrophyte cover. Female GCN require aquatic vegetation for egg-laying.

3.2.3.3 An HSI score between 0 and 1 is then determined by scoring the pond against these 10 criteria. HSI scores give an approximate indication of habitat suitability as shown in **Table 2**:

Table 2: HSI score definitions.

HSI score	Definition
< 0.5	Poor
0.5-0.59	Below average
0.6-0.69	Average
0.7-0.79	Good
≥ 0.8	Excellent

3.2.3.4 Where a pond was not suitable to support GCN, no HSI assessment was undertaken and no eDNA survey was conducted. Reasons for this include the following:

- Pond not existing and/or no longer present;
- Pond fully dry at the time of the survey; and
- Fishing ponds are not deemed suitable for GCN due to high concentration of ducks, geese or other water fowl.

3.2.4 eDNA Survey Methodology

3.2.4.1 Water samples were collected by Royal HaskoningDHV ecologists from all 42 ponds subject to an HSI Assessment in April and June 2019. The survey was undertaken by two GCN licenced ecologists, Charlotte Clements (survey class licence (Level 1) – Licence reference:

2016-25773-CLS-CLS) and Paul Hiscocks (survey class licence (Level 1) – Licence reference: 2015-18845-CLS-CLS).

- 3.2.4.2 All samples were sent to Fera Science Limited for analysis for eDNA in accordance with the approved laboratory protocols (Briggs et al. 2014). No ponds were entered by the surveyors during collection of the water sample, in accordance with the eDNA survey methodology, and new sterile equipment supplied by Fera Science Limited was used to collect each water sample, to prevent contamination between samples. General site biosecurity measures were also adhered to during the survey, including boot disinfection (using Virkon S biosecurity tablets) and hand sanitisation.
- 3.2.4.3 The presence or absence of GCN from each of the surveyed ponds was determined based on the results of the eDNA analysis. If eDNA is detected, this provides confirmation of presence and the relevant ponds could represent a constraint that requires further consideration. If eDNA is not detected then it is considered that there is no reasonable likelihood of GCN being present in the relevant ponds, and they therefore require no further assessment with regards to GCN.

3.3 Limitations

- 3.3.1.1 The eDNA sampling technique does not enable an estimate of population size class. Instead the eDNA technique provides confirmation of presence or likely absence of GCN in the pond concerned. Pre-construction surveys will then be undertaken using traditional survey methods in order understand population sizes, for which the appropriate mitigation will apply. For further information on pre-construction surveys see [Volume F2, Chapter 3: Outline Ecological Management Plan \(OEMP\)](#). This approach was discussed and agreed with Natural England during the second Hornsea Four Ecology and Nature Conservation Evidence Plan Meeting held on 8th January 2019.
- 3.3.1.2 Based on Natural England's standing advice on GCN, the window for collecting eDNA samples is between the 15th April until the 30th June. The eDNA samples for the 42 ponds surveyed in April and June 2019 were collected during this period and therefore are compliant with the eDNA methodology and analysis.
- 3.3.1.3 A total of 11 ponds remain to be surveyed for the presence of GCN. Access to these 11 ponds was not granted at the time of the 2019 eDNA survey effort. [Figure 2 - Figure 26](#) show the ponds which were not accessible at the time of the survey. Hornsea Four is in discussion with Natural England in relation to proposed mitigation for these ponds (see [Section 6](#)). For three ponds (Pond_A30, Pond_A31 and Pond_A83, [Appendix B](#)) the photographs taken

during the survey were corrupted beyond recovery. Additional photographs of these ponds can be obtained if requested by consultees prior to submission of the ES.

4 Results

4.1 HSI Assessment Results

4.1.1.1 The results of the 2019 HSI assessment are shown in [Table 3](#). For completeness, all 85 ponds have been included in the table and where a pond was not subject to the HSI assessment (or subsequent eDNA sampling) a justification is provided. All ponds were subject to the HSI assessment. Those ponds subject to subsequent eDNA sampling are highlighted in green in and are shown on [Figure 2 - Figure 26](#) in this report.

Table 3: Summary of 2019 HSI Assessment.

Pond reference	Description	HSI Score	Scoped in to eDNA Survey (Yes/No)
Pond_A01	Small pond on field margin closed with vegetation. Very little open water. No feed, rainwater only.	0.68	Yes
Pond_A02	Large pond, dry to edges. Set in BL woodland, only centre of pond has water (30 x 15). Rain water fed.	0.88	Yes
Pond_A03	Dry pond. Small scrape set in middle of pasture field – heavily scraped and no water.	n/a	No
Pond_A04	Dry pond. Small scrape set in middle of pasture field – heavily scraped.	n/a	No
Pond_A05	Dry pond. Small scrape set in middle of pasture field – heavily scraped, no water.	n/a	No
Pond_A06	Small pond in garden. Detritus and leaf litter on pond bottom. Lily,, bramble, hawthorn, beech, field maple, ash and dock leaf were all present.	0.66	Yes
Pond_A07	Large pond within gardens. Bulrush and glyceria present around edges, soft rush. Fenced, mowed short sward grass.	0.76	Yes
Pond_A08	Small pond with limited aquatic vegetation; willow, dock leave, soft rush, fenced through middle of pond.	0.63	Yes
Pond_A09	Dry pond.	n/a	No
Pond_A10	Dry pond.	n/a	No
Pond_A11	Long narrow pond surrounded by vegetation, including hawthorn, willow, bramble, nettle, soft rush. Within grassland used for silage.	0.57	Yes
Pond_A12	Small crescent shaped pond, very shallow in small wooded copse. Fed by drainage ditches from arable fields.	0.50	Yes
Pond_A13	Ornamental pond next to farmhouse. Set in amenity grassland with woodland to north east and arable fields.	0.72	Yes
Pond_A14	Dry pond. Small ditch joins A-13 to A-14, completely dry and dense with vegetation.	n/a	No

Pond reference	Description	HSI Score	Scoped in to eDNA Survey (Yes/No)
Pond_A15	No access possible due to livestock being present in field at the time of the survey.	n/a	n/a
Pond_A16	Dry pond. Small vegetated scrape in the ground poached by cattle under ash trees, no water.	n/a	No
Pond_A17	Dry pond. Small vegetated scrape in the ground poached by cattle under ash trees, no water.	n/a	No
Pond_A18	Large pond within grassland, majority of bank covered in glyceria, island in middle of pond. Good connectivity to A_06 and A_07. 2 eDNA kits used.	0.85	Yes
Pond_A19	No pond present.	n/a	No
Pond_A20	Large pond in small woodland. Highly overgrown and therefore difficult to access. Very little marginal vegetation.	0.74	Yes
Pond_A21	Round ornamental pond in garden surrounded by iris and rush pond weed. Pond and duck weed also present.	0.75	Yes
Pond_A22	Large pond with a gravel base, remnant from sand/gravel extraction. Clear water with little vegetation. Edges dominated by iris, bulrush and willow scrub. Connecting habitat between this pond and other ponds in the wider area.	0.89	Yes
Pond_A23	L shaped pond remnant from sand/gravel extraction (as Pond_A22). Surrounded by semi-improved grassland comprising birds foot trefoil, cock's foot and teasel. Good connectivity to other ponds.	0.65	Yes
Pond_A24	Pond in centre of woodland. Pondweed, iris and bulrush. Water quality good, excellent connectivity to surrounding terrestrial habitats.	0.85	Yes
Pond_A25	Fishing pond with high concentration of ducks and geese, fenced and locked. Deemed to be unsuitable for GCN.	n/a	n/a
Pond_A26	Fishing pond with high concentration of ducks and geese, fenced and locked. Deemed to be unsuitable for GCN.	n/a	n/a
Pond_A27	No access granted at the time of the survey.	n/a	n/a
Pond_A28	Dry pond. Large pond fed by adjacent river. Bone dry when surveyed.	n/a	No
Pond_A29	Small remnant of A-28 divided by mound of vegetated earth, choked with water lily, water quality okay, surrounded by chest high vegetation. Good connectivity	0.78	Yes
Pond_A30	Dry pond. Middle of arable fields planted with crop, owner says may contain small amounts of water over winter.	n/a	No
Pond_A31	Dry pond. Middle of arable fields planted with crop, owner says may contain small amounts of water over winter.	n/a	No
Pond_A32	Round ornamental pond in front of factory. Steep sided naturally fed with lots of invertebrates, small fish present and water lily abundant.	0.71	Yes
Pond_A33	Large pond once stocked with trout, little vegetation in bottom, spring fed with a gravel bottom.	0.92	Yes

Pond reference	Description	HSI Score	Scoped in to eDNA Survey (Yes/No)
Pond_A33a	Small man-made pond approximately 100 m from onshore cable route. Overgrown with duckweed, iris and bulrush.	0.70	Yes
Pond_A34	Dry pond. Small scrape in ground full of grasses.	n/a	No
Pond_A35	Dry pond. Small scrape in the ground, no water grasses only.	n/a	No
Pond_A36	Scape in the ground in the centre of a field completely covered in vegetation. Small areas of standing water.	0.52	Yes
Pond_A37	No pond, small scrape in ground choked with vegetation. Looks like flood plain for the Bryan Mills Beck.	n/a	No
Pond_A38	No pond, small scrape in ground choked with vegetation. Looks like flood plain for the Bryan Mills Beck.	n/a	No
Pond_A39	No pond, small scrape in ground choked with vegetation. Looks like flood plain for the Bryan Mills Beck.	n/a	No
Pond_A40	No pond, small scrape in ground choked with vegetation. Looks like flood plain for the Bryan Mills Beck.	n/a	No
Pond_A41	No pond, small scrape in ground choked with vegetation. Looks like flood plain for the Bryan Mills Beck.	n/a	No
Pond_A42	No pond, small scrape in ground choked with vegetation. Looks like flood plain for the Bryan Mills Beck.	n/a	No
Pond_A43	Linear spring fed stream, slight slow flow towards Bealeys Beck within a plantation woodland.	0.58	Yes
Pond_A44	Small, heavily shaded pond at edge of arable fields, adjacent to hedgerow and public right of way, feeds into ditch, partially dry.	0.69	Yes
Pond_A45	Dry pond.	n/a	No
Pond_A46	No pond present.	n/a	No
Pond_A47	Small pond/hollow in side of arable field completely covered in vegetation/buttercup and dogrose. Water 2" to 3" deep.	0.70	Yes
Pond_A48	This is not a pond but a wide section of ditch that is currently dry. However, a garden pond is present, and this is referenced Pond_A48 and therefore surveyed. Small pond, glyceria throughout, fish pond adjacent (not connected).	0.77	Yes
Pond_A49	Dry pond	n/a	No
Pond_A50	Small pond within woodland (habitat rich, potentially ancient woodland); glyceria duckweed, sycamore, field maple, willow, ramsons, nettle, bramble, red-dead nettle, white-dead nettle, bluebells, red campion, forget me not, lesser celandine, ground ivy	0.61	Yes
Pond_A51	Pear shaped ornamental pond (lined). Few iris and bulrush planted around edge. Bitumen lined with large fish ad moorhen present. Mowed lawns surround.	0.67	Yes

