Tween Bridge Solar Farm

A Nationally Significant Infrastructure Project in the Energy Sector

Preliminary Environmental Information Report

Technical Appendix 7.6 – Amphibian Baseline Report

October 2023

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Tween Bridge Solar Farm

on behalf of Pegasus Planning Limited

Technical Appendix 7.7: Amphibian Baseline Report

avian ecology

Document Control	
Project Name:	Tween Bridge Solar Farm
Project / Report Number:	Pegas-075-1622
Report Title	Technical Appendix 7.7: Amphibian Baseline Report

Issue	Date	Notes	Prepared	Reviewed
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V2	26/07/2023	Updated Draft	K Love *BSc (Hons.)*	B.Walker *MSC MCIEEM*

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1 INTRODUCTION

1.1 Background

1.1.1 Avian Ecology Limited (AEL) was commissioned by Pegasus Planning Limited to undertake great crested newt (GCN) presence/absence surveys adopting the environmental DNA (eDNA) sampling methodology.

1.1.2 The surveys were undertaken in relation to The Scheme of a renewable energy generating project; consisting of ground-mounted solar photovoltaic ('PV') arrays, together with on-site energy storage and associated infrastructure. The Scheme is located on land to the east of the town of Thorne and to the west of the town of Crowle (the 'Draft Order Limits') as illustrated on **Figure 1.**

1.1.3 This report subsequently provides detailed survey methodology and results and should be read with reference to the Ecology and Nature Conservation Chapter 7 of the Preliminary Environmental Information Report (PEIR) and the corresponding chapter within the Environmental Statement (ES).

1.2 Survey Area

1.2.1 Ponds were identified from aerial images and Ordnance Survey (OS) maps on or within 250m of the Draft Order Limits. Due to the low impact of solar energy developments on GCN habitats, and reflecting guidance published by Natural England (NE)[1], ponds beyond 250m from the Draft Order Limits were not considered within the ecological assessment process.

1.2.2 Following from changes to the Draft Order Limits during the project design process, two ponds (Ponds P18 and P42) originally located within 250m of the Draft Order Limit boundaries are now located beyond 250m. For context these have been referred to within the report and associated figures.

1.2.3 Pond locations are provided within **Figures 1** to **5**.

1.3 Legislation

1.3.1 GCN and their habitat are fully protected under national (Wildlife & Countryside Act 1981 (as amended))[2] and European law (The Habitats and Species Regulations 2017)[3]. The legislation makes it illegal to:

- Intentionally or deliberately capture, kill or injure a GCN;
- intentionally or recklessly damage, destroy or obstruct access to any place used for shelter and protection including resting and breeding places, whether occupied or not;
- deliberately, intentionally or recklessly disturb a GCN when in a place of shelter;
- possess a GCN, or any part of it, unless acquired lawfully; or,
- sell, barter, exchange or transport or offer for sale GCN or parts of them.

1.3.2 Anyone carrying out activities which may affect European Protected Species (EPS) must consider the presence of EPS, their breeding sites and resting places. Good practice guidance is available from NE[4],

[1] Available at: https://www.gov.uk/guidance/great-crested-newts-advice-for-making-planning-decisions#when-to-ask-for-a-survey (accessed 27th February 2023)

[2] Available at: https://www.legislation.gov.uk/ukpga/1981/69 (accessed 21st February 2023)

[3] Available at: https://www.legislation.gov.uk/uksi/2017/1012/contents/made (accessed 21st February 2023)

[4] Available at: https://www.gov.uk/guidance/european-protected-species-policies-for-mitigation-licences (accessed 10th July 2023)

which advises on assessing for the presence of EPS, and the possible impact of operations (including strategies for avoiding committing offences). If an offence cannot be avoided, then an EPS Mitigation Licence or District Level Licence (DLL) should be sought from NE.

1.3.3 GCN and common toad are listed as priority species under Section 41 of the Natural Environment and Rural Communities (NERC) Act 2006[5]. Natterjack toad, GCN, smooth newt and palmate newt are further listed under the Lincolnshire Biodiversity Action Plan[6], with common toad, common frog and all three newt species listed under the Doncaster Biodiversity Action Plan[7]. These species are therefore a material consideration within the planning process.

[5] Available at: https://www.legislation.gov.uk/ukpga/2006/16/contents (accessed 10th July 2023)

[6] Available at: http://www.southkesteven.gov.uk/CHttpHandler.ashx?id=7371&p=0 (accessed 10th July 2023)

[7] Available at: https://www.doncaster.gov.uk/services/environmental/doncaster-biodiversity-action-plan (accessed 10th July 2023)

2 METHODOLOGY

2.1 Desk Study

2.1.1 A desk study was undertaken to inform the approach to field survey work and provide context for subsequent assessment.

2.1.2 The desk study has included:

- A review of the Multi-Agency Geographic Information for the Countryside (MAGIC)[8] website to identify the proximity of the Site to any national or internationally designated sites for nature conservation, designated for amphibian species.
- A review of existing amphibian records within 2km of the Draft Order Limits, obtained from the following key sources:
  - Records request to Greater Lincolnshire Nature Partnership (GLNP)[9];
  - Records request to Doncaster Biological Records Centre (DoBRC)[10]; and,
  - A review of Magic Map for EPS licence records relating to GCN.

2.1.3 Only recent records dated from 2013 onwards were used unless historic records (pre-2013) were received from within (or within close proximity to) the Draft Order Limits and/or historic records were considered otherwise pertinent to The Scheme.

2.2 Survey Overview

2.2.1 Potential ponds which could be used by GCN for breeding, if present and suitable, were identified within a 250m radius of the Draft Order Limits using OS and aerial mapping and during extended habitat surveys.

2.2.2 An extensive watercourse network is present within and adjacent to the Draft Order Limits. A number of small sections of watercourses were identified as standing water during the mapping process. Due to the scale of the watercourse network, not all watercourses within and adjacent to the Draft Order Limits could be surveyed. As a result, the small selection identified during the mapping process were included within surveys to provide a sample across the Draft Order Limits. As a result, all sampled waterbodies will be referred to as ponds henceforth.

2.2.3 Prior to the survey, forty-four possible ponds were originally identified within 250m of the Draft Order Limits, with eight present within the Draft Order Limits itself (as seen in **Figure 1**).

2.2.4 Following from changes to the Draft Order Limits during the project design process, two additional ponds (Ponds P18 and P42) originally located within 250m of the Draft Order Limit boundaries are now located beyond 250m.

2.2.5 During the eDNA survey from the 27th to 28th June 2023, five ponds identified from aerial review were found to be absent (Ponds P7, P8, P13, P36 and P37). Four additional ponds were found during

[8] Available at: https://magic.defra.gov.uk/magicmap.aspx (accessed 27th February 2023)

[9] Available at: https://glnp.org.uk/ (accessed 18th July 2023)

[10] Available at: https://www.doncaster.gov.uk/services/planning/local-record-centre (accessed 18th July 2023)

surveys, including ponds P4A, P4B, P36A and P39A. A total of forty-three ponds were therefore identified within 250m of the Draft Order Limits.

