



**HELIOS** RENEWABLE  
ENERGY  
PROJECT

# **Preliminary Environmental Information Report**

**Volume 3: Technical Appendices**  
Appendix 8.5: Amphibian Baseline  
Report

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# Helios Renewable Energy Project

on behalf of Enso Green Holdings D Limited

## Technical Appendix 8.5: Amphibian Baseline Report



<b>Document Control</b>				
Project Name:		Helios Renewable Energy Project		
Project / Report Number:		EnsoE-517-1435		
Report Title		Technical Appendix 8.5: Amphibian Baseline Report		
<b>Issue</b>	<b>Date</b>	<b>Notes</b>	<b>Prepared</b>	<b>Reviewed</b>
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# 1 INTRODUCTION

## 1.1 Background

- 1.1.1 Avian Ecology Ltd. was commissioned by Enso Green Holdings D Limited to undertake a great crested newt (GCN) *Triturus cristatus* presence/absence surveys adopting the environmental DNA (eDNA) sampling methodology.
- 1.1.2 The surveys were undertaken in relation to the proposed development of a renewable energy generating project; consisting of ground-mounted solar photovoltaic arrays, together with on-site energy storage, associated infrastructure and grid connection (the 'Proposed Development'), on land to the south-west of the village of Camblesforth and to the north of the village of Hirst Courtney in North Yorkshire (the 'Site'), 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 Biodiversity Chapter 8 of the Preliminary Environmental Information Report ('PEIR') and the corresponding chapter within the Environmental Statement ('ES') to be submitted in support of the application for development consent.

## 1.2 Survey Area

- 1.2.1 Ponds were identified from aerial images and Ordnance Survey ('OS') maps on or within 250m of the Site boundary. Due to the low impact of solar energy developments on GCN habitats, and reflecting guidance published by Natural England<sup>1</sup>, ponds beyond 250m from the Site were not considered within the ecological assessment process.
- 1.2.2 Following from changes to the Site boundary during the project design process, eight ponds originally located within 250m of the Site boundary are now located beyond 250m of the Site, for context (and due to laboratory testing having been undertaken on two of these) 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))<sup>2</sup> and European law (The Habitats and Species Regulations 2017)<sup>3</sup>. 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;
  - sell, barter, exchange or transport or offer for sale GCN or parts of them.

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

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

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

- 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 Natural England<sup>4</sup>, 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 Natural England.
- 1.3.3 GCN and common toad *Bufo bufo* are listed as priority species under Section 41 of the Natural Environment and Rural Communities ('NERC') Act 2006<sup>5</sup>, and GCN are also listed as a local priority species within the Selby Biodiversity Action Plan ('BAP')<sup>6</sup> and are therefore, a material consideration within the planning process.

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<sup>4</sup> Available at: <https://www.gov.uk/guidance/european-protected-species-policies-for-mitigation-licences> (accessed 21st February 2023)

<sup>5</sup> Available at: <https://www.legislation.gov.uk/ukpga/2006/16/contents> (accessed 21st February 2023)

<sup>6</sup> Available at: <https://www.selby.gov.uk/sites/default/files/Documents/Biodiversity%20Action%20Plan%20Aug%202004.pdf> (accessed 21<sup>st</sup> February 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')<sup>7</sup> 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 Site, obtained from the following key sources:
  - Records request to North & East Yorkshire Ecological Data Centre ('NEYEDC')<sup>8</sup>;
  - A review of Magic Map for EPS licence records relating to GCN.

2.1.3 Only recent records dated from 2005 onwards were used unless historic records (pre-2005) were received from within (or within close proximity to) the Site and/or historic records were considered otherwise pertinent to the Proposed Development.

### 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 Site using OS and aerial mapping and during extended habitat surveys within the Site.

2.2.2 37 ponds were originally identified within and surrounding the Site, including a single pond present within the Site itself (P4) (as seen in **Figure 1**). Due to Site boundary changes, eight of these ponds (P3, P11, P18, P19, P20, P21, P22, and P28) are now located beyond the 250m buffer boundary. In addition, during the eDNA surveys, an additional pond was found within 250m of the Site (i.e., a chain of small, interlinked garden ponds) in close proximity to P3, and subsequently recorded as P3a. In addition, two static ditches with suitable emergent/aquatic vegetation were recorded within the Site and considered suitable to support GCN (ditches D1 and D2), all ponds and suitable ditch networks are provided within **Figure 1**.

2.2.3 Access was granted on 17<sup>th</sup> and 18<sup>th</sup> June 2021 to five ponds (ponds P1, P2, P4, P5 and P6) and eight ditch sections (D1: sections 1 - 4, and D2: sections 1 - 4). Three of the ponds (ponds P1, P2 and P5) and ditches D1 (sections 1 - 4), and D2 (sections 1 - 4) were subject to eDNA survey sampling to determine the presence or likely absence of GCN. Ponds P4 and P6 were found to be dry and therefore not subject to survey in 2021.