Pond reference	Description	HSI Score	Scoped in to eDNA Survey (Yes/No)
Pond_A52	Small round pond fed by pipe under the road/runoff. Steep bank with no vegetation.	0.54	Yes
Pond_A53	Pond is a wide section of a (dry) ditch, between an arable field (ploughed) and grassland, fenced. Sycamore, nettle, bramble, dock, cleavers, white dead nettle and hawthorn. Grassland potentially used as grazing for farm	0.66	Yes
Pond_A54	Medium sized pond in middle of arable field (in crop - oilseed), fenced and gated (gate sign stated 'pollution control'). Manhole cover visible at bottom of pond. Surrounded by grass, broad leaf dock and hawthorn, some pond scum on surface, could potential be SUDS for A164. Disconnected from wider habitat by arable crops and roads	0.79	Yes
Pond_A55	Small pond in woodland adjacent to grassland. Sycamore, bramble, ribwort plantain, nettle, cow parsley, cleavers, dog mercury. Shallow pond approximately 15cm deep	0.54	Yes
Pond_A56	Dry pond	n/a	No
Pond_A57	Small pond adjacent to woodland. Bulrush and glyceria. Tadpoles, arable crop adjacent	0.73	Yes
Pond_A58	Medium sized fish pond within animal paddock surrounded by alder, bramble and nettle	0.33	Yes
Pond_A59	No pond present.	n/a	No
Pond_A60	Medium pond within garden and fenced horse paddock. Willow, hawthorn, broad leaf dock, glyceria, bulrush, soft rush. Common frogs present	0.85	Yes
Pond_A61	Small pond in woodland adjacent to grassland. Sycamore, bramble, ribwort plantain, nettle, cow parsley, cleavers, dog mercury. Shallow pond approx 15cm deep	0.74	Yes
Pond_A62	Small pond in woodland, some aquatic vegetation at margins	0.72	Yes
Pond_A63	Small ephemeral pond between arable fields and PRow. Water mint present, broad leaf dock, nettle, bramble, hawthorn and sycamore	0.56	Yes
Pond_A64	Medium pond at corner of arable field adjacent to PRow, hedgerows, grassy field margins. Bulrush dominant throughout, bramble, nettle and hawthorn	0.86	Yes
Pond_A65	Small pond at edge of arable field with wide field margins and hedgerow, tadpoles present. Bulrush, glyceria, hawthorn, soft rush and dandelion	0.7	Yes
Pond_A66	Small pond at edge of arable field with wide grassy margins and scrub vegetation. Bulrush and glyceria throughout.	0.81	Yes
Pond_A67	No pond present.	n/a	No
Pond_A68	Large lagoon within ruderal/scrub habitat, borders railway line and major road (A1079). Potentially SUDS from road so potentially polluted. Steep sides. Willow, ash, water mint, yellow flag iris, bramble, nettle, gorse	0.8	Yes

Pond reference	Description	HSI Score	Scoped in to eDNA Survey (Yes/No)
Pond_A69	No pond present.	n/a	No
Pond_A70	Large concrete pond with bitumen liner. Surrounded by overhanging paving slabs set in amenity grassland and concrete.	0.53	Yes
Pond_A71	No access granted at the time of the survey.	n/a	n/a
Pond_A72	Small pond at confluence of arable fields, ditch and PRow. Yellow flag iris, cherry trees, hawthorn, nettle, cow parsley	0.76	Yes
Pond_A73	No access granted at the time of the survey.	n/a	n/a
Pond_A74	No access granted at the time of the survey.	n/a	n/a
Pond_A75	No access granted at the time of the survey.	n/a	n/a
Pond_A76	No access granted at the time of the survey.	n/a	n/a
Pond_A77	No access granted at the time of the survey.	n/a	n/a
Pond_A78	No access granted at the time of the survey.	n/a	n/a
Pond_A79	No access granted at the time of the survey.	n/a	n/a
Pond_A80	No access granted at the time of the survey.	n/a	n/a
Pond_A81	No access granted at the time of the survey.	n/a	n/a
Pond_A82	Concrete ornamental pond with no vegetation. Pump visible and no fish.	0.44	Yes
Pond_A83	No pond – removed and re-landscaped in 2004.	n/a	No
Pond_A84	Dry pond	n/a	No
Pond_A85	No access granted at the time of the survey.	n/a	n/a

4.2 eDNA Survey Results

4.2.1.1 All 42 ponds subject to an HSI assessment (as listed in [Table 3](#)) were then subject to the collection of water samples which were sent to Fera Science Limited for laboratory eDNA analysis. A summary of the results of the eDNA analysis for each pond is summarised in [Table 4](#), with full laboratory analysis results provided in [Appendix A - eDNA Laboratory Analysis](#). A total of four sample analysis results were not available at the time of writing this report, this information will be provided in an updated version of this technical report which will be produced to support the development of the Environmental Statement (ES). [Table 4](#) should be read in conjunction with [Figure 2 - Figure 26](#).

Table 4: Summary of eDNA Laboratory Analysis for ponds subject to an eDNA sample as listed in Table 3.

Pond reference	eDNA Result	FERA reference
Pond_A01	Negative	S19-015705
Pond_A02	Negative	S19-015703

Pond reference	eDNA Result	FERA reference
Pond_A06	Negative	S19-015702
Pond_A07	Negative	S19-015688
Pond_A08	Positive	S19-015694
Pond_A11	Positive	S19-015692
Pond_A12	Negative	S19-015717
Pond_A13	Negative	S19-015780
Pond_A18	Negative	S19-015704
Pond_A20	Negative	S19-015714
Pond_A21	Negative	S19-015783
Pond_A22	Negative	S19-015786
Pond_A23	Negative	S19-015781
Pond_A24	Awaiting analysis results	S19-015787
Pond_A29	Negative	S19-015701
Pond_A32	Positive	S19-015680
Pond_A33	Negative	S19-015682
Pond_A33a	Inconclusive	S10-015697
Pond_A36	Awaiting analysis results	S19-015681
Pond_A43	Awaiting analysis results	S19-015689
Pond_A44	Negative	S19-015698
Pond_A47	Negative	S19-015684
Pond_A48	Negative	S19-015696
Pond_A50	Negative	S19-015690
Pond_A51	Negative	S19-015691
Pond_A52	Negative	S19-015695
Pond_A53	Negative	S19-015720
Pond_A54	Negative	S19-015723
Pond_A55	Negative	S19-015726
Pond_A57	Negative	S19-015791
Pond_A58	Negative	S19-015686
Pond_A60	Negative	S19-015711
Pond_A61	Negative	S19-015788
Pond_A62	Negative	S19-015785
Pond_A63	Negative	S19-015800
Pond_A64	Negative	S19-015708

Pond reference	eDNA Result	FERA reference
Pond_A65	Negative	S19-015799
Pond_A66	Negative	S19-015779
Pond_A68	Negative	S19-015794
Pond_A70	Awaiting analysis results	S19-015683
Pond_A72	Negative	S19-015793
Pond_A82	Negative	S19-015693

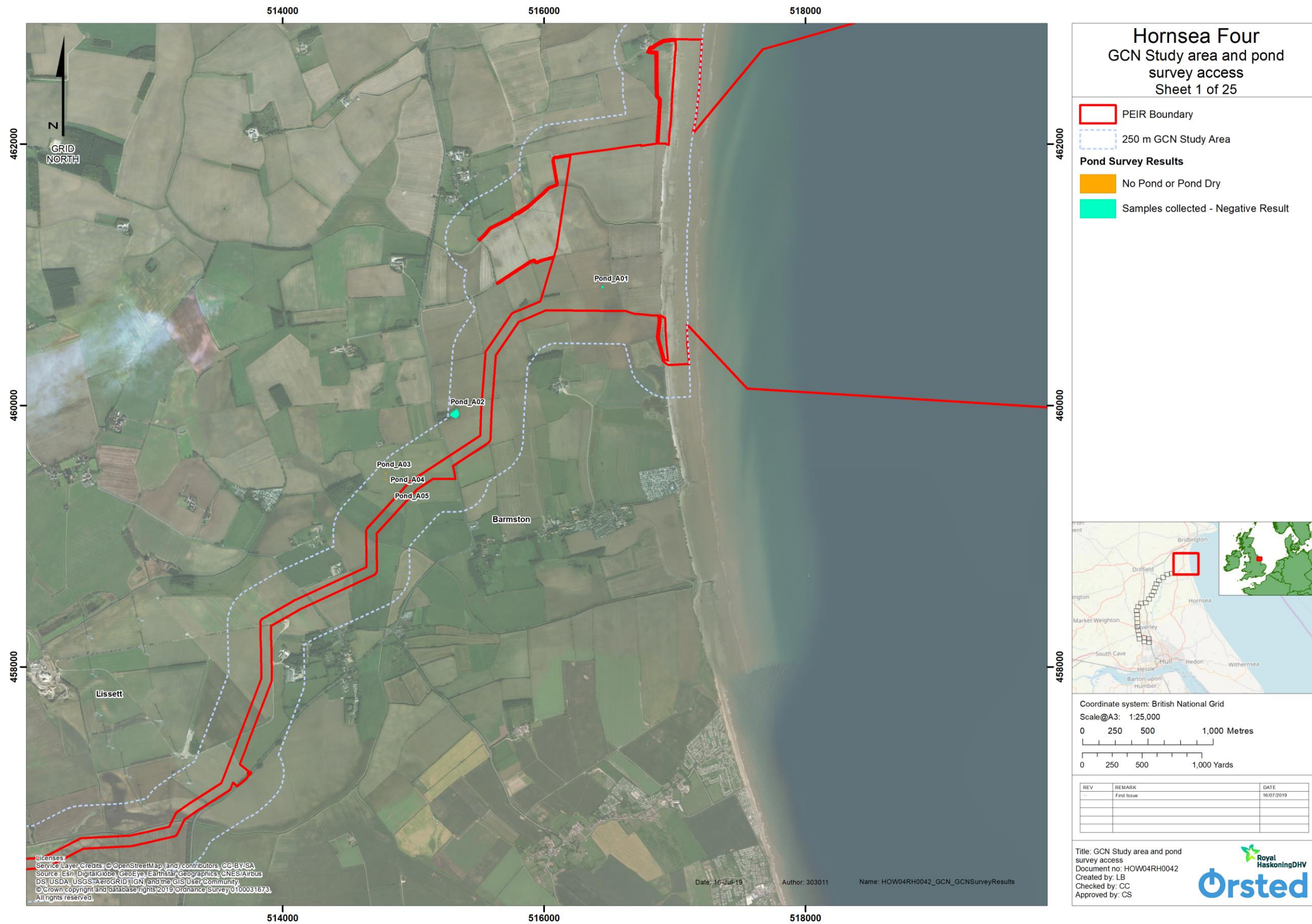


Figure 2: GCN Survey Results (Not to Scale).



Figure 3: GCN Survey Results (Not to Scale).



Figure 4: GCN Survey Results (Not to Scale).

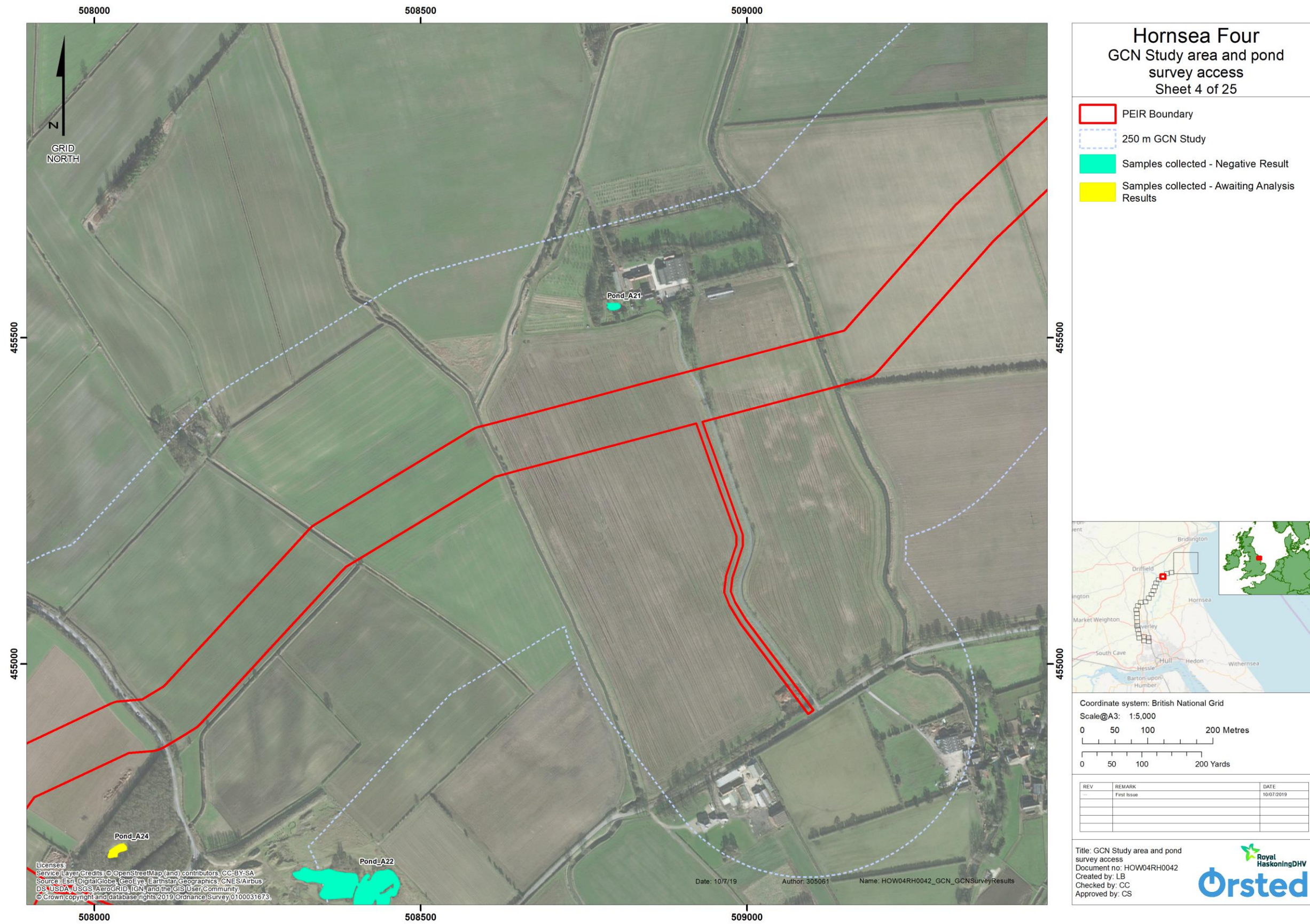


Figure 5: GCN Survey Results (Not to Scale).