2.2.6 Twenty-three ponds (Ponds P1-P6, P9, P11-P12, P14, P20-P22, P24, P29, P31, P33-P34, P35, P36A, P39A, P40 and P46) were accessed and subject to eDNA survey sampling to determine the presence or likely absence of GCN. Pond P35 was surveyed using two eDNA kits due to the extensive size of the waterbody exceeding over 1ha.

2.2.7 As detailed in Section 2.5, twenty ponds identified within 250m were not subject to eDNA surveys.

2.2.8 Please refer to **Annex 1**, which provides a summary of access and methodologies used for each pond set out within a table.

2.3 Habitat Suitability Index (HSI) Assessments

2.3.1 All accessible ponds, as well as visible ponds not subject to an eDNA survey (P4A, P4B, P19, P28 and P41), were also assessed for their suitability to support GCN via the Habitat Suitability Index (HSI) process. Dry ponds (P17, P32 and P38) were also subject to HSI assessment should they contain water at other times of the year. The assessment took place on the same dates as the eDNA surveys and followed the methodology detailed within ARG UK guidance (ARG UK, 2010[11]); which is a refined version of the Oldham et al. 2000[12] methodology. The assessment calculates a habitat suitability score for each pond based on a series of indices generated from variables including pond size and the presence/absence of wildfowl. Final scores relate to suitability and range from 'poor' to 'excellent' suitability.

2.3.2 The results of the HSI assessment can be used to provide a useful indication of GCN suitability and help assess any likely impacts of a development, but do not represent a substitute for presence/absence surveys.

2.4 eDNA Surveys

2.4.1 eDNA is nuclear or mitochondrial DNA that is released from an organism into the environment. Sources of eDNA include secreted faeces, mucous, gametes, shed skin and carcasses. In aquatic environments, eDNA is diluted and distributed in the water where it persists for 7–21 days, depending on the conditions (Biggs *et al*., 2014a[13]). The technique for determining presence/absence of GCN uses Polymerase Chain Reaction (PCR) laboratory techniques to detect the species eDNA within water samples.

2.4.2 Recent research by the Department for Environment Food and Rural Affairs (Defra) Project WC1067, concludes that the sampling of waterbodies collecting eDNA appears to be a highly effective method for determining whether great crested newts are present or absent during the breeding season, even where eDNA is present in very low concentrations (Biggs *et al.,* 2014b[14]).

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[11] ARG UK (2010). ARG UK Advice Note 5: Great Crested Newt Habitat Suitability Index. Amphibian and Reptile Groups of the United Kingdom.

[12] Oldham R.S., Keeble J., Swan M.J.S. and Jeffcote M. (2000) Evaluating the suitability of habitat for the Great Crested Newt (Triturus cristatus). Herpetological Journal, 10(4), pp. 143-155.

[13] Biggs J., Ewald N., Valentini A., Gaboriaud C, Griffiths R.A., Foster J., Wilkinson J., Arnett A., Williams P and Dunn F (2014a). Analytical and methodological development for improved surveillance of the Great Crested Newt. Defra Project WC1067. Freshwater Habitats Trust: Oxford.

[14] Biggs J, et.al. (2014b). Analytical and methodological development for improved surveillance of the Great Crested Newt. Appendix 4. Technical advice note for field and laboratory sampling of great crested newt (Triturus cristatus) environmental DNA. Freshwater Habitats Trust, Oxford.

2.4.3 NE accepts the use of eDNA surveys as evidence of presence or absence of GCN, provided samples are taken when newts are likely to be present (this depends on location and conditions like the weather)[15]. NE will only accept eDNA survey results undertaken between mid-April and 30th June, in strict accordance with the published technical advice note, by suitably trained, experienced, and licensed/accredited GCN surveyors.

Field Sampling Technique

2.4.4 Twenty-three ponds were accessed and sampled by suitably experienced and licensed surveyors.

2.4.5 The protocol for sampling followed that outlined within the technical advice note for field and laboratory sampling of great crested newts (Biggs *et al*., 2014b), which required the collection of 20 x 30ml subsamples from each pond, spaced as evenly as possible around the pond margin.

2.4.6 Each sample was then placed within a Whirl-Pak bag and shaken for 10 seconds, before a 15ml sample was pipetted from the bag and placed in a specimen tube for laboratory analysis. Following collection, samples were refrigerated prior to laboratory dispatch.

Laboratory Analysis

2.4.7 Laboratory analysis was undertaken by SureScreen Scientifics[16]; the laboratory follows the analysis methodology outlined within the Defra Project WC1067 research note (Biggs *et al*., 2014a) using the q-PCR test conducted in two phases.

2.4.8 eDNA laboratory analysis results are provided in **Annex 2.**

2.5 Limitations of Survey

2.5.1 Access was not permitted or confirmed for ponds P4A, P4B, P10, P15, P16, P23, P25-P27, P30, P43 or P44 and therefore, these were not surveyed for GCN. In addition, ponds P13, P19, P28, P39, P41 and P45 were inaccessible due to health and safety relating to steep banks and/or deep water and/or dense vegetation. It is considered that with the survey of 23 ponds within the survey area alongside the predominantly unsuitable intensively managed agricultural habitats within the Draft Order Limits; the lack of survey information is not considered to represent a significant constraint to the ecological assessment process.

2.5.2 Ponds P17, P32 and P38, were found to have insufficient water levels for eDNA and therefore were not subject to survey. The lack of survey information for these potential ponds is not considered a significant constraint to the ecological assessment process.

2.5.3 Pond P39, which could not be accurately surveyed for HSI assessment due to dense vegetation, was assumed to be consistent with pond P39A. Pond P45 was permitted visual access without photography, however an accurate HSI assessment was not possible due to inaccessibility from a field boundary ditch and dense vegetation limiting the view of the waterbody.

[15] Available at: https://www.gov.uk/guidance/great-crested-newts-surveys-and-mitigation-for-development-projects (accessed 21st February 2023)

[16] Available at: https://surescreenscientifics.com/edna/gcn-edna/ (accessed 21st February 2023)

3 RESULTS

3.1 Desk Study

Legislation

3.1.1 Great crested newts and their habitats are protected under the Wildlife and Countryside Act 1981 (as amended) and the Conservation of Habitats and Species Regulations 2017 (as amended) and the Conservation of Habitats and Species (Amendment) (EU Exit) Regulations 2019. The Act and Regulations make it an offence to;

- kill, injure, or disturb a great crested newt;
- damage or destroy a breeding or resting place; or,
- obstruct access to any place that is used for shelter or protection.

3.1.2 Great crested newt and common toad are also listed as priority species in England under Section 41 of the NERC Act 2006. Natterjack toad, great crested newt, smooth newt and palmate newt are further listed under the Lincolnshire LBAP, with common toad, common frog and all three newt species listed under the Doncaster LBAP. These species are therefore a material consideration within the planning process.

Desk Study

3.1.3 The combined data returned 34 records of amphibian within 2km of the Draft Order Limits during the last ten years. Multiple records were situated near the Draft Order Limits although none are located within the Draft Order Limits boundary. Records returned include 15 great crested newt, four common toad, three smooth newt and 12 common frog.