2.2.4 Access was granted on 28<sup>th</sup> and 29<sup>th</sup> June 2022 to an additional 18 ponds (ponds P3, P3a, P10, P11, P14, P15, P16, P17, P18, P19, P20, P22, P30, P31, P32, P33, P34, P35); eight of these additional ponds (ponds P3, P3a, P10, P11, P14, P32, P33, and P34) were also subject to eDNA survey sampling to determine the presence or likely absence of GCN. Ponds P15, P16, P17, P19, P20, P22, P31, and P35

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<sup>7</sup> Available at: <https://magic.defra.gov.uk/magicmap.aspx> (accessed 27th February 2023)

<sup>8</sup>Non-statutory designated sites are all provided by the North & East Yorkshire Ecological Data Centre <https://www.neyedc.org.uk/>

were found to be dry and therefore not subject to survey during this survey period. Ponds P3, P11, P18, P19, P20, P21, and P22 are now located beyond the 250m buffer boundary.

- 2.2.5 Ponds P18 (now located beyond the 250m buffer boundary) and P30 which are both marked on OS maps were found to be no longer present (either holding water or as a dry pond) and therefore not subject to survey.
- 2.2.6 Access was later granted on 13<sup>th</sup> and 14<sup>th</sup> May 2023 to 13 ponds which were either dry or not surveyed in previous years (ponds P4, P6, P9, P15, P16, P17, P22, P24, P29, P31, P35, P36, P37); eight of these ponds were subject to eDNA survey sampling to determine the presence or likely absence of GCN (P4, P6, P9, P22<sup>9</sup>, P24, P29, P36, P37). Ponds P15, P16, P17, P31 and P35 were found to be dry at the time of survey, and consequently not subject to eDNA survey.
- 2.2.7 Access was not permitted to survey ponds P7, P8, P12, P13, P21, P23, P25, P26, P27 and P28 (although P21 and P28 are now located beyond the 250m buffer boundary following Site boundary changes).

### 2.3 Habitat Suitability Index (HSI) Assessments

- 2.3.1 All accessible wet ponds/areas of suitable standing water were assessed for their suitability to support GCN via the HSI process. The assessment took place on the same dates as the eDNA surveys and followed the methodology detailed within ARG UK guidance (ARG UK, 2010<sup>10</sup>); which is a refined version of the Oldham et al. 2000<sup>11</sup> 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<sup>12</sup>). 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

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<sup>9</sup> Although P22 is now located beyond the 250m buffer zone, it is located in immediate proximity to P13 (located within the 250m buffer), which was not accessible; P22 was surveyed in order to provide presence or absence data, which would indicate the likelihood of GCN presence/absence within the adjacent P13.

<sup>10</sup> ARG UK (2010). ARG UK Advice Note 5: Great Crested Newt Habitat Suitability Index. Amphibian and Reptile Groups of the United Kingdom.

<sup>11</sup> 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.

<sup>12</sup> 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.



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<sup>13</sup>).

- 2.4.3 Natural England 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)<sup>14</sup>. Natural England will only accept eDNA survey results undertaken between mid-April and 30<sup>th</sup> 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 Nineteen ponds and eight ditch sections 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<sup>15</sup>; 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 1**.

## **2.5 Limitations of Survey**

- 2.5.1 Access was not permitted or confirmed for ponds P7, P8, P12, P13, P23, P25, P26, P27 (plus P21 and P28 which are now located beyond the 250m Site boundary) and therefore, these were not surveyed for GCN. However, it is considered that due to the very low number of ponds which tested positive for GCN eDNA, the low number of GCN records within the wider environment and the predominantly unsuitable intensively managed agricultural habitats within the Site; the lack of survey information is not considered to represent a significant constraint to the ecological assessment process.
- 2.5.2 Ponds P15, P16, P17, P31, and P35 (plus P19, P20 which are now located beyond the 250m Site boundary) were found to be dry, P18 and P30 were recorded to be not present, and therefore these were not subject to survey. The lack of survey information for these ponds is not considered a significant constraint to the ecological assessment process.

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<sup>13</sup> 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.

<sup>14</sup> Available at: <https://www.gov.uk/guidance/great-crested-newts-surveys-and-mitigation-for-development-projects> (accessed 21st February 2023)

<sup>15</sup> Available at: <https://surescreenscientifics.com/edna/gcn-edna/> (accessed 21st February 2023)

### 3 RESULTS

#### 3.1 Desk Study

##### *Designated Sites*

3.1.1 No statutory or non-statutory designated sites designated for GCN are located within 2km of the Site.

##### *Species Records*

3.1.2 NEYEDC returned a total of thirty recent records relating to amphibian species from within a 2km radius of the Site; specifically, these records related to GCN, common toad *Bufo bufo*, common frog *Rana temporaria* and smooth newt *Lissotriton vulgaris*.

3.1.3 No amphibian records received from NEYEDC were located directly within the Site; the closest records in proximity to the Site relate to GCN, located approximately 0.8km north-east.

3.1.4 A data review of MAGIC identified a single record of a GCN class license return within 2km of the Site, relating to an area approximately 1.8km to the north-east of the Site boundary.