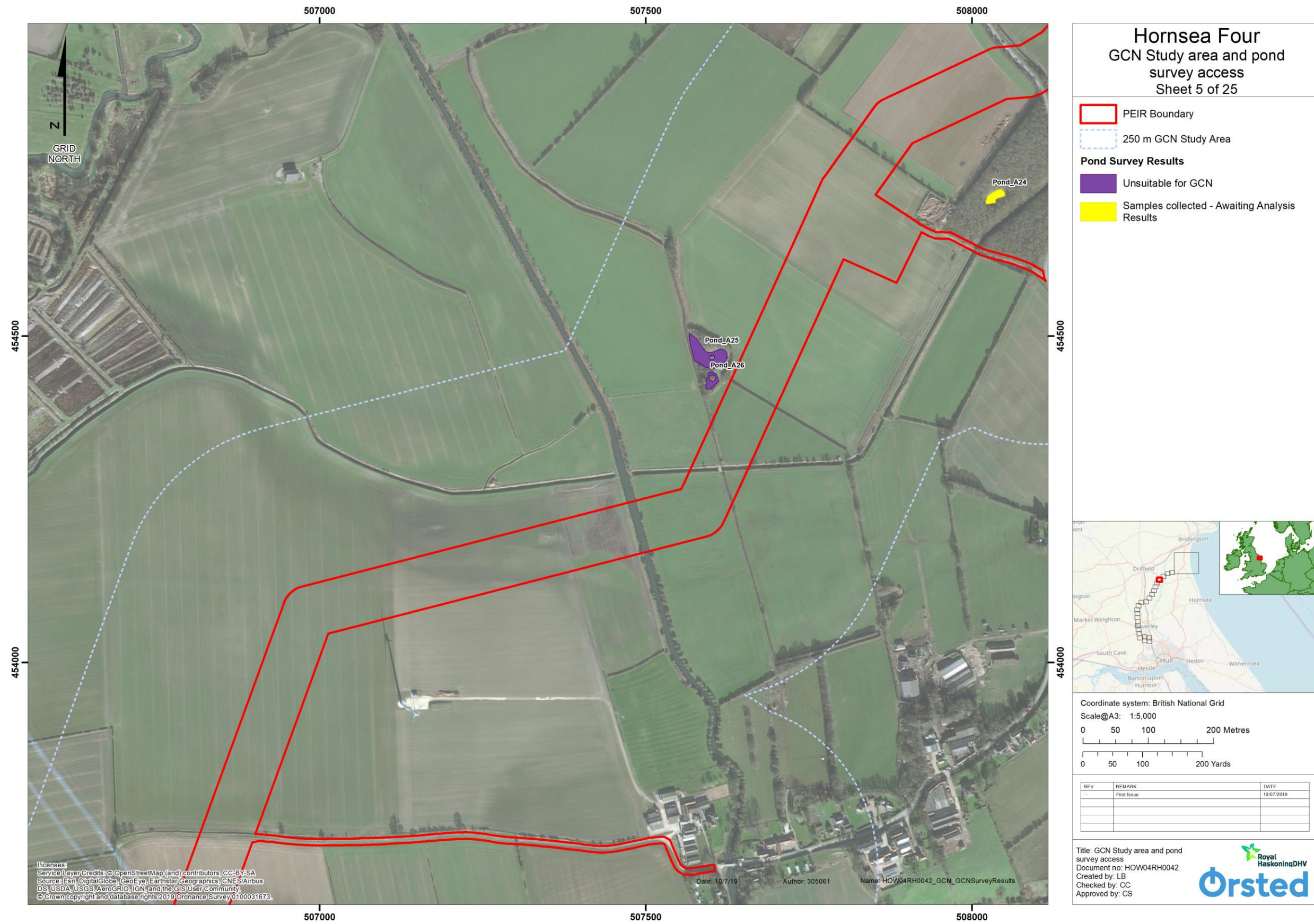


Figure 6: GCN Survey Results (Not to Scale).

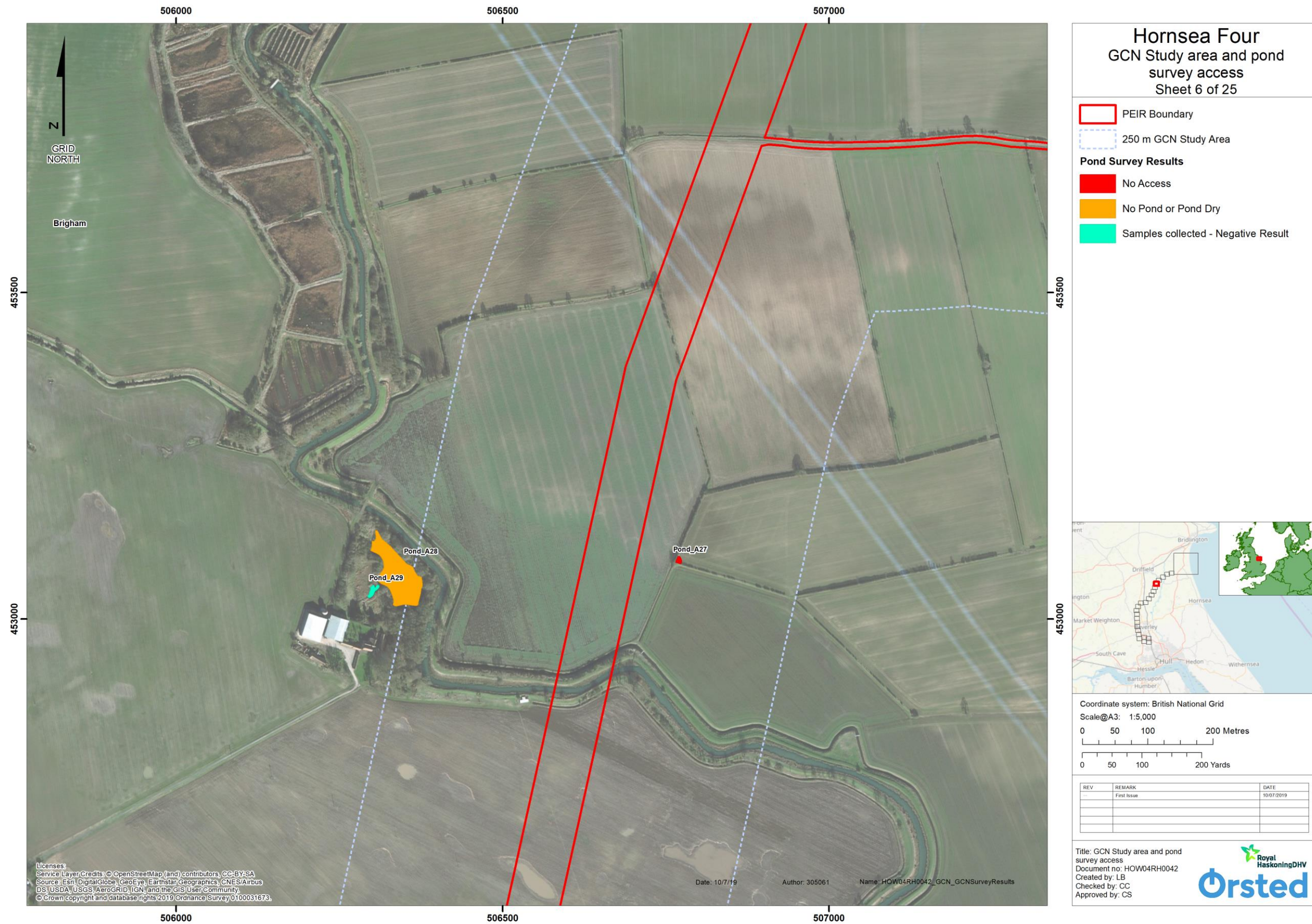


Figure 7: GCN Survey Results (Not to Scale).



Figure 8: GCN Survey Results (Not to Scale).



Figure 9: GCN Survey Results (Not to Scale).



Figure 10: GCN Survey Results (Not to Scale).

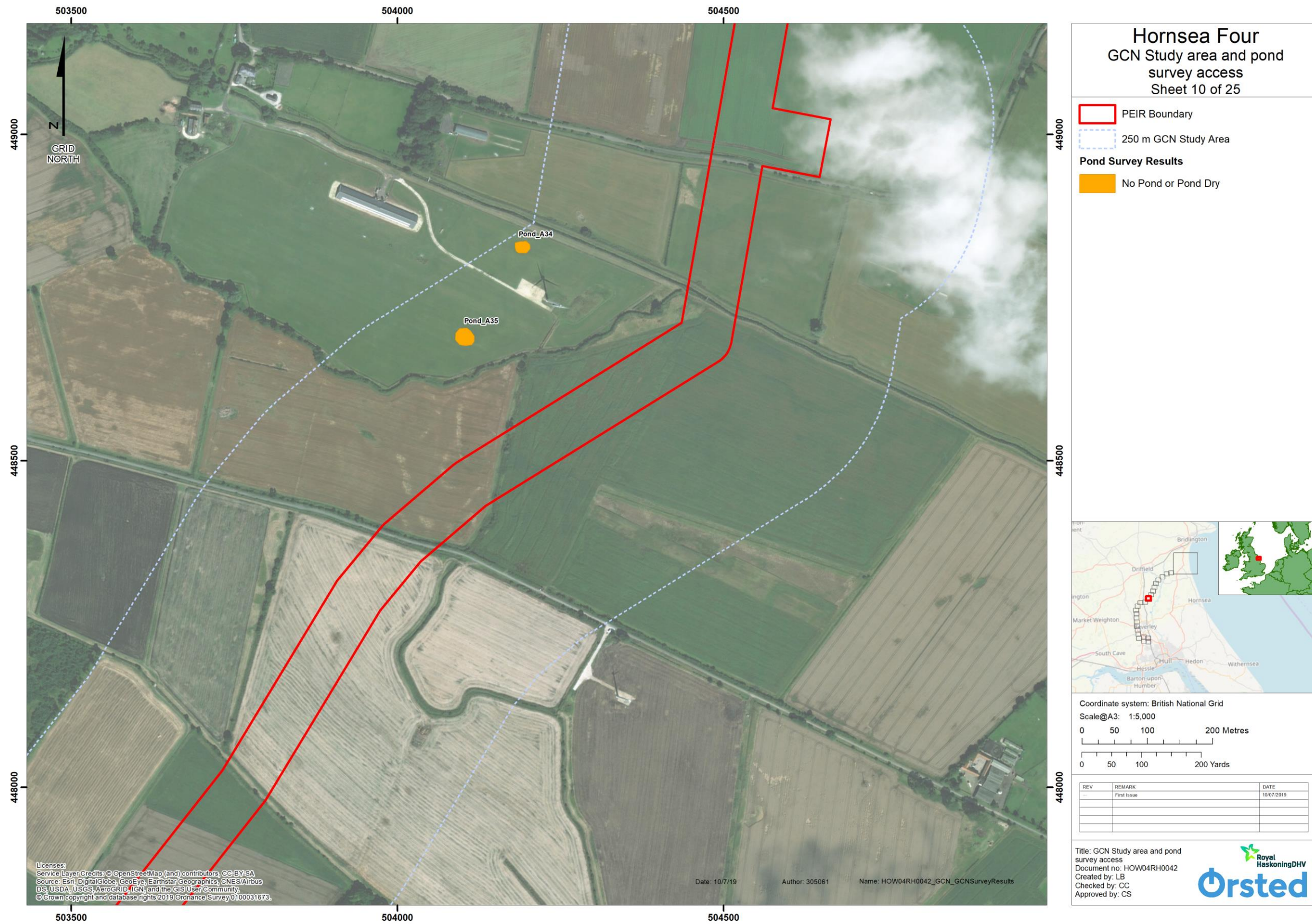


Figure 11: GCN Survey Results (Not to Scale).