3.1.4 A review of MAGIC identified five Natural England licences granted for great crested newt in three locations within 2km of the Draft Order Limits:

- 2017-32435-EPS-MIT; damage and destruction of a great crested newt resting place and breeding site between 2017 and 2019. Located c.1.5km west of the Draft Order Limits.
- 2017-31327-EPS-MIT-2; damage and destruction of a great crested newt resting place between 2019 and 2028. Located c.1.5km west of the Draft Order Limits.
- 2017-31327-EPS-MIT and 2017-31327-EPS-MIT-1; damage and destruction of great crested newt resting place between 2017 to 2028. Located c. 1.75km north of the Draft Order Limits.
- 2017-27924-EPS-MIT-1; damage and destruction of great crested newt resting place between 2017 to 2022. Located c. 1.8km west of the Draft Order Limits.

3.1.5 Review of MAGIC did not identify any great crested newt class licence returns within 2km of the Draft Order Limits.

3.1.6 As illustrated in in **Figure 2** and **3** of the *Baseline Habitat & Desk Study Report*[17] presented within Chapter 7 of the PEIR, Buntings Wood Thorne Local Nature Reserve (LNR) and Thorne Railway Delves

[17] Avian Ecology Ltd (2023) Technical Appendix 7: Baseline Habitat & Desk Study Report.

Local Wildlife Site (LWS) are both designated for supporting amphibian populations. GCN in particular, are listed within the citation for Thorne Railway Delves LWS.

3.2 Habitat Suitability Index (HSI) Assessments

3.2.1 All accessible and visible ponds were assessed for their suitability to support GCN following the HSI assessment methodology outlined above.

3.2.2 Features surveyed showed variation in individual HSI scores based on the indices assessed; HSI scores ranged from 'Poor' to 'Excellent' habitat suitability.

3.2.3 The HSI results for all features surveyed are presented within **Table 3.1** below, whilst pond photographs and descriptions are provided in **Annex 3**, and pond locations outlined within **Figures 1** to **5**.

Table 3.1 – Habitat Suitability Index Assessment Results

HSI Criteria / Pond ref.	Zone	Area (m^2)	Drying	Water Quality	Shade	Fowl present?	Fish present?	Pond count	Terrestrial habitat	Macrophytes	HSI Score	GCN habitat suitability
Pond 1	1	0.88	0.9	1	1	0.67	0.67	0.95	1	0.4	0.82	Excellent
Pond 2	1	N/A	0.9	1	1	0.67	0.67	0.95	1	0.8	0.89	Excellent
Pond 3	1	N/A	0.9	0.67	1	0.67	0.67	0.95	1	0.7	0.84	Excellent
Pond 4	1	1	0.5	0.67	1	0.67	0.67	0.7	0.33	0.8	0.70	Average
Pond 4A	1	0.8	0.5	0.33	1	1	1	0.7	0.33	0.8	0.69	Average
Pond 4B	1	0.6	0.5	0.01	1	1	1	0.7	0.33	0.3	0.43	Poor
Pond 5	1	0.88	0.9	0.33	1	1	0.67	0.7	0.33	0.6	0.69	Average
Pond 6	1	0.8	1	0.67	0.4	0.67	0.67	0.7	0.67	0.5	0.68	Average
Pond 9	1	0.92	0.9	0.67	1	0.67	0.67	0.5	0.67	0.3	0.69	Average
Pond 11	1	0.4	1	0.67	0.4	1	0.67	0.6	0.33	0.4	0.60	Below Average
Pond 12	1	0.4	0.5	0.67	1	0.67	0.67	0.62	0.67	0.8	0.68	Average
Pond 14	1	N/A	0.9	0.67	1	0.67	0.01	0.62	1	0.5	0.51	Below Average
Pond 17	1	N/A	0.1	0.67	1	1	1	0.1	0.67	0.3	0.52	Below Average
Pond 19	1	0.98	1	0.67	0.2	1	0.67	0.1	1	0.7	0.60	Average
Pond 20	1	0.1	0.5	0.67	1	0.01	1	0.65	1	0.3	0.38	Poor
Pond 21	1	0.5	0.5	0.33	1	0.67	1	0.65	1	0.3	0.64	Average
Pond 22	1	1	1	1	1	1	0.67	0.55	0.33	0.8	0.79	Good
Pond 24	1	0.2	0.1	0.01	0.2	1	1	0.55	1	0.3	0.30	Poor
Pond 28	1	0.4	1	0.67	1	1	1	0.55	0.67	0.8	0.78	Good
Pond 29	1	1	1	0.67	1	0.67	0.67	0.65	0.67	0.3	0.72	Good
Pond 31	1	0.05	0.9	0.67	1	0.67	0.67	0.4	0.67	0.3	0.51	Below Average

HSI Criteria / Pond ref.	Zone	Area (m²)	Drying	Water Quality	Shade	Fowl present?	Fish present?	Pond count	Terrestrial habitat	Macrophytes	HSI Score	GCN habitat suitability
Pond 32	1	0.4	0.5	0.67	0.8	1	1	0.65	0.33	0.85	0.67	Average
Pond 33	1	0.4	0.5	0.67	0.8	1	1	0.65	0.33	0.85	0.75	Good
Pond 34	1	Omit	0.9	1	1	0.67	0.33	0.65	1	0.6	0.42	Poor
Pond 35	1	0.8	0.9	0.33	1	0.67	0.01	0.55	0.67	0.3	0.29	Poor
Pond 36A	1	Omit	0.9	0.67	1	0.01	0.01	0.725	1	0.3	0.43	Poor
Pond 38	1	0.05	0.1	0.01	0.2	1	1	0.55	0.33	0.8	0.26	Poor
Pond 39A	1	0.05	0.9	1	1	1	0.01	0.65	0.67	1	0.64	Average
Pond 40	1	0.05	0.1	0.01	0.8	1	1	0.55	0.67	0.3	0.67	Average
Pond 41	1	0.05	0.1	0.01	0.2	1	1	0.55	0.33	0.8	0.50	Below Average
Pond 46	1	0.3	0.5	0.67	0.5	1	1	0.4	0.67	0.8	0.84	Excellent

3.3 eDNA Survey Results

3.3.1 Of ponds, all returned **negative** results for the presence of GCN based on eDNA sampling.

3.3.2 A summary of eDNA results is presented in **Table 3.2**. below, whilst detailed laboratory reports produced by SureScreen Scientifics are reproduced in **Annex 2**.

Table 3.2: eDNA survey results.