3.1.5 GCN eDNA pond surveys undertaken in 2017, 2018, and 2019<sup>16</sup> to inform the provision of District Level Licencing, included the survey of six ponds within 2km of the Site. Of these ponds, a single pond was located directly on-Site (P4), and another directly adjacent (P34). P34 was stated to have GCN eDNA present at the time of survey (2019), whilst survey results for P4 were stated to be inconclusive (2019). A further three ponds were recorded as positive for GCN eDNA in the wider landscape, all of which are located beyond 500m from the Site boundaries, the closest of which is located approximately 0.8km northeast of the Site boundary within Barlow Common Local Nature Reserve ('LNR').

#### 3.2 Habitat Suitability Index (HSI) Assessments

3.2.1 All accessible ponds (containing water) and suitable ditches 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 2**, and pond locations outlined within **Figures 1** to **5**.

**Table 3.1 – Habitat Suitability Index Assessment Results**

HSI Criteria Pond ref.	Zone	Area (m <sup>2</sup> )	Drying	Water Quality	Shade	Fowl present?	Fish present?	Pond count	Terrestrial habitat	Macrophytes	HSI Score	GCN habitat suitability
Pond 1	1	0.95	0.5	0.33	1	0.01	1	0.9	1	0.3	0.46	Poor
Pond 2	1	0.95	1	0.67	1	0.67	0.67	0.5	0.67	0.3	0.70	Good

<sup>16</sup> Available at: <https://www.data.gov.uk/dataset/8643f1b9-b419-4ee8-8e9c-18200e0edc31/great-crested-newt-edna-habitat-suitability-index-pond-surveys-for-district-level-licensing-2017-2018-2019> (accessed 27th February 2023)

HSI Criteria Pond ref.	Zone	Area (m <sup>2</sup> )	Drying	Water Quality	Shade	Fowl present?	Fish present?	Pond count	Terrestrial habitat	Macrophytes	HSI Score	GCN habitat suitability
Pond 3*	1	1	0.1	0.67	1	0.67	1	0.6	0.67	0.3	0.59	Below Average
Pond 3a	1	0.05	1	0.67	1	1	0.33	0.6	1	1	0.61	Average
Pond 4	1	0.2	1	0.33	0.4	1	1	0.55	0.67	0.4	0.57	Below Average
Pond 5	1	0.7	0.5	0.33	0.6	1	1	0.85	0.67	0.3	0.64	Average
Pond 6	1	0.05	0.5	0.01	0.2	1	1	0.67	1	0.3	0.39	Poor
Pond 9	1	0.8	0.9	0.33	0.4	1	1	0.7	0.33	0.4	0.59	Below Average
Pond 10	1	0.1	1	0.67	1	1	0.33	0.6	0.67	0.9	0.62	Average
Pond 11*	1	1	1	0.67	1	1	0.33	0.5	1	0.7	0.77	Good
Pond 14	1	0.4	1	0.67	1	0.67	1	0.75	0.33	0.8	0.72	Good
Pond 22*	1	0.05	0.5	0.01	0.2	1	1	0.9	1	0.3	0.33	Poor
Pond 24	1	0.2	1	0.01	0.2	1	1	0.67	1	0.3	0.39	Poor
Pond 29	1	0.3	0.9	0.67	1	0.67	1	0.55	0.67	0.6	0.70	Average
Pond 32	1	1	0.1	0.33	1	1	1	0.72	0.33	0.3	0.55	Below Average
Pond 33	1	0.3	0.1	0.33	1	0.67	1	0.72	0.67	0.3	0.50	Poor
Pond 34	1	0.95	0.5	0.33	1	0.67	1	0.65	0.67	0.3	0.65	Average
Pond 36	1	0.6	1	1	0.8	1	1	0.55	1	0.7	0.84	Excellent
Pond 37	1	1	0.9	1	1	0.67	1	0.55	1	0.7	0.86	Excellent
Ditch 1 – S1	1	0.8	1	0.67	1	1	0.67	0.28	0.33	0.8	0.70	Good
Ditch 1 – S2	1	0.8	1	0.67	1	1	0.67	0.28	0.33	0.3	0.63	Average
Ditch 1 – S3	1	0.8	1	0.67	1	1	0.67	0.28	0.33	0.3	0.63	Average
Ditch 1 – S4	1	0.8	1	0.67	1	1	0.67	0.28	0.33	0.8	0.70	Good
Ditch 2 – S1	1	0.9	0.9	0.67	1	1	0.67	0.85	0.33	0.5	0.74	Good
Ditch 2 – S2	1	0.9	0.9	0.67	1	1	0.67	0.85	0.33	0.7	0.77	Good
Ditch 2 – S3	1	0.9	0.9	0.67	1	1	0.67	0.85	0.33	0.45	0.73	Good
Ditch 2 – S4	1	0.9	0.9	0.67	1	1	0.67	0.85	0.67	0.45	0.79	Good

\* No longer within 250m of the Site boundaries

### 3.3 eDNA Survey Results

3.3.1 Of ponds and ditches surveyed, all returned **negative** results for the presence of GCN based on eDNA sampling, with the exception of P34 which returned a **positive** result for the presence of GCN.