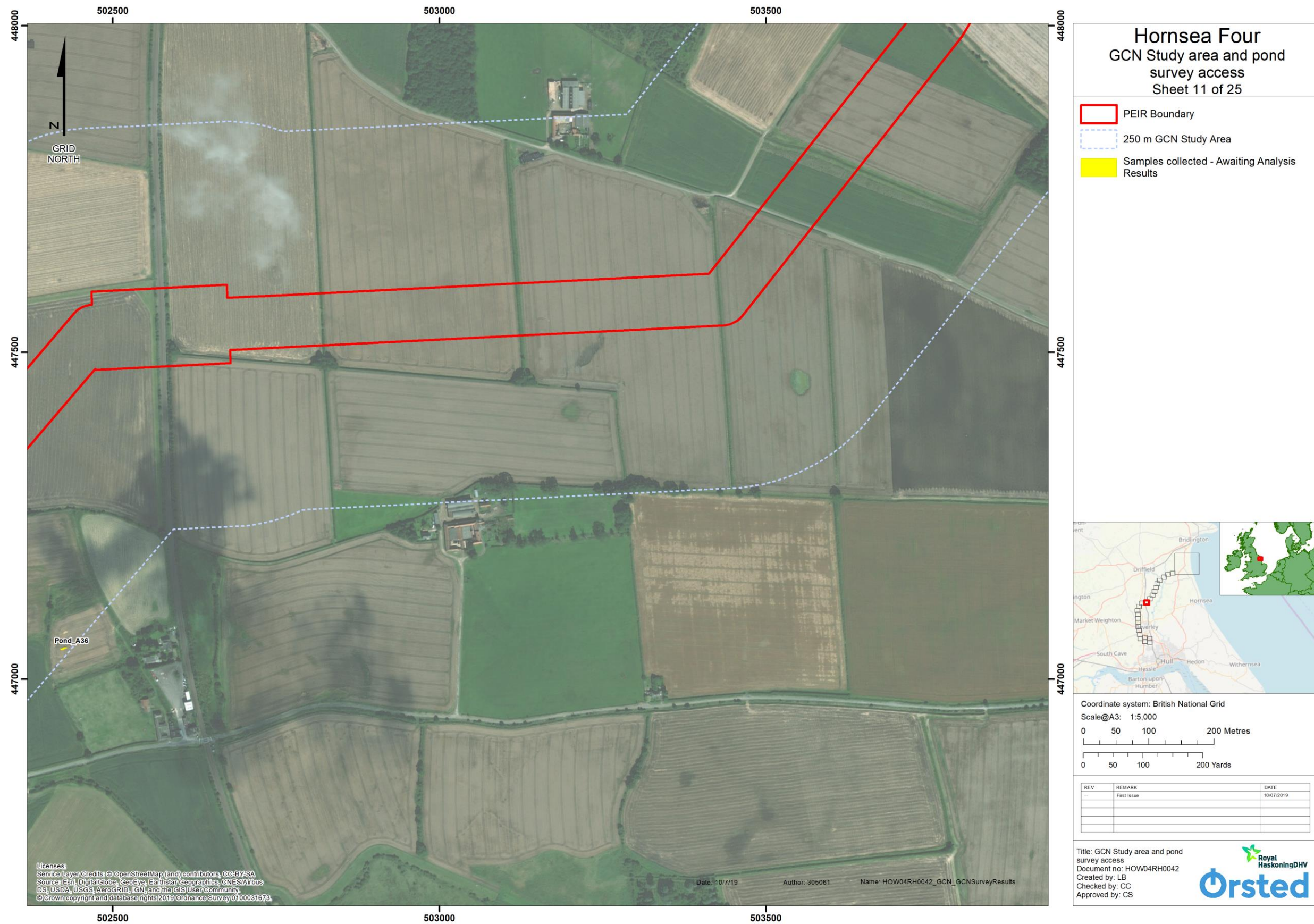


Figure 12: GCN Survey Results (Not to Scale).

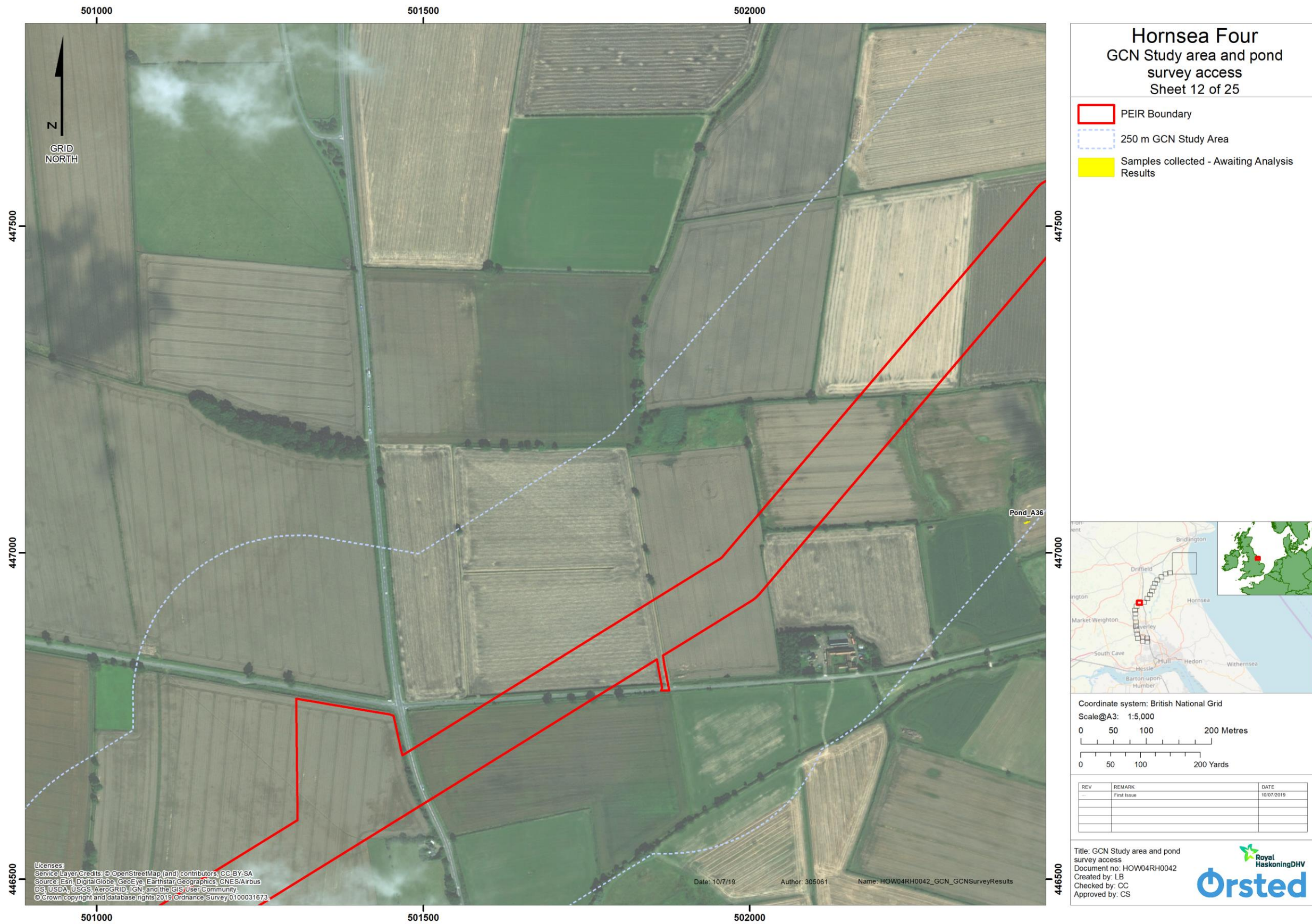


Figure 13: GCN Survey Results (Not to Scale).

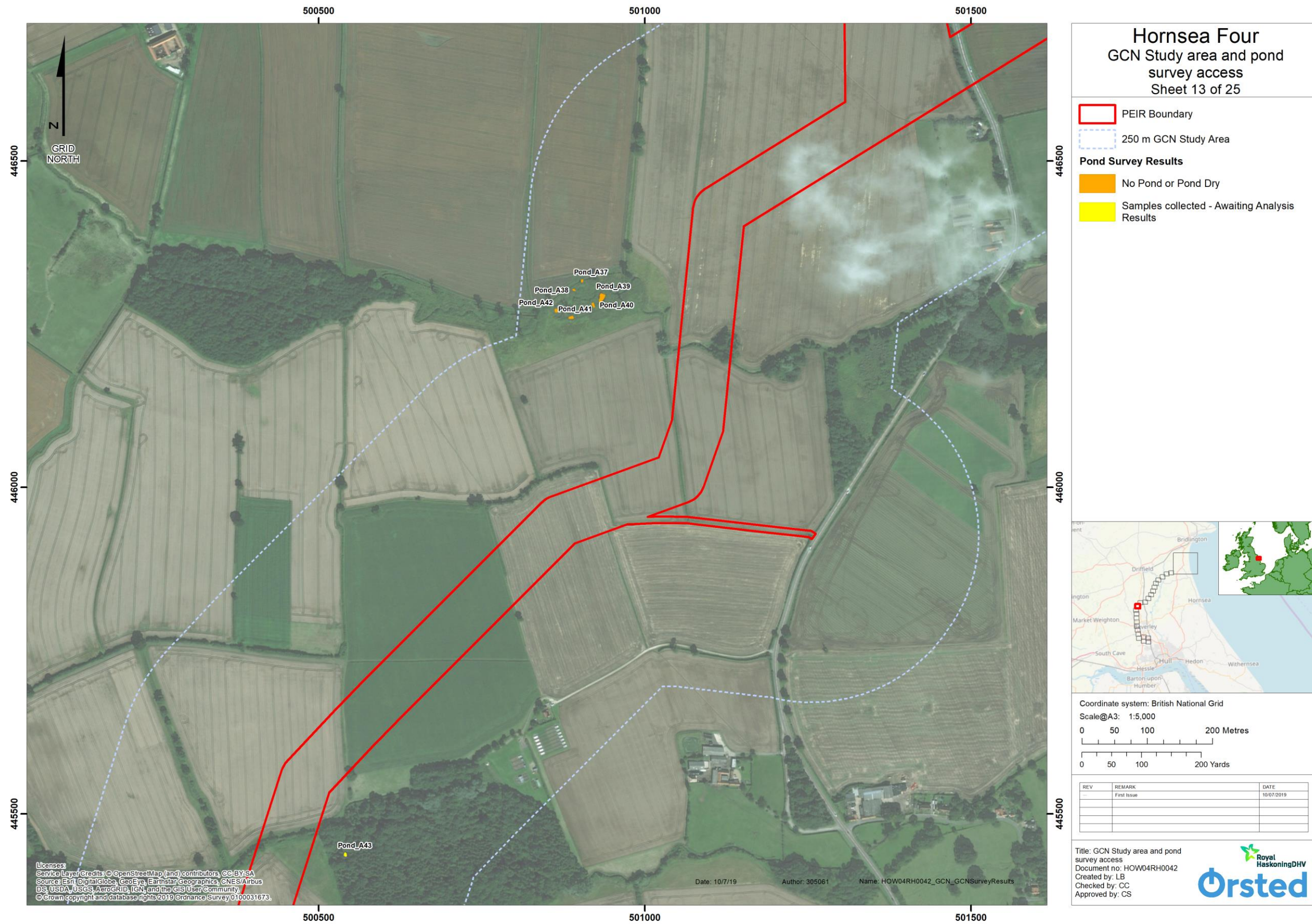


Figure 14: GCN Survey Results (Not to Scale).