Pond	Sample Ref.	Inhibition Check	Degradation Check	Sample Integrity Score	Result
Pond 1	6242	Pass	Pass	Pass	Negative
Pond 2	6239	Pass	Pass	Pass	Negative
Pond 3	6240	Pass	Pass	Pass	Negative
Pond 4	6257	Pass	Pass	Pass	Negative
Pond 5	6236	Pass	Pass	Pass	Negative
Pond 6	6244	Pass	Pass	Pass	Negative
Pond 9	6238	Pass	Pass	Pass	Negative
Pond 11	6233	Pass	Pass	Pass	Negative
Pond 12	6234	Pass	Pass	Pass	Negative
Pond 14	5642	Pass	Pass	Pass	Negative
Pond 20	6264	Pass	Pass	Pass	Negative
Pond 21	6262	Pass	Pass	Pass	Negative
Pond 22	5273	Pass	Pass	Pass	Negative
Pond 24	6243	Pass	Pass	Pass	Negative
Pond 29	6246	Pass	Pass	Pass	Negative
Pond 31	6255	Pass	Pass	Pass	Negative
Pond 33	6256	Pass	Pass	Pass	Negative

Pond	Sample Ref.	Inhibition Check	Degradation Check	Sample Integrity Score	Result
Pond 34	6258	Pass	Pass	Pass	Negative
Pond 35	6247 6253	Pass Pass	Pass Pass	Pass Pass	Negative Negative
Pond 36A	6263	Pass	Pass	Pass	Negative
Pond 39A	6232	Pass	Pass	Pass	Negative
Pond 40	6251	Pass	Pass	Pass	Negative
Pond 46	6237	Pass	Pass	Pass	Negative

4 CONCLUSIONS

4.1.1 EDNA sampling of ponds all returned negative results for GCN DNA at the time of survey.

4.1.2 Records identified via the desk study indicate the presence of GCN at Thorne Railway Delves LWS and several ponds in the search area, indicating that GCN populations are present within the wider environment (i.e., a 2km radius).

4.1.3 Habitat enhancement measures, which will be informed by a detailed Landscape and Ecological Management Plan (LEMP), which will include creation of grassland, planting of hedgerows and installation of hibernacula which will all provide increased opportunities for foraging, commuting and sheltering amphibians.

4.1.4 An assessment of potential impacts of The Scheme on amphibians is provided in the Preliminary Environmental Information report (PEIR) and subsequent ES Chapter.