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 1.**

**Table 3.2: eDNA survey results**

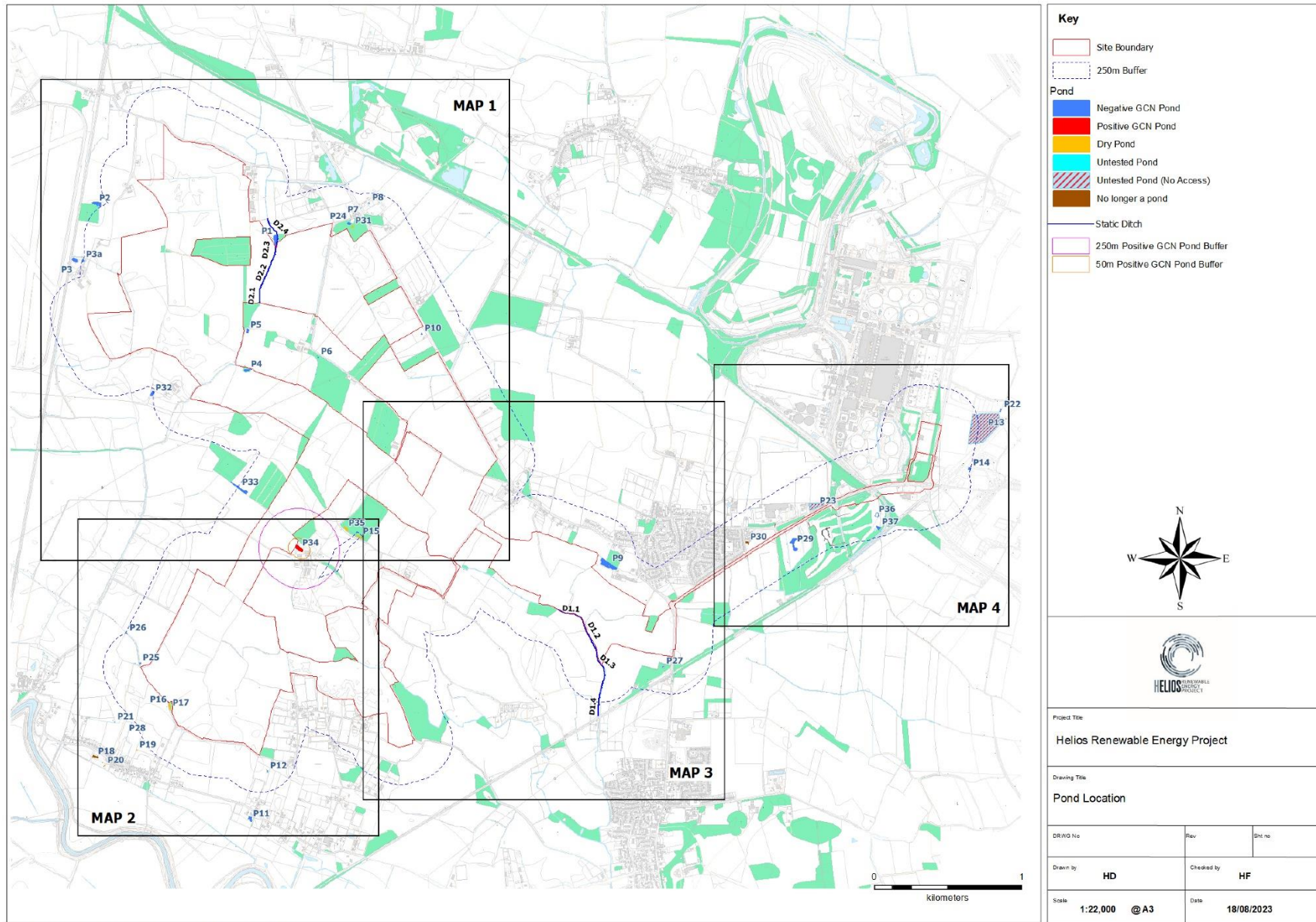
Pond	Sample Ref.	Inhibition Check	Degradation Check	Sample Integrity Score	Result
Pond 1*	6584	Pass	Pass	Pass	Negative
Pond 2	6583	Pass	Pass	Pass	Negative
Pond 3*	6588	Pass	Pass	Pass	Negative
Pond 3a	6587	Pass	Pass	Pass	Negative
Pond 4	R409	Pass	Pass	Pass	Negative
Pond 5	6586	Pass	Pass	Pass	Negative
Pond 6	R414	Pass	Pass	Pass	Negative
Pond 9	R409	Pass	Pass	Pass	Negative
Pond 10	6589	Pass	Pass	Pass	Negative
Pond 11*	6568	Pass	Pass	Pass	Negative
Pond 14	6579	Pass	Pass	Pass	Negative
Pond 22*	R406	Pass	Pass	Pass	Negative
Pond 24	R420	Pass	Pass	Pass	Negative
Pond 29	R405	Pass	Pass	Pass	Negative
Pond 32	6590	Pass	Pass	Pass	Negative
Pond 33	6578	Pass	Pass	Pass	Negative
Pond 34	6585	Pass	Pass	Pass	<b>Positive</b>
Pond 36	R408	Pass	Pass	Pass	Negative
Pond 37	R417	Pass	Pass	Pass	Negative
Ditch 1 – S1	4123	Pass	Pass	Pass	Negative
Ditch 1 – S2	5396	Pass	Pass	Pass	Negative
Ditch 1 – S3	5389	Pass	Pass	Pass	Negative
Ditch 1 – S4	4110	Pass	Pass	Pass	Negative
Ditch 2 – S1	6528	Pass	Pass	Pass	Negative
Ditch 2 – S2	6529	Pass	Pass	Pass	Negative
Ditch 2 – S3	6530	Pass	Pass	Pass	Negative
Ditch 2 – S4	4121	Pass	Pass	Pass	Negative