Figure 15: GCN Survey Results (Not to Scale).



Figure 16: GCN Survey Results (Not to Scale).

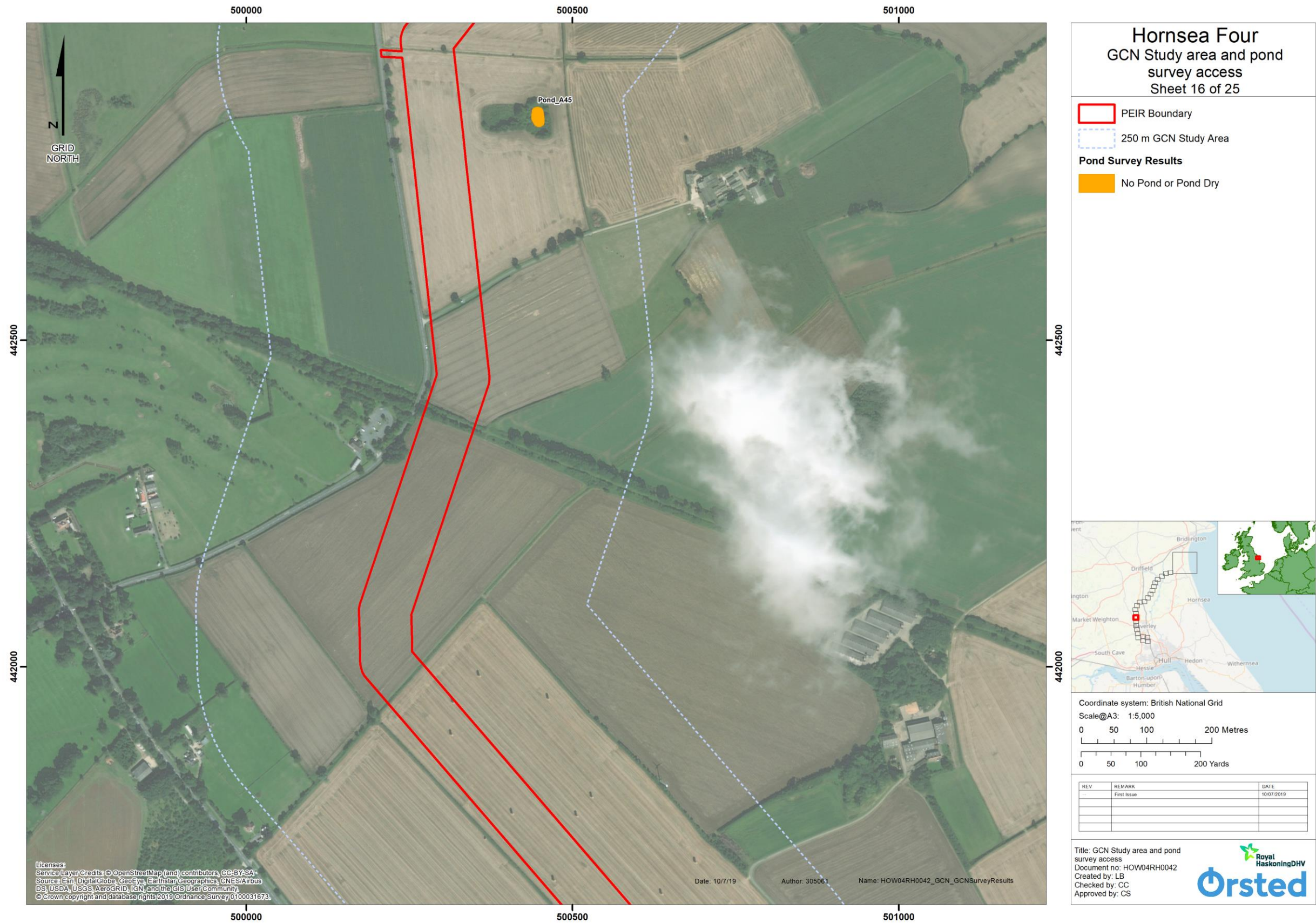


Figure 17: GCN Survey Results (Not to Scale).

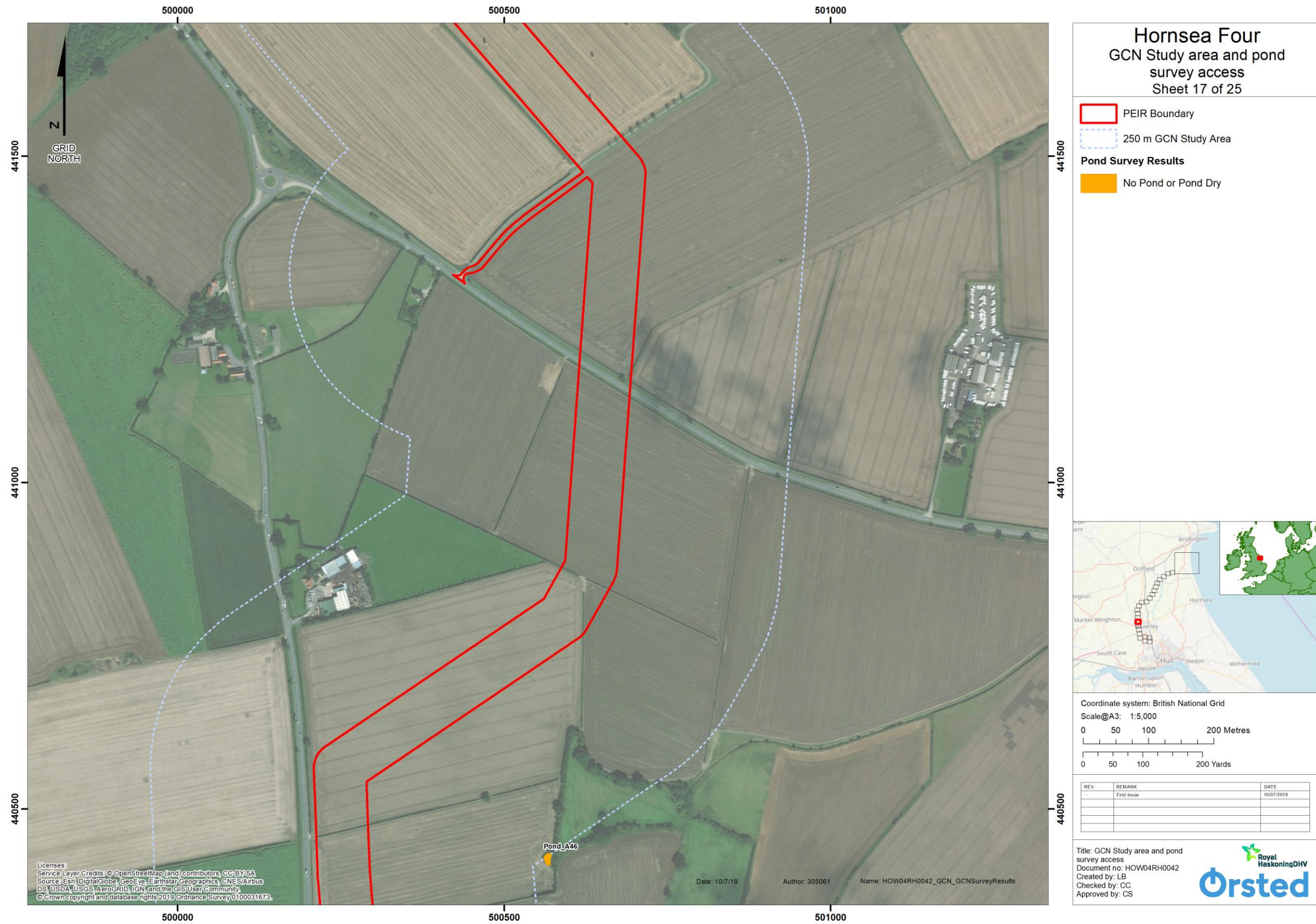


Figure 18: GCN Survey Results (Not to Scale).

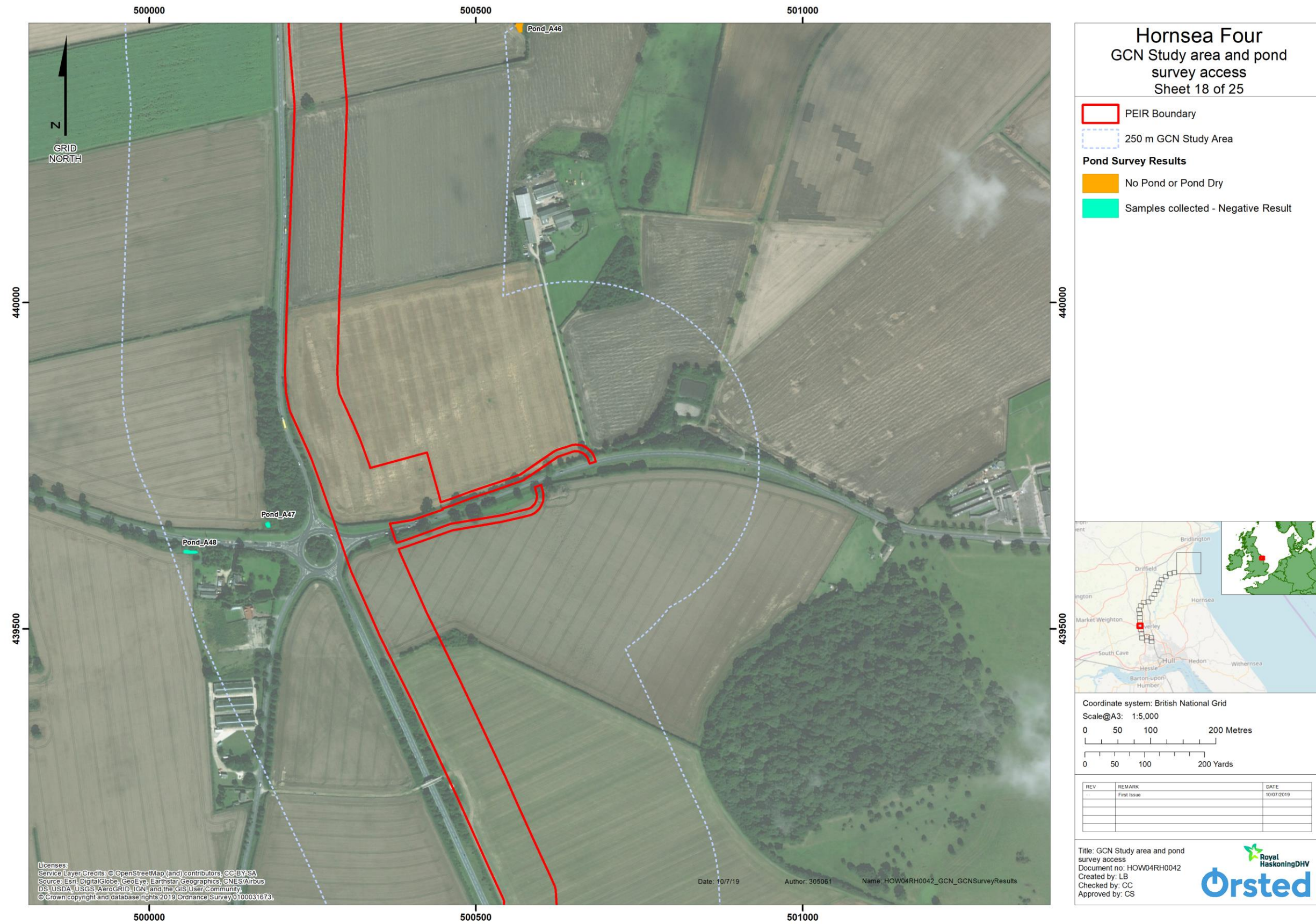


Figure 19: GCN Survey Results (Not to Scale).