Figure 1: Pond Location Plan - Overview

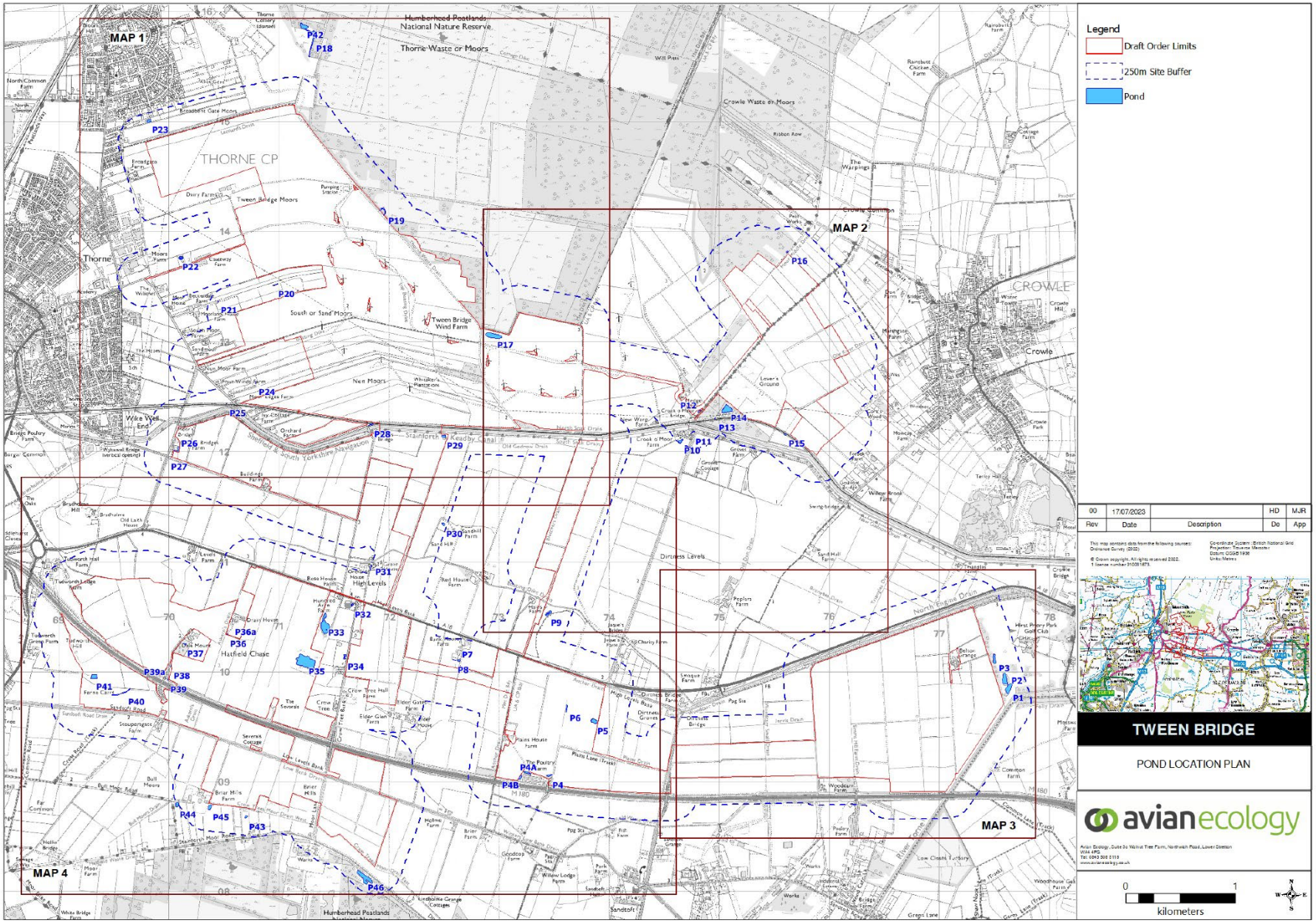


Figure 2: Pond Location Plan – Map 1

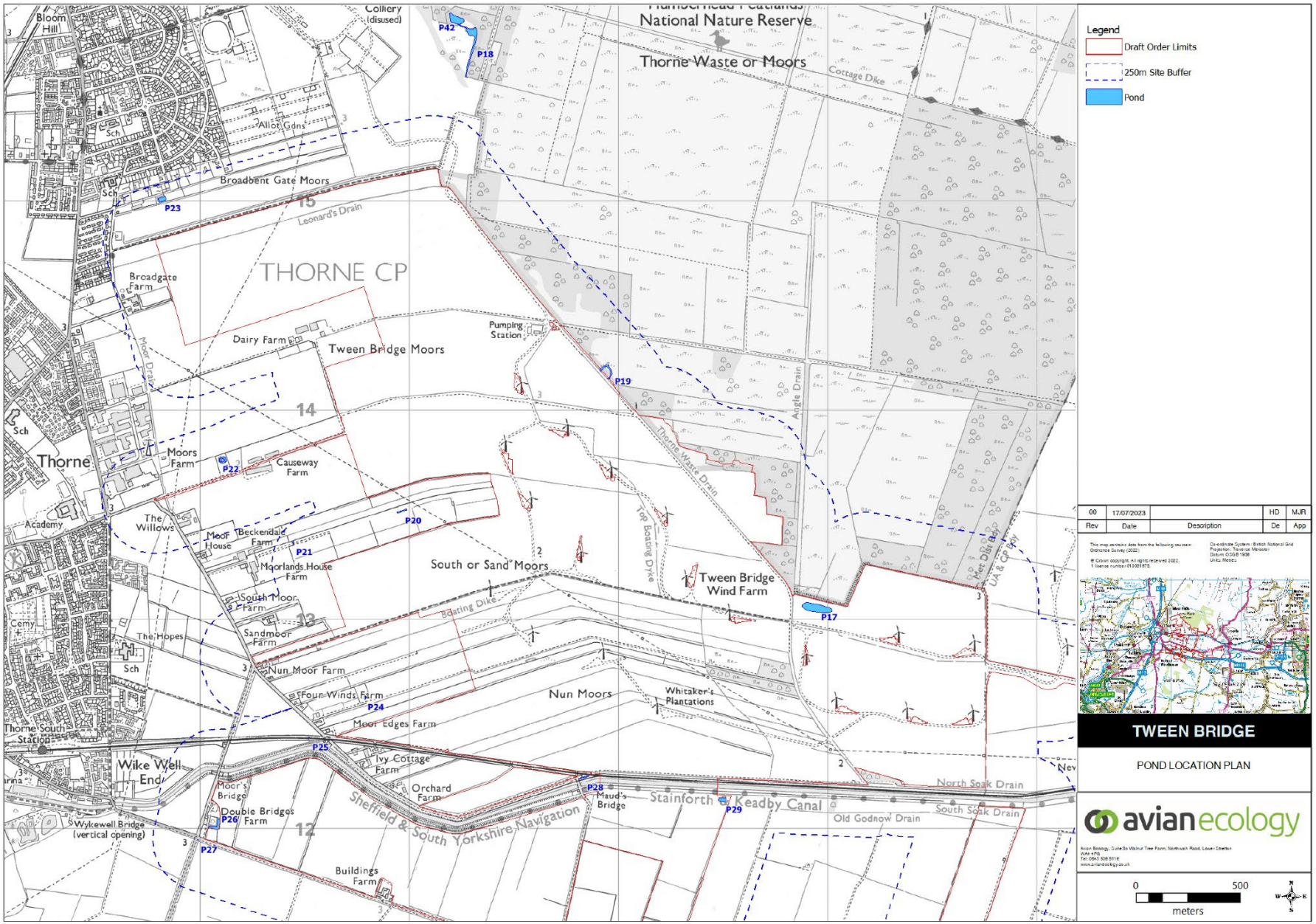