\* No longer within 250m of the Site boundary

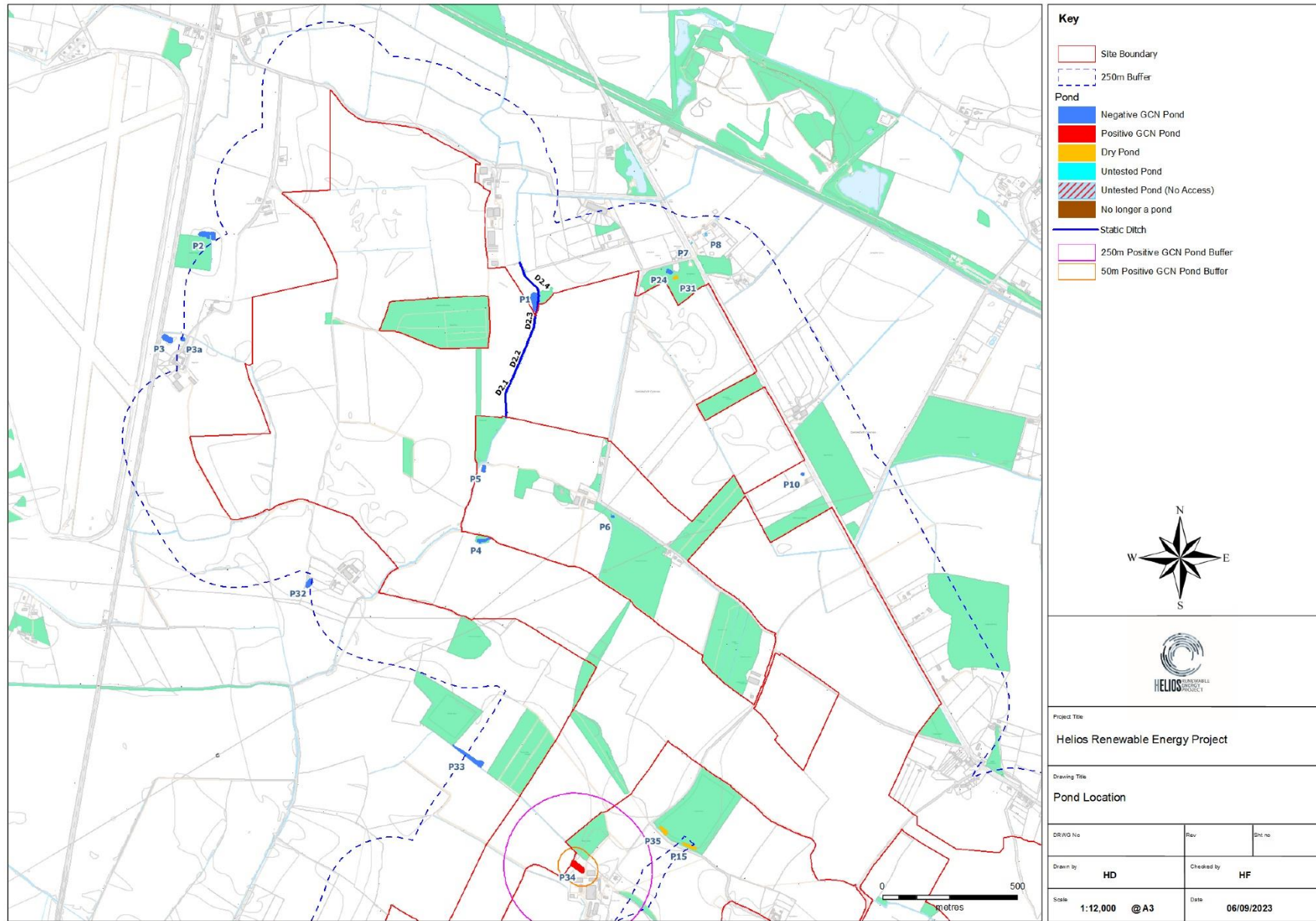
## 4 CONCLUSIONS

- 4.1.1 eDNA sampling of ponds and ditches surveyed returned a single positive result for **P34**, whilst the remaining features surveyed all returned negative results based on the presence of GCN eDNA at the time of survey.
- 4.1.2 Consequently, a positive result for **P34** suggests that breeding GCN are present directly adjacent to Site, although wider survey results would indicate that the geographical terrestrial spread of the species within the Site is limited to a localised area surrounding **P34**.
- 4.1.3 Additionally, records identified via the desk study also indicated the presence of GCN at **P34** suggesting a relatively stable population within the pond and further populations within the wider environment (i.e., a 2km radius).