Figure 20: GCN Survey Results (Not to Scale).

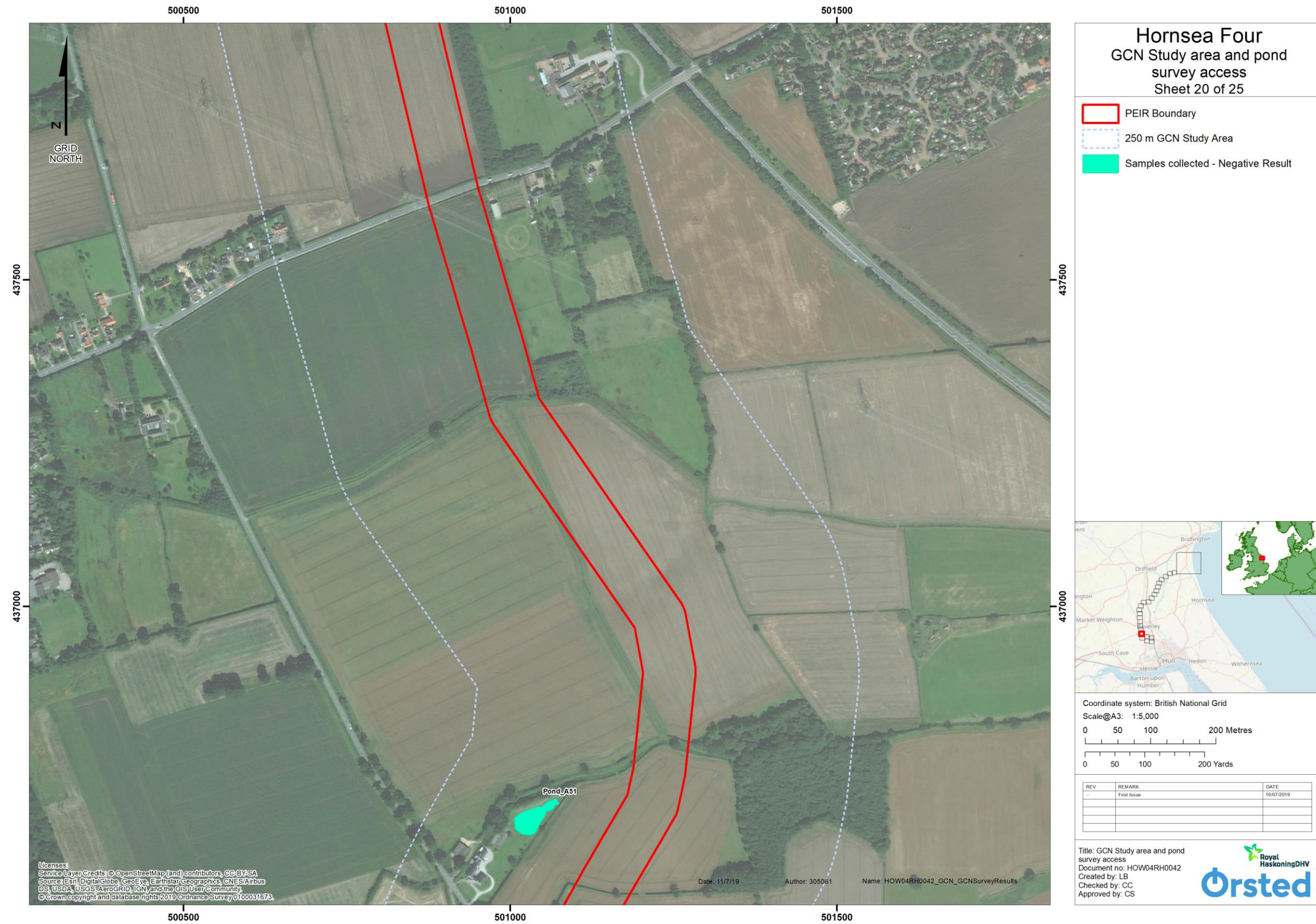


Figure 21: GCN Survey Results (Not to Scale).



Figure 22: GCN Survey Results (Not to Scale).



Figure 23: GCN Survey Results (Not to Scale).



Figure 24: GCN Survey Results (Not to Scale).

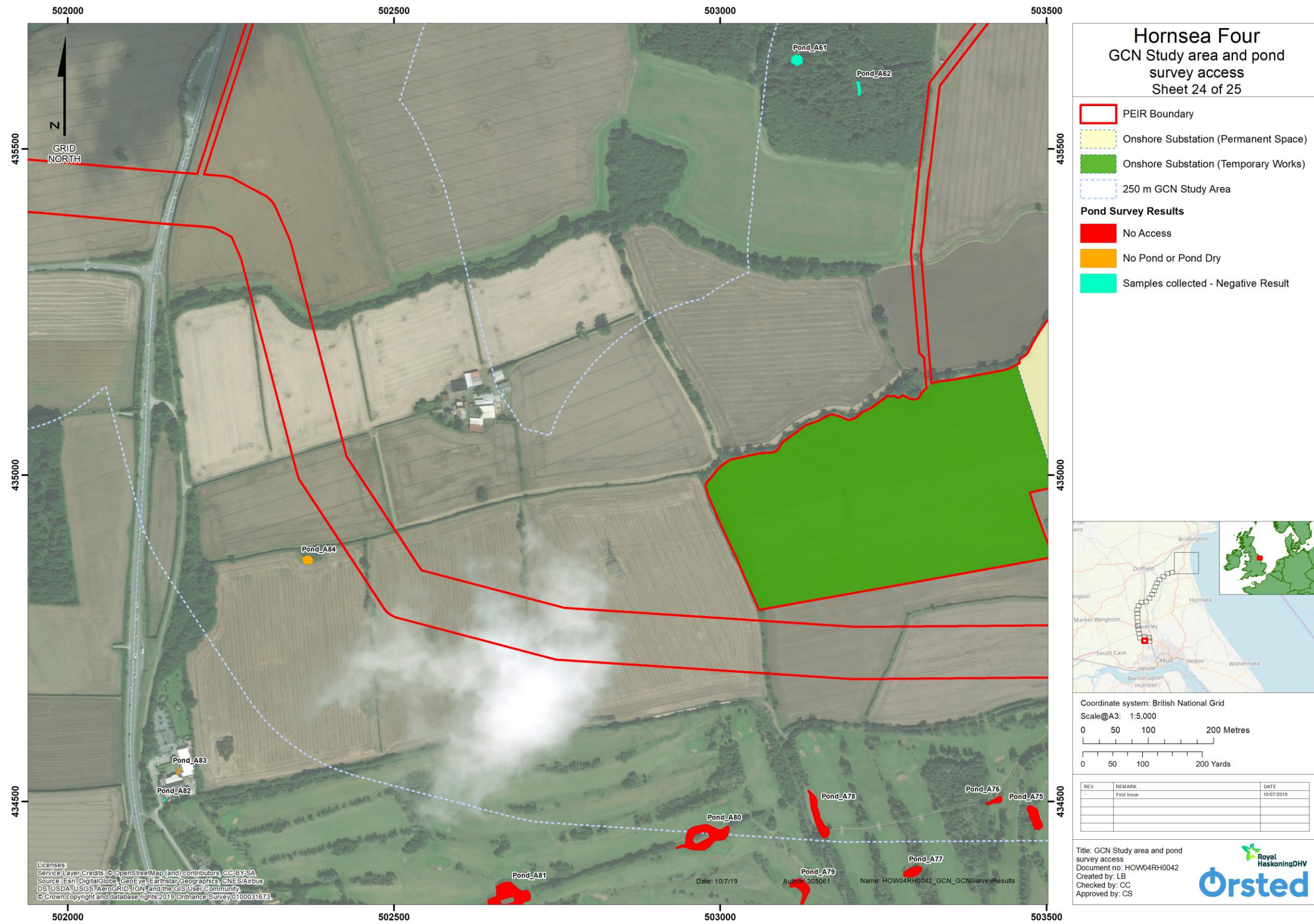


Figure 25: GCN Survey Results (Not to Scale).

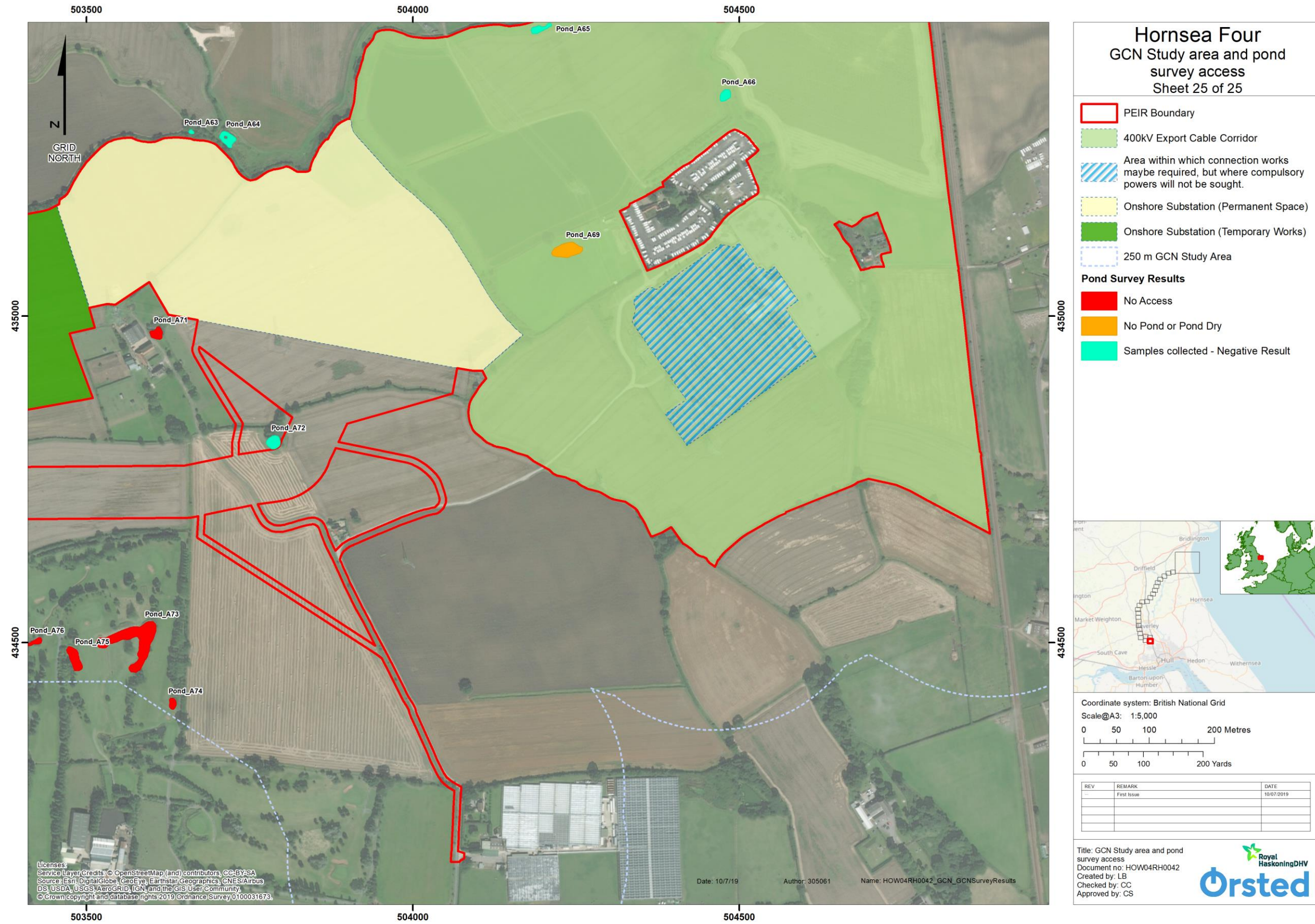


Figure 26: GCN Survey Results (Not to Scale).