Figure 3: Pond Location Plan – Map 2

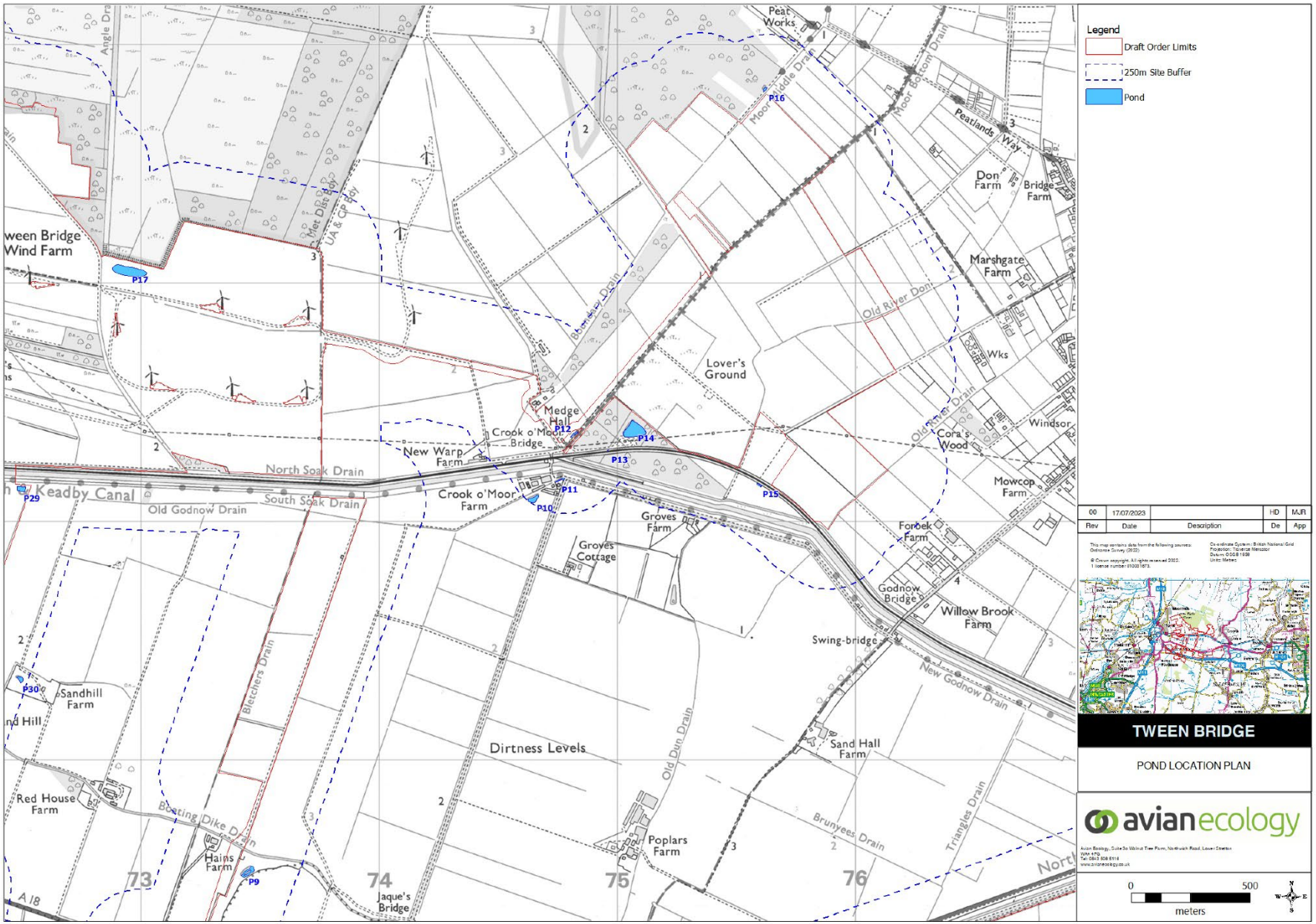


Figure 4: Pond Location Plan – Map 3

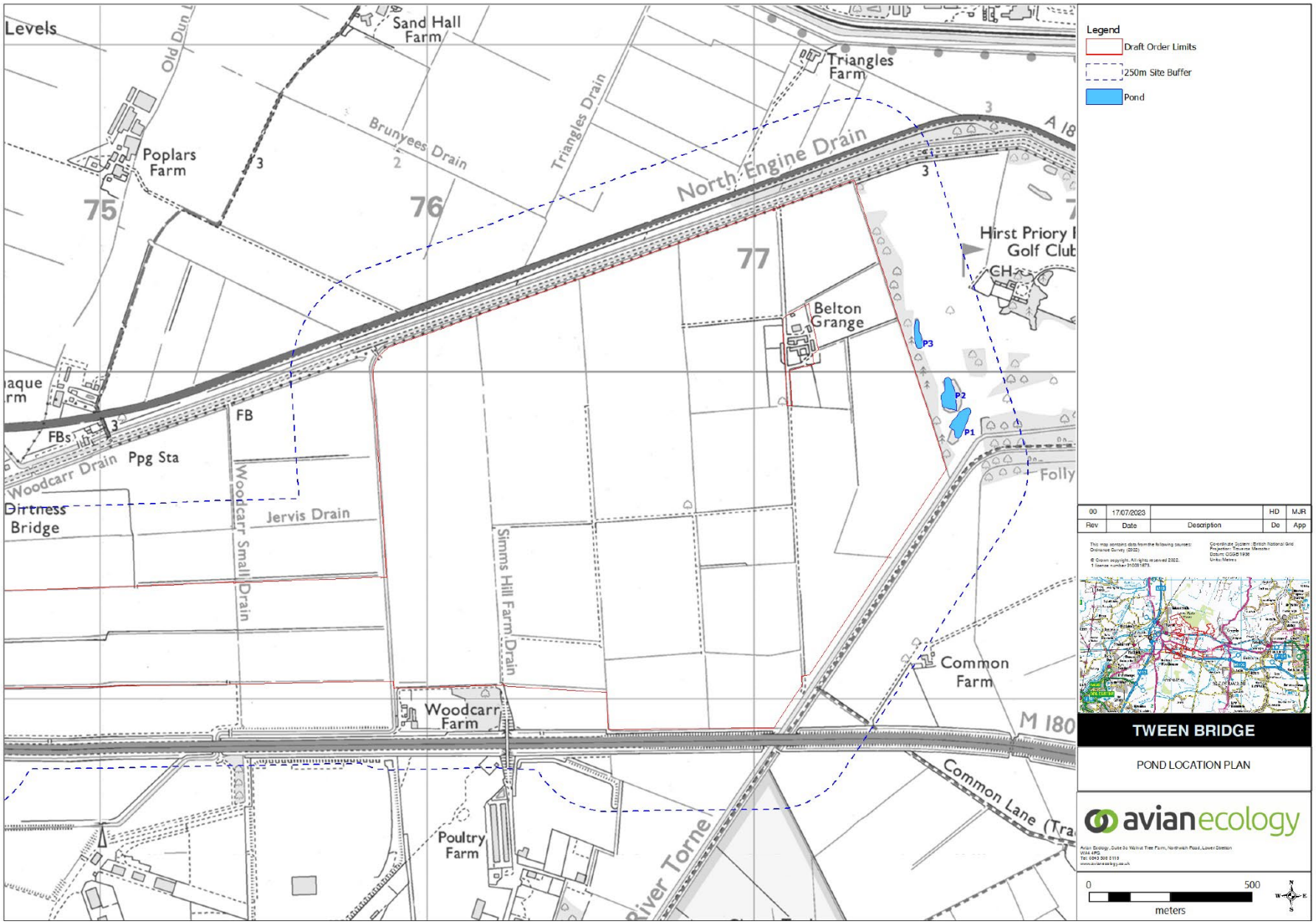
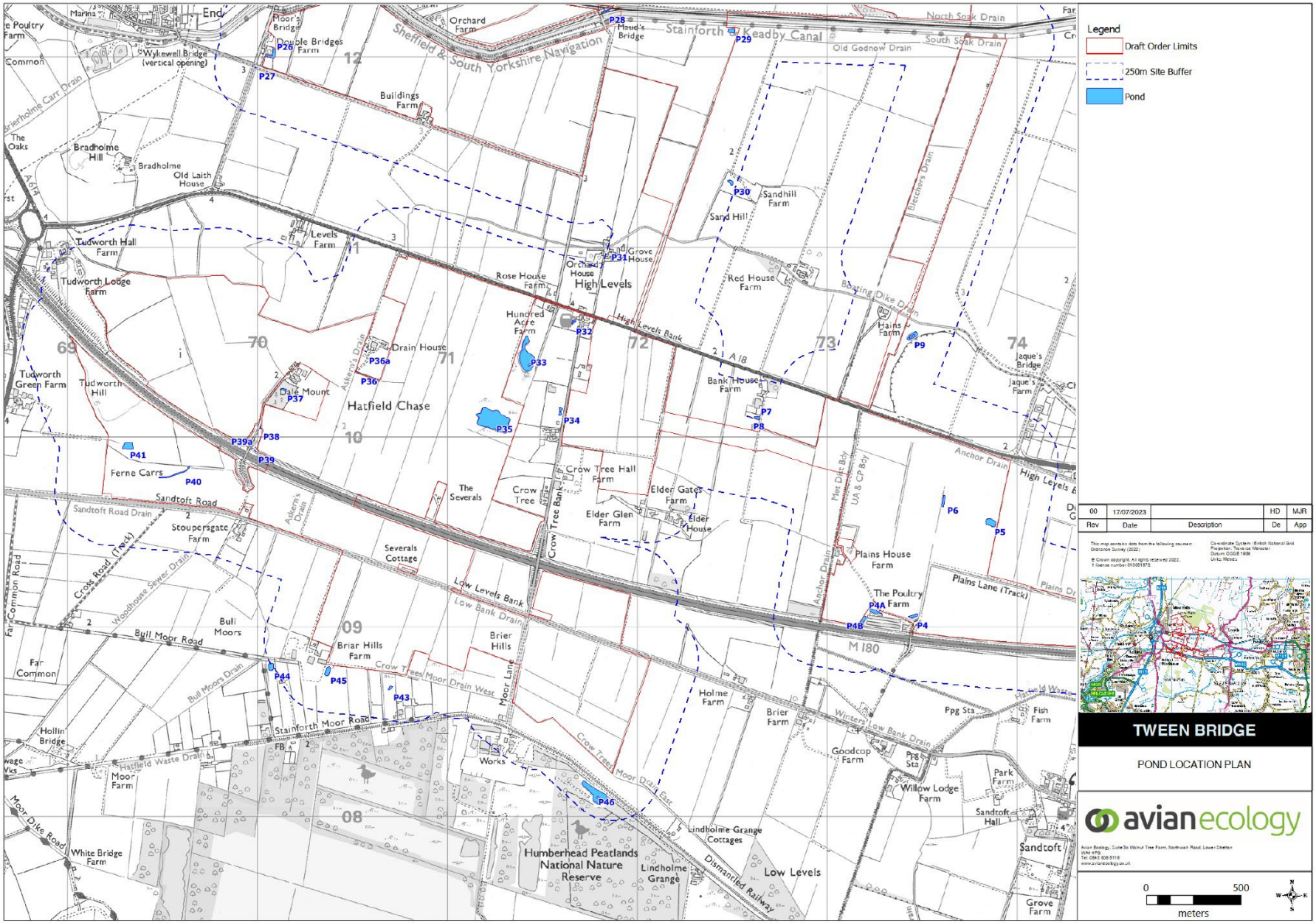