- 4.1.4 Habitat enhancement measures, which will be informed by a detailed Landscape and Ecological Management Plan, will include native shrub, hedgerow, and tree planting, and new grassland to be managed to provide suitable habitat for amphibians (including GCN), creating structurally diverse grassland and hedgerow and scrub areas to provide shelter and foraging and opportunities and connectivity around the Proposed Development peripheries.
- 4.1.5 An assessment of potential impacts of the Proposed Development on amphibians will be provided within the PEIR and ES Chapter and include the provision of a suitable mitigation strategy.

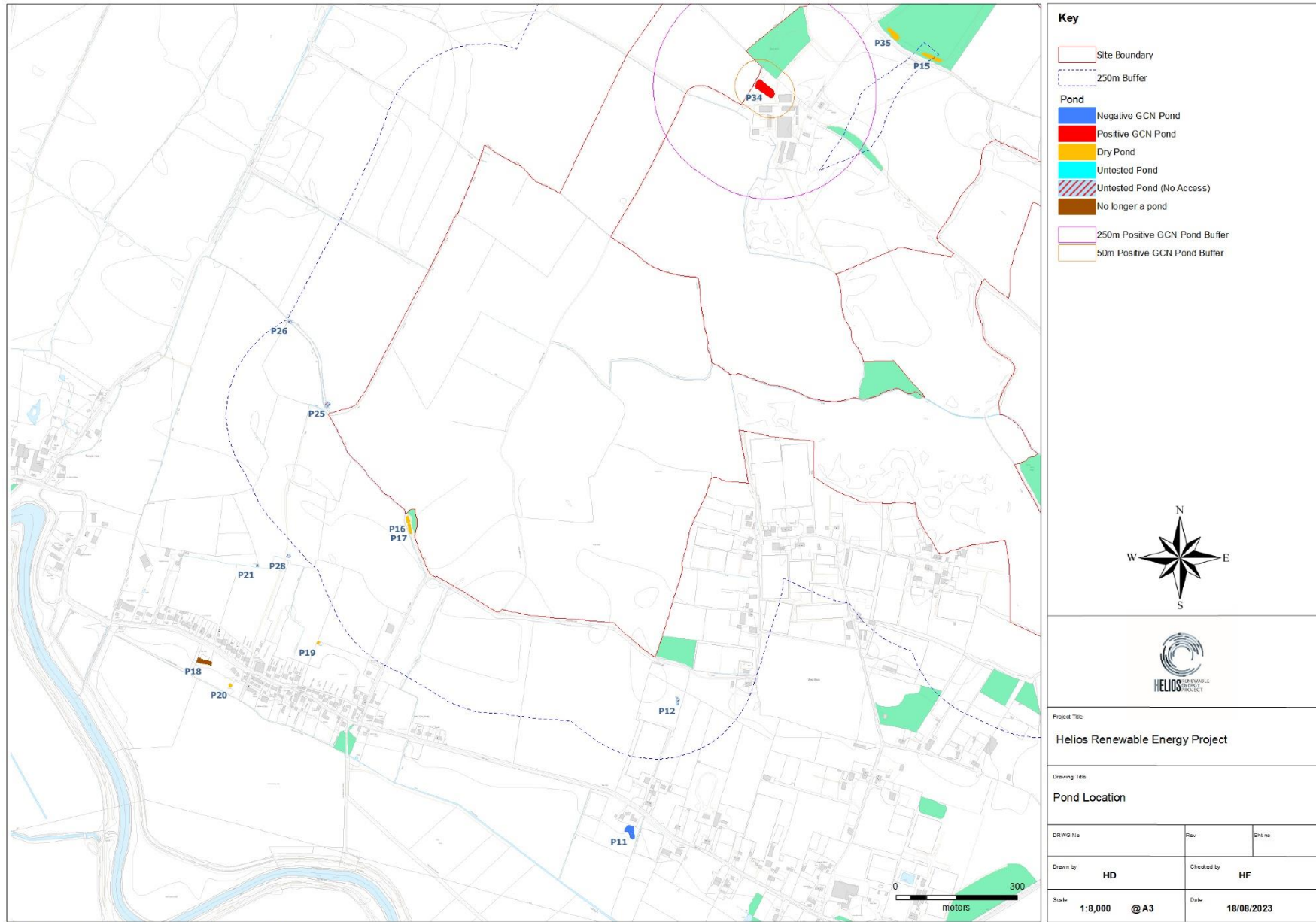
# Figure 1: Pond Location Plan - Overview