5 Conclusion

- 5.1.1.1 A GCN eDNA survey was undertaken over two survey visits in April and June 2019. A total of 85 ponds were identified, with access granted to a total of 74 ponds. Out of these 74 ponds, a total of 42 ponds were subject to an HSI assessment and subsequent eDNA sampling survey (see [Figure 2 – Figure 26](#)). Of the remaining ponds surveyed, a total of 29 were either dry or no longer present, and three ponds were inaccessible due to livestock or electric fencing (See [Figure 2 – Figure 26](#) and [Appendix B - 2019 GCN eDNA Survey Pond Descriptions and Photographs](#)). Eleven ponds (of the 85 ponds identified) remain to be surveyed for the presence of GCN due to landowner access restrictions at the time of survey.
- 5.1.1.2 Three of the 42 ponds subject to the 2019 GCN eDNA survey returned a positive result for GCN, 38 ponds returned a negative result and one pond returned an inconclusive result. The ponds where GCN presence has been confirmed are as follows:
- Pond_A08 ([Figure 3](#));
 - Pond_A11 ([Figure 3](#)); and
 - Pond_A32 ([Figure 10](#)).
- 5.1.1.3 GCN are known to exist as meta-populations within small aggregations or clusters of ponds (within a 500 m area), i.e. individual GCN may travel between several ponds that are in proximity to one another (English Nature, 2001). This allows genetic diversity within the breeding populations and allows the population to move freely, depending on changing conditions. Based on this information, clusters of ponds within distinct areas (500 m) can be classified as potentially supporting genetically distinct meta-populations of GCN.
- 5.1.1.4 Pond_A08 and Pond_A11 ([Figure 3](#)) are situated within approximately 130 m of each other, within an area of grassland that is maintained for the production of silage. The surrounding habitat consists of semi-improved grassland with standalone trees and patches of scrub, offering both hibernation and foraging opportunities for GCN.
- 5.1.1.5 These two ponds are approximately 450 m from the onshore ECC with no clear connecting ecological corridors linking the ponds to the onshore ECC. These two ponds are also approximately 100 m from an existing road that is proposed as a temporary access track for Hornsea Four.
- 5.1.1.6 Pond_A32 ([Figure 10](#)) is an ornamental pond within the grounds of a water bottling factory, situated approximately 200m from the onshore ECC. The habitat between Pond_A32 and the onshore ECC is an arable field (in crop at the time of the survey). The arable field is bounded by hedgerows and ditches, meaning that there is the possibility of GCN movement between the two locations.
- 5.1.1.7 Of the remaining ponds that were subject to the 2019 GCN eDNA survey, a total of 38 returned a negative result for GCN, and therefore it is concluded that there is no reasonable likelihood of this species being present within these ponds. Consequently, there are likely to

be no impacts on GCN associated with these 38 ponds and therefore no mitigation measures are required. One pond returned an inconclusive result for the presence of GCN, therefore this pond (Pond_A33a) will require a further eDNA survey prior to the commencement of Hornsea Four construction to determine the presence of likely absence of GCNs from this pond. Further information relating to proposed mitigation can be found in [Volume F2, Chapter 3: Outline Ecological Management Plan](#).

6 Proposed Mitigation

- 6.1.1.1 Consultation with Natural England (NE) has been undertaken as part of the Hornsea Four Ecology and Nature Conservation Evidence Plan process, to discuss the eDNA results and mitigation measures for GCN that will be required for those ponds where a positive eDNA result has been returned. These discussions remain ongoing but a full GCN mitigation strategy will be developed and agreed with NE. Mitigation strategies will be in line with current industry guidance and standards. The GCN mitigation strategy will accompany the Hornsea Four DCO submission, alongside an Outline GCN European Protected Species (EPS) mitigation licence application.

7 References

Briggs, J., Ewald, N., Valentini, A., Gaboriaund, C., Griffiths, R.A., Foster, J., Wilkinson, J., Arnett, A., Williams, P. & 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.

Chartered Institute of Ecology and Environmental Management (CIEEM) (2017) Guidelines for Ecological Report Writing (2nd Edition). Technical Guidance Series.

The Conservation of Habitats and Species Regulations 2017 (as amended). Available at <http://www.legislation.gov.uk/ukxi/2017/1012/contents/made> (accessed: July 2019).

Cresswell and Whitworth (2004) An assessment of the efficiency of capture techniques and the value of different habitats for the great crested newt *Triturus cristatus*. English Nature Research Reports.

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

JNCC and Defra (on behalf of the Four Countries' Biodiversity Group). 2012. UK Post-2010 Biodiversity Framework. July 2012. Available from: <http://jncc.defra.gov.uk/page-6189>. (Accessed: July 2019)

Natural England (2015). Great crested newts: surveys and mitigation for development projects. Natural England Standing Advice.

Natural Environmental and Rural Communities (NERC) Act 2006. Available at <https://www.legislation.gov.uk/ukpga/2006/16/contents> (accessed: July 2019).

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

Wildlife and Countryside Act (WCA) 1981 (as amended). Available at <https://www.legislation.gov.uk/ukpga/1981/69> (accessed: July 2019).

Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora. Available at <https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:31992L0043> (accessed: July 2019).

Appendix A - eDNA Laboratory Analysis

DNA Analysis Report - Commercial in Confidence



Customer: Royal Haskoning DHV
Address: 2 Abbey Gardens,
Great College Street
London
SW1P 3NL

Contact: Charlotte Clements
Email: charlotte.clements@rhdhv.com
Tel: 02073402892

Report date: 08-May-2019

Order Number: GCN19-0999

Samples: Pond Water

Analysis requested: Detection of Great Crested Newt eDNA from pond water.

Thank you for submitting your samples for analysis with the Fera eDNA testing service. The details of the analysis are as follows:

Method:

The method detects pond occupancy from great crested newts (GCN) using traces of DNA shed into the pond environment (eDNA). The detection of GCN eDNA is carried out using real time PCR to amplify part of the cytochrome 1 gene found in mitochondrial DNA. The method followed is detailed in Biggs J., et al, (2014). Analytical and methodological development for improved surveillance of the Great Crested Newt. Appendix 5. Technical advice note for field and laboratory sampling of great crested newt (*Triturus cristatus*) environmental DNA. Freshwater Habitats Trust, Oxford.

The limits of this method are as follows: 1) the results are based on analyses of the samples supplied by the client and as received by the laboratory, 2) any variation between the characteristics of this sample and a batch will depend on the sampling procedure used. 3) the method is qualitative and therefore the levels given in the score are for information only, they do not constitute the quantification of GCN DNA against a calibration curve, 4) a 'not detected' result does not exclude presence at levels below the limit of detection.

The results are defined as follows:

Positive: DNA from the species was detected.
eDNA Score: Number of positive replicates from a series of twelve.
Negative: DNA from the species was not detected; in the case of negative samples the DNA extract is further tested for PCR inhibitors and degradation of the sample.
Inconclusive: Controls indicate degradation or inhibition of the sample, therefore the lack of detection of GCN DNA is not conclusive evidence for determining the absence of the species in the sample provided.

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CustomerReference	Fera Reference	GCN Detection	eDNA Score	Inhibition	Degradation
A63	S19-015800	Negative	0	No	No
A65	S19-015799	Negative	0	No	No
A68	S19-015794	Negative	0	No	No
A72	S19-015793	Negative	0	No	No
A57	S19-015791	Negative	0	No	No
A61	S19-015788	Negative	0	No	No
A62	S19-015785	Negative	0	No	No
A66	S19-015779	Negative	0	No	No
A55	S19-015726	Negative	0	No	No
A54	S19-015723	Negative	0	No	No
A53	S19-015720	Negative	0	No	No
A60	S19-015711	Negative	0	No	No
A64	S19-015708	Negative	0	No	No
A18	S19-015704	Negative	0	No	No
A06	S19-015702	Negative	0	No	No
A18	S19-015700	Negative	0	No	No
A44	S19-015698	Negative	0	No	No
A48	S19-015696	Negative	0	No	No
A50	S19-015690	Negative	0	No	No
A07	S19-015688	Negative	0	No	No
A58	S19-015686	Negative	0	No	No
A08	S19-015694	Positive	1	n/a	n/a
A11	S19-015692	Positive	2	n/a	n/a

The results indicate that eDNA for great crested newts was detected in two of the samples and in the remaining samples eDNA was not detected (as detailed in the table above). Analysis was conducted in the presence of the following controls: 1) extraction blank, 2) appropriate positive and negative PCR controls for each of the TaqMan assays (GCN, Inhibition, and Degradation). All controls performed as expected.

This test procedure was developed using research funded by the Department of Environment, Food and Rural Affairs.

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DNA Analysis Report - Commercial in Confidence



Customer: Royal Haskoning DHV
Address: 2 Abbey Gardens,
 Great College Street
 London
 SW1P 3NL

Contact: Charlotte Clements
Email: charlotte.clements@rhdhv.com
Tel: 02073402892

Report date: 04-Jul-2019

Order Number: GCN19-0999

Samples: Pond Water

Analysis requested: Detection of Great Crested Newt eDNA from pond water.

Thank you for submitting your samples for analysis with the Fera eDNA testing service. The details of the analysis are as follows:

Method:

The method detects pond occupancy from great crested newts (GCN) using traces of DNA shed into the pond environment (eDNA). The detection of GCN eDNA is carried out using real time PCR to amplify part of the cytochrome 1 gene found in mitochondrial DNA. The method followed is detailed in Biggs J., et al, (2014). Analytical and methodological development for improved surveillance of the Great Crested Newt. Appendix 5. Technical advice note for field and laboratory sampling of great crested newt (*Triturus cristatus*) environmental DNA. Freshwater Habitats Trust, Oxford.

The limits of this method are as follows: 1) the results are based on analyses of the samples supplied by the client and as received by the laboratory, 2) any variation between the characteristics of this sample and a batch will depend on the sampling procedure used. 3) the method is qualitative and therefore the levels given in the score are for information only, they do not constitute the quantification of GCN DNA against a calibration curve, 4) a 'not detected' result does not exclude presence at levels below the limit of detection.

The results are defined as follows:

Positive: DNA from the species was detected.
eDNA Score: Number of positive replicates from a series of twelve.
Negative: DNA from the species was not detected; in the case of negative samples the DNA extract is further tested for PCR inhibitors and degradation of the sample.
Inconclusive: Controls indicate degradation or inhibition of the sample, therefore the lack of detection of GCN DNA is not conclusive evidence for determining the absence of the species in the sample provided.

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Customer Reference	Fera Reference	GCN Detection	eDNA Score	Inhibition	Degradation
-	S19-015786	Negative	0	No	No
-	S19-015783	Negative	0	No	No
-	S19-015781	Negative	0	No	No
-	S19-015780	Negative	0	No	No
-	S19-015717	Negative	0	No	No
-	S19-015714	Negative	0	No	No
-	S19-015705	Negative	0	No	No
-	S19-015703	Negative	0	No	No
-	S19-015701	Negative	0	No	No
-	S19-015697	Inconclusive	0	YES	YES
-	S19-015695	Negative	0	No	No
-	S19-015693	Negative	0	No	No
-	S19-015691	Negative	0	No	No
-	S19-015684	Negative	0	No	No
-	S19-015682	Negative	0	No	No
-	S19-015680	Positive	12	n/a	n/a

The results indicate that eDNA for great crested newts was detected in one of the samples and in the remaining samples eDNA was not detected (as detailed in the table above). However, with sample S19-015697 we detected PCR inhibitors and degradation of the internal control. Therefore, due to the risk of inhibition of the eDNA assay and any eDNA also being degraded resulting in a false negative, we have issued an inconclusive result for this sample. We did note a substantial amount of sediment in this sample which may have contributed to this result.

Analysis was conducted in the presence of the following controls: 1) extraction blank, 2) appropriate positive and negative PCR controls for each of the TaqMan assays (GCN, Inhibition, and Degradation). All controls performed as expected.

This test procedure was developed using research funded by the Department of Environment, Food and Rural Affairs.

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Appendix B - 2019 GCN eDNA Survey Pond Descriptions and Photographs


Table 5: 2019 GCN eDNA Survey Pond Descriptions and Photographs.



Pond Reference	Pond Description	Photograph
Pond_A01	Small pond on field margin closed with vegetation. Very little open water. No feed, rainwater only.	
Pond_A02	Large pond, dry to edges. Set in BL woodland, only centre of pond has water (30 m x 15 m). Rain water fed.	