Figure 5: Pond Location Plan – Map 4



Annex 1

Summary table

Pond	Access	HSI	eDNA	Pond	Access	HSI	eDNA
1	Y	Y	Y	24	Y	Y	Y
2	Y	Y	Y	25	N		
3	Y	Y	Y	26	N		
4	Y	Y	Y	27	N		
4A	N	Y (viewed from a distance)		28	Y	Y	Inaccessible
4B	N	Y (viewed from a distance)		29	Y	Y	Y
5	Y	Y	Y	30	N		
6	Y	Y	Y	31	Y	Y	Y
7	Y	Not a pond		32	Y	Y	Dry
8	Y	Not a pond		33	Y	Y	Y
9	Y	Y	Y	34	Y	Y	Y
10	N			35	Y	Y	Y
11	Y	Y	Y	36	Y	Not a pond	
12	Y	Y	Y	36A	Y	Y	Y
13	Y	Not a pond		37	Y	Not a pond	
14	Y	Y	Y	38	Y	Y	Dry
15	N			39	Y	Assumed to be the same as P39A	Inaccessible
16	N			39A	Y	Y	Y
17	Y	Y	Dry	40	Y	Y	Y
18	Outside of 250m			41	Y	Y	Inaccessible
19	Y	Y	Inaccessible	42	Outside of 250m		
20	Y	Y	Y	43	N		
21	Y	Y	Y	44	N		
22	Y	Y	Y	45	Y	N	Inaccessible
23	N			46	Y	Y	Y

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Pond Description and Photograph Panel

SureScreen Scientifics

Folio No: E18885
Report No: 1
Purchase Order: AESS-23-059
Client: AVIAN ECOLOGY LTD
Contact: Beth Walker

TECHNICAL REPORT

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

SUMMARY

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

RESULTS

Date sample received at Laboratory: 11/07/2023
Date Reported: 21/07/2023
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
5273	P22 Tween Bridge		Pass	Pass	Pass	Negative	0
5642	P14 Tween Bridge	SE 75033 12389	Pass	Pass	Pass	Negative	0
6232	P39A Tween Bridge		Pass	Pass	Pass	Negative	0
6233	P11 Tween Bridge	SE 74757 12178	Pass	Pass	Pass	Negative	0
6234	P12 Tween Bridge	SE 74813 12369	Pass	Pass	Pass	Negative	0
6236	P5 Tween Bridge	SE 73857 09551	Pass	Pass	Pass	Negative	0
6237	P46 Tween Bridge	SE 71791 08120	Pass	Pass	Pass	Negative	0

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6238	P9 Tween Bridge	SE 73446 10533	Pass	Pass	Pass	Negative	0
6239	P2 Tween Bridge	SE 77393 09927	Pass	Pass	Pass	Negative	0
6240	P3 Tween Bridge	SE 77501 10101	Pass	Pass	Pass	Negative	0
6242	P1 Tween Bridge	SE 77626 09848	Pass	Pass	Pass	Negative	0
6243	P24 Tween Bridge		Pass	Pass	Pass	Negative	0
6244	P6 Tween Bridge	SE 73610 09666	Pass	Pass	Pass	Negative	0
6246	P29 Tween Bridge		Pass	Pass	Pass	Negative	0
6247	P35 Tween Bridge		Pass	Pass	Pass	Negative	0
6251	P40 Tween Bridge		Pass	Pass	Pass	Negative	0
6253	P35 Tween Bridge		Pass	Pass	Pass	Negative	0
6255	P31 Tween Bridge		Pass	Pass	Pass	Negative	0
6256	P33 Tween Bridge		Pass	Pass	Pass	Negative	0
6257	P4 Tween Bridge	SE 73455 09050	Pass	Pass	Pass	Negative	0
6258	P34 Tween Bridge		Pass	Pass	Pass	Negative	0
6262	P21 Tween Bridge		Pass	Pass	Pass	Negative	0
6263	P36A Tween Bridge		Pass	Pass	Pass	Negative	0
6264	P20 Tween Bridge		Pass	Pass	Pass	Negative	0

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

Reported by: Lauryn Jewkes **Approved by:** Chris Troth

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METHODOLOGY

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

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

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

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

INTERPRETATION OF RESULTS

SIC: **Sample Integrity Check** [Pass/Fail]
When samples are received in the laboratory, they are inspected for any tube leakage, suitability of sample (not too much mud or weed etc.) and absence of any factors that could potentially lead to inconclusive results.

DC: **Degradation Check** [Pass/Fail]
Analysis of the spiked DNA marker to see if there has been degradation of the kit or sample between the date it was made to the date of analysis. Degradation of the spiked DNA marker may lead indicate a risk of false negative results.

IC: **Inhibition Check** [Pass/Fail]
The presence of inhibitors within a sample are assessed using a DNA marker. If inhibition is detected, samples are purified and re-analysed. Inhibitors cannot always be removed, if the inhibition check fails, the sample should be re-collected.

Result: **Presence of GCN eDNA** [Positive/Negative/Inconclusive]
Positive: GCN DNA was identified within the sample, indicative of GCN presence within the sampling location at the time the sample was taken or within the recent past at the sampling location.
Positive Replicates: Number of positive qPCR replicates out of a series of 12. If one or more of these are found to be positive the pond is declared positive for GCN presence. It may be assumed that small fractions of positive analyses suggest low level presence, but this cannot currently be used for population studies. In accordance with Natural England protocol, even a score of 1/12 is declared positive. 0/12 indicates negative GCN presence.
Negative: GCN eDNA was not detected or is below the threshold detection level and the test result should be considered as evidence of GCN absence, however, does not exclude the potential for GCN presence below the limit of detection.

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Pond Description and Photograph Panel

Photograph	Description
	Photo 1: P1 – A large pond situated close to P2. Located in the Lincolnshire Golf Course. Surrounded by grassland of varying sward height, some tall herb, scattered scrub/trees and woodland. Banksides consist of earth and vary between being shallow and a 50° gradient. Pond base is sandy. Marginal vegetation includes grasses, herbs and scattered willow scrub. Emergent vegetation is limited to margins and comprised solely of common reed. Water clarity is clear and water quality is considered to be good from terrestrial invertebrate presence. No fish were observed, but minor waterfowl numbers are present.
	Photo 2: P2 – A large pond situated between ponds P1 and P3 in the Lincolnshire Golf Course. Surrounded by grassland of varying sward height, some tall herb, scattered scrub/trees and woodland. Banksides comprise of earth and are steep and undercut in places, with some shallow areas. The pond has a sediment base and is mainly sandy. Pond margins comprise grass swards of varying heights and tall herbs. Scattered scrub and trees also frequent around the margin, composed mainly of willow, with some elder, horse chestnut and hawthorn. Emergent vegetation includes common reed, with large areas of pond lily. Submerged vegetation is potentially present but rain impacted visibility. Fish presence is possible but none were observed. Mallard and moorhen were present, but impacts are considered minor. Water clarity is clear and quality is considered to be good due to terrestrial and aquatic invertebrates recorded.
	Photo 3: P3 – An oblong pond located in Lincolnshire Golf Course An adjacent treeline separates the pond from an arable landscape. The pond is partially enclosed within a woodland stand and is connected to the treeline. Surrounding habitat includes sheep grazed grassland of varying sward heights in addition to tall herbs Scattered scrub and trees are also present. Trees overhang in places creating shaded areas. Emergent vegetation includes willow, common reed and pond lily, with unidentified submerged vegetation present towards the centre of the pond. The bank gradient is generally steep/undercut. The pond base is sandy and is considered to potentially be an old bunker relic of the golf course. Water clarity is clear and invertebrate presence implied good quality. No fish observed and waterfowl impact is considered minor.