# Figure 2: Pond Location Plan – Map 1



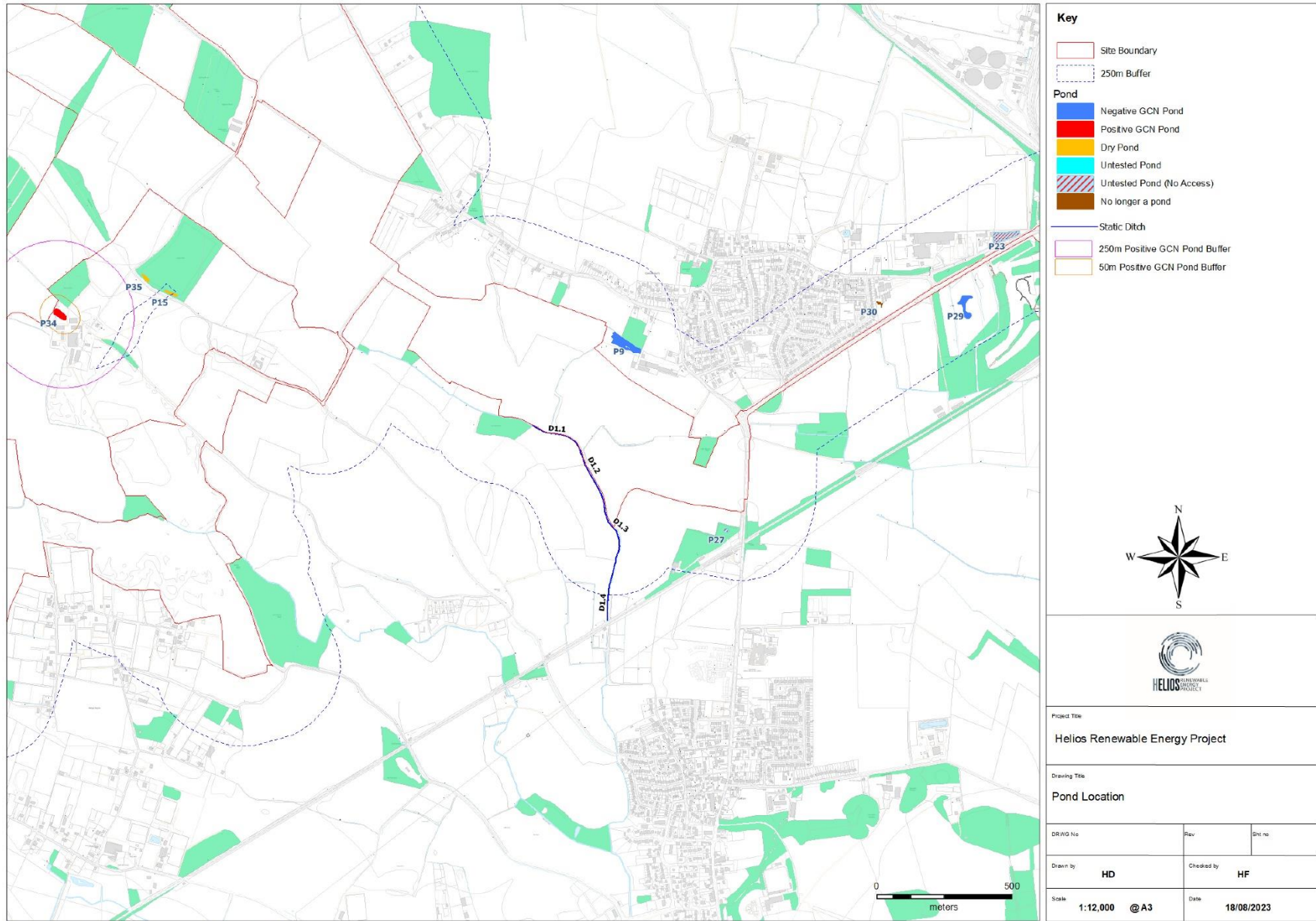
# Figure 3: Pond Location Plan – Map 2



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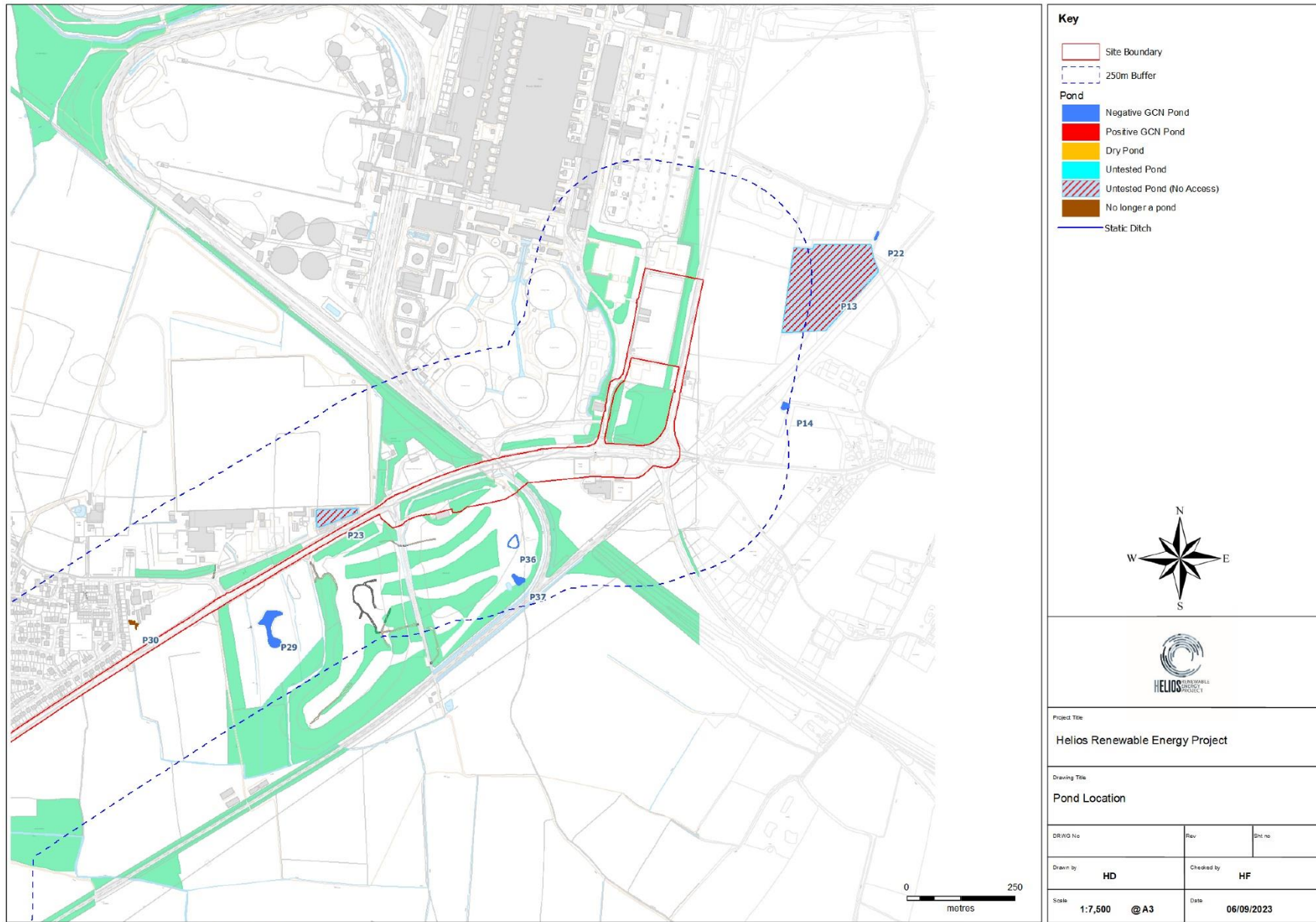


# Figure 4: Pond Location Plan – Map 3



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**Figure 5: Pond Location Plan – Map 4**



# Annex 1

## eDNA Laboratory Results



Folio No: E14959  
Report No: 1  
Purchase Order: AE-22-142  
Client: AVIAN ECOLOGY LTD  
Contact: Dan Foy

### 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:** 06/07/2022  
**Date Reported:** 20/07/2022  
**Matters Affecting Results:** None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
6568	P11, HELIOS (DRAX)		Pass	Pass	Pass	Negative	0
6578	P33, HELIOS (DRAX)		Pass	Pass	Pass	Negative	0
6579	P14, HELIOS (DRAX)		Pass	Pass	Pass	Negative	0
6583	P2, HELIOS (DRAX)		Pass	Pass	Pass	Negative	0
6584	P1, HELIOS (DRAX)		Pass	Pass	Pass	Negative	0
6585	P34, HELIOS (DRAX)		Pass	Pass	Pass	Positive	4
6586	P5, HELIOS (DRAX)		Pass	Pass	Pass	Negative	0



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Company Registration No. 08950940