Pond Reference	Pond Description	Photograph
Pond_A03	Dry pond. Small scrape set in middle of pasture field – heavily scraped and no water.	



Pond Reference	Pond Description	Photograph
Pond_A04	Dry pond. Small scrape set in middle of pasture field – heavily scraped.	



Pond Reference	Pond Description	Photograph
Pond_A05	Dry pond. Small scrape set in middle of pasture field – heavily scraped, no water.	
Pond_A06	Small pond in garden. Detritus and leaf litter on pond bottom. Lily, bramble, hawthorn, beech, field maple, ash and dock leaf were all present.	



Pond Reference	Pond Description	Photograph
Pond_A07	Large pond within gardens. Bulrush, glyceria and soft rush present. Fenced and surrounded by maintained short sward grass.	



Pond Reference	Pond Description	Photograph
Pond_A08	Small pond with limited aquatic vegetation; willow, dock leaf, soft rush, fenced through middle of pond.	
Pond_A09	Dry pond.	

Pond Reference	Pond Description	Photograph
Pond_A10	Dry pond.	
Pond_A11	Long narrow pond surrounded by vegetation including; hawthorn, willow, bramble, nettle, soft rush. Within grassland used for silage.	



Pond Reference	Pond Description	Photograph
Pond_A12	Small crescent shaped pond, very shallow in small wooded copse. Fed by drainage ditches from arable fields.	
Pond_A13	Ornamental pond next to farmhouse. Set in amenity grassland with woodland to north east and arable fields.	

Pond Reference	Pond Description	Photograph
Pond_A14	Dry pond. Small ditch joins Pond_A13 and Pond_A14, completely dry and dense vegetation.	
Pond_A15	No access to pond possible due to livestock present in field.	No photograph available as access was not possible due to presence of livestock at the time of the survey.
Pond_A16	Dry pond. Small vegetated scrape in the ground poached by cattle under ash trees, no water.	

Pond Reference	Pond Description	Photograph
Pond_A17	Dry pond. Small vegetated scrape in the ground poached by cattle under ash trees, no water.	
Pond_A18	Large pond within grassland, majority of bank covered in glyceria, island in middle of pond. Good connectivity to Pond_A06 and Pond_A07. Due to size of pond, two eDNA kits were used.	



Pond Reference	Pond Description	Photograph
Pond_A19	No pond present.	 A photograph showing a field of tall, dense green grass and weeds. In the background, there are several trees with green foliage and a glimpse of a building.
Pond_A20	Large pond in small woodland. Highly overgrown and therefore difficult to access. Very little marginal vegetation.	 A photograph of a narrow, shallow pond or stream flowing through a dense woodland. The water is dark and reflects the surrounding trees. The banks are heavily overgrown with green vegetation, making the pond difficult to access.



Pond Reference	Pond Description	Photograph
Pond_A21	Round ornamental pond in garden surrounded by iris and rush pond weed. Duckweed also present.	
Pond_A22	Large pond with a gravel base, remnant from sand/gravel extraction. Clear water with little vegetation. Edges dominated by iris, bulrush and willow scrub. Connecting habitat between this pond and other ponds in the surrounding area.	

Pond Reference	Pond Description	Photograph
Pond_A23	L shaped pond remnant from sand/gravel extraction (as Pond_A22). Surrounded by semi-improved grassland comprising cock's foot, birds foot trefoil and teasel. Good connectivity to other ponds.	
Pond_A24	Pond in centre of woodland. Pondweed, iris and bulrush. Water quality good, excellent connectivity to surrounding terrestrial habitats.	

Pond Reference	Pond Description	Photograph
Pond_A25	Fishing pond with high concentration of ducks and geese, fenced and locked. Deemed to be unsuitable for GCN	
Pond_A26	Fishing pond with high concentration of ducks and geese, fenced and locked. Assessed as being unsuitable for GCN.	
Pond_A27	No access granted at the time of the survey.	No photograph available as no access granted at the time of the survey.



Pond Reference	Pond Description	Photograph
Pond_A28	Dry pond. Large pond fed by adjacent river. However, dry at time of survey.	
Pond_A29	Small remnant of Pond_A28 divided by mound of vegetated earth, choked with water lily, water quality okay, surrounded by chest high vegetation. Good connectivity	
Pond_A30	Dry pond. Middle of arable fields planted with crop, owner says may contain small amounts of water over winter.	No photograph available as photograph was corrupted beyond recovery.
Pond_A31	Dry pond. Middle of arable fields planted with crop, owner says may contain small amounts of water over winter.	No photograph available as photograph was corrupted beyond recovery.



Pond Reference	Pond Description	Photograph
Pond_A32	Round ornamental pond in front of factory. Steep sided naturally fed with rain water. Lots of invertebrates, small fish present and water lily abundant.	
Pond_A33	Large pond once stocked with trout, little vegetation in bottom, spring fed with a gravel bottom.	

Pond Reference	Pond Description	Photograph
Pond_A33a	Small man-made pond 100 m from onshore cable route. Overgrown with iris and bulrush.	
Pond_A34	Dry pond. Small scrape in ground full of grasses.	

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Pond Reference	Pond Description	Photograph
Pond_A35	Dry pond. Small scrape in the ground, no water grasses only.	
Pond_A36	Scrape in the ground in the centre of a field completely covered in vegetation. Small areas of standing water.	


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Pond Reference	Pond Description	Photograph
Pond_A37	No pond, small scrape in ground choked with vegetation. Looks like flood plain for the Bryan Mills Beck.	
Pond_A38	No pond, small scrape in ground choked with vegetation. Looks like flood plain for the Bryan Mills Beck.	



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Pond Reference	Pond Description	Photograph
Pond_A39	No pond, small scrape in ground choked with vegetation. Looks like flood plain for the Bryan Mills Beck.	
Pond_A40	No pond, small scrape in ground choked with vegetation. Looks like flood plain for the Bryan Mills Beck.	
Pond_A41	No pond, small scrape in ground choked with vegetation. Looks like flood plain for the Bryan Mills Beck.	
Pond_A42	No pond, small scrape in ground choked with vegetation. Looks like flood plain for the Bryan Mills Beck.	



Pond Reference	Pond Description	Photograph
Pond_A43	Linear spring fed stream, slight slow flow towards Bealeys Beck in area of plantation woodland.	

Pond Reference	Pond Description	Photograph
Pond_A44	Small, heavily shaded pond at edge of arable fields, adjacent to hedgerow and public right of way, feeds into ditch, partially dry.	



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Pond Reference	Pond Description	Photograph
Pond_A45	Dry pond.	
Pond_A46	No pond present.	


Pond Reference	Pond Description	Photograph
Pond_47	Small pond/hollow in side of arable field completely covered in vegetation/buttercup and dog rose. Water 2" to 3" deep.	
Pond_A48	<p>This is not a pond but a wide section of ditch that is currently dry. However, a garden pond is present, and this is referenced Pond_A48 and therefore surveyed.</p> <p>Small pond, glyceria throughout, fish pond adjacent (not connected).</p>	

Pond Reference	Pond Description	Photograph
Pond_A49	Dry pond.	
Pond_A50	Small pond within woodland (habitat rich, potentially ancient woodland); glyceria duckweed, sycamore, field maple, willow, ramsons, nettle, bramble, red-dead nettle, white-dead nettle, bluebells, red campion, forget me not, lesser celandine, ground ivy	



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Pond Reference	Pond Description	Photograph
Pond_A51	Pear shaped ornamental pond (lined). Few iris and bulrush planted around edge. Bitumen lined with large fish ad moorhen present. Mowed lawns surround.	
Pond_A52	Small round pond fed by pipe under the road/runoff. Steep bank with no vegetation.	



Pond Reference	Pond Description	Photograph
Pond_A53	Pond is a wide section of a (dry) ditch, between an arable field (ploughed) and grassland, fenced. Sycamore, nettle, bramble, dock, cleavers, white dead nettle and hawthorn. Grassland potentially used as grazing for farm	
Pond_A54	Medium sized pond in middle of arable field (in crop - oilseed), fenced and gated (gate sign stated 'pollution control'). Manhole cover visible at bottom of pond. Surrounded by grass, broad leaf dock and hawthorn, some pond scum on surface, could potential be SUDS for A164. Disconnected from wider habitat by arable crops and roads	


Pond Reference	Pond Description	Photograph
Pond_A55	Small pond in woodland adjacent to grassland. Sycamore, bramble, ribwort plantain, nettle, cow parsley, cleavers, dog mercury. Shallow pond approximately 15cm deep	



Pond Reference	Pond Description	Photograph
Pond_A56	Dry pond.	
Pond_A57	Small pond adjacent to woodland. Bulrush and glyceria. Tadpoles, arable crop adjacent	

Pond Reference	Pond Description	Photograph
Pond_A58	Medium sized fish pond within animal paddock surrounded by alder, bramble and nettle	
Pond_A59	No pond present.	



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Pond Reference	Pond Description	Photograph
Pond_A60	Medium pond within garden and fenced horse paddock. Willow, hawthorn, broad leaf dock, glyceria, bulrush, soft rush. Common frogs present	
Pond_A61	Small pond in woodland adjacent to grassland. Sycamore, bramble, ribwort plantain, nettle, cow parsley, cleavers, dog mercury. Shallow pond approximately 15cm deep	

Pond Reference	Pond Description	Photograph
Pond_A62	Small pond in woodland, some aquatic vegetation at margins	



Pond Reference	Pond Description	Photograph
Pond_A63	Small ephemeral pond between arable fields and PRow. Water mint present, broad leaf dock, nettle, bramble, hawthorn and sycamore	
Pond_A64	Medium pond at corner of arable field adjacent to PRow, hedgerows, grassy field margins. Bulrush dominant throughout, bramble, nettle and hawthorn	

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Pond Reference	Pond Description	Photograph
Pond_A65	Small pond at edge of arable field with wide field margins and hedgerow, tadpoles present. Bulrush, glyceria, hawthorn, soft rush and dandelion	
Pond_A66	Small pond at edge of arable field with wide grassy margins and scrub vegetation. Bulrush and glyceria throughout.	

Pond Reference	Pond Description	Photograph
Pond_A67	No pond present.	

Pond Reference	Pond Description	Photograph
Pond_A68	Large lagoon within ruderal/scrub habitat, borders railway line and major road (A1079). Potentially SUDS from road so potentially polluted. Steep sides. Willow, ash, water mint, yellow flag iris, bramble, nettle, gorse	

Pond Reference	Pond Description	Photograph
Pond_A69	No pond present.	
Pond_A70	Large concrete pond with bitumen liner. Surrounded by overhanging paving slabs set in amenity grassland and concrete.	
Pond_A71	No access granted at the time of the survey.	No photograph available as no access granted at the time of the survey.

Pond Reference	Pond Description	Photograph
Pond_A72	Small pond at confluence of arable fields, ditch and PRow. Yellow flag iris, cherry trees, hawthorn, nettle, cow parsley	
Pond_A73	No access granted at the time of the survey.	No photograph available as no access granted at the time of the survey.
Pond_A74	No access granted at the time of the survey.	No photograph available as no access granted at the time of the survey.
Pond_A75	No access granted at the time of the survey.	No photograph available as no access granted at the time of the survey.
Pond_A76	No access granted at the time of the survey.	No photograph available as no access granted at the time of the survey.
Pond_A77	No access granted at the time of the survey.	No photograph available as no access granted at the time of the survey.
Pond_A78	No access granted at the time of the survey.	No photograph available as no access granted at the time of the survey.
Pond_A79	No access granted at the time of the survey.	No photograph available as no access granted at the time of the survey.
Pond_A80	No access granted at the time of the survey.	No photograph available as no access granted at the time of the survey.
Pond_A81	No access granted at the time of the survey.	No photograph available as no access granted at the time of the survey.

Pond Reference	Pond Description	Photograph
Pond_A82	Concrete ornamental pond with no vegetation. Pump visible and no fish.	
Pond_A83	No pond – removed and re-landscaped in 2004.	No photograph available as photograph was corrupted beyond recovery. Buildings and areas of hard standing present.

Pond Reference	Pond Description	Photograph
Pond_A84	Dry pond.	
Pond_A85	No access granted at the time of the survey.	No photograph available as no access granted at the time of the survey.