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Pond Description and Photograph Panel

	Photo 24: P31 – A steep sided ditch with static water and presence of duckweed. Water depth is considered deep and earth banksides are steep. Southern banks are dominated by dense nettles, with elder scrub dominating the northern bank. Mallard and moorhen are present in low numbers. The wider area includes sheep grazed pastures and farm buildings.
	Photo 25: P32 – A dry residential pond located in a garden area of an adjourning pub. The earth banks have a steep gradient at 70°. The banks are vegetated with a medium sward-length of grass and herbs. The dry pond is enclosed in a woodland copse, which creates a high amount of shading. A vegetated island is present in the centre should the pond seasonally contain water. The local landscape includes grazed pastures, tree lines, scattered trees and urban areas with roads. Iris is prominent in the dry pond area. No notable invertebrates were recorded or any signs of waterfowl presence.
	Photo 26: P33 - A large, deep waterbody with no shade. Algae is present along the edges. A narrow, northern section of the pond is dominated by bulrush. Sedge, bulrush and tall herb dominates the remaining banksides, providing good egg laying opportunities for GCN. Banksides are shallow and mostly accessible. The surrounding area comprises grassland and scattered scrub considered good habitat for amphibians. The pond likely contains fish and is considered good for waterfowl.
	Photo 27: P34 – A fishing pond located close to a main road and caravan park. Banksides are vertical and approximately 0.5m deep. Approximately 80% of the bankside being accessible for survey. Banks are dominated by mown amenity grassland. Limited common reed and iris is present. Scattered willow trees and bramble scrub are adjacent. The recreational pond contains a high density of fish that are frequently fed, creating turbid and enriched water. A water fountain is also present.

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Pond Description and Photograph Panel

	Photo 28: P35 – A large, deep waterbody that was surveyed with two eDNA kits due to the ponds expansive surface area. Each eDNA kit sampled approximately 50% of the pond perimeter. Waterfowl are present and frequent, with fish likely present. The pond contains an expansive area of open water with little shade. It is surrounded by dense common reed that limit access in places, as well as scattered scrub, grassland, areas of mown tall herb and arable fields adjacent. Egg laying opportunities are present on the marginal vegetation. Banksides vary between being shallow and steep.
	Photo 29: P36 - An absent pond comprised of tall herb and scattered scrub. On inspection and additional pond (P36A) was identified in close proximity.
	Photo 30: P36A - A new pond added during the survey visit. The pond is a small ornamental pond located in a garden. The pond is approximately 5m^2 and contains shallow water. Emergent vegetation is abundant and includes sedge, lily and ornamental plants. Algae is present, but the surface includes some open areas. The pond has artificial banks and lining. It is surrounded by the garden itself, grazed pastures and arable land. Tree lines and hedgerow are present in close proximity to the pond. There is no shade and fish are present.
	Photo 31: P37 - An absent pond located in a shallow woodland depression.

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Pond Description and Photograph Panel

	Photo 32: P38 – A dry section of ditch. Located along a shaded tree line with some common reed present. Contains shallow banksides with short grassland. Has potential to contains seasonal water levels. Located adjacent to arable land and a single-track road.
	Photo 33: P39 – An inaccessible section of widened ditch located directly adjacent to the M180 motorway. Dense bramble and fencing prevented access. Banksides are considered to be steep and the water quality is likely poor from potential motorway pollution. Approximately 10m north is an adjacent and accessible ditch which was included in the survey as it was considered that any potential GCN population would be found in both waterbodies. The surrounding land includes arable land, with a tree line and woodland in the vicinity.
	Photo 34: P39A – A static wet ditch approximately 10m from P39A. It was included in the survey as P39 could not be accessed and it was expected that any GCN population potentially present in P39 could also be present in P39A. The 2m wide ditch contains relatively deep water, as well as steep banks that include dense common reed. Reed presence restricts access to the ditch. In order to collect a sampled most representative and similar to P39, the survey was focused only at the southern end of the ditch closest to P39. Water quality was considered poor. There was no evidence of fish or water fowl. The surrounding area was the same as P39, although the ditch itself runs adjacent to a tree line.
	Photo 35: P40 – A suitable, static wet ditch measuring approximately 2-3m wide. The water quality was considered poor, with signs of enrichment and turbidity. Water levels are deep, with banksides steep and vertical at 0.5m deep. Banksides are dominated by bulrush, nettle and common thistle. The surrounding area includes arable land, with the M180 motorway nearby. The water surface includes some areas of duckweed, as well as egg laying opportunities.

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Pond Description and Photograph Panel

	Photo 36: P41 - An inaccessible, large area of grassland, scrub and tall herb in the location of a remnant pond. A steep earth mound dominated in dense nettle and scrub prevented close examination. Although considered to no longer be a pond from the landowner, a small pool of water was identified from the earth mound vantage point. The water levels appeared to be shallow and dominated by algae in a shallow earth depression. The pool of water likely dries and may only be temporarily present due to recent heavy rainfall. Further areas of pooled water may be present in the expansive area. The surrounding area includes arable land which isolates P41. The localised area of grassland, tall herb and scrub is excellent terrestrial amphibian habitat.
No photo permitted	P45 is a slurry lagoon that was denied inclusion in the survey by the landowner. No photos of the pond were permitted. Close inspection was also not possible due to an adjacent ditch, with dense surrounding scrub further limiting visibility. Excellent tall herb and scrub terrestrial habitat surrounds the waterbody, with the wider area dominated by arable land. The pond is artificially lined an has an open surface with no shading. No emergent vegetation was identified, and the 45° lined banksides contain no vegetation. No egg laying vegetation is therefore present. Some log piles are located in the adjacent recycling centre, however such piles are likely subject to activity disturbance. Water quality is assumed to be poor due to being a slurry lagoon, however invertebrates over the pond are abundant with high levels of foraging house martin activity. No fish or waterfowl are expected.
	Photo 37: P46 - A large oblong pond enclosed within a woodland reserve. The pond is surrounded by dense bracken, scrub and trees. Earth banks vary in gradient with both shallow and steep areas. The local landscape comprises primarily of woodland. Emergent vegetation includes pond lily, willow and common reed. The pond has deep water, with a turbid clarity. Moderate invertebrate activity was recorded and fish presence was considered possible. Waterfowl activity was considered to be likely, although no impact was observed. Overhanging trees provide areas of shading and submerged leaves that likely cause enrichment.