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**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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## eDNA Laboratory Results



Folio No: E11100  
Report No: 1  
Purchase Order: AE-21-135  
Client: AVIAN ECOLOGY  
Contact: Dan Foy

### 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:** 21/06/2021  
**Date Reported:** 30/06/2021  
**Matters Affecting Results:** None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
4110	Drax D1 - Sec 4		Pass	Pass	Pass	Negative	0
4114	Drax Pond 2		Pass	Pass	Pass	Negative	0
4121	Drax D2 - Sec 4		Pass	Pass	Pass	Negative	0
4122	Drax Pond 24		Pass	Pass	Pass	Negative	0
4123	Drax D1 - Sec 1		Pass	Pass	Pass	Negative	0
5387	Drax Pond 9		Pass	Pass	Pass	Negative	0
5389	Drax D1 - Sec 3		Pass	Pass	Pass	Negative	0
5396	Drax D1 - Sec 2		Pass	Pass	Pass	Negative	0
6524	Drax Pond 1		Pass	Pass	Pass	Negative	0



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6527	Drax Pond 5		Pass	Pass	Pass	Negative	0
6528	Drax D2 Sec 1		Pass	Pass	Pass	Negative	0
6529	Drax D2 - Sec 11		Pass	Pass	Pass	Negative	0
6530	Drax D2 - Sec 3		Pass	Pass	Pass	Negative	0

If you have any questions regarding results, please contact us: [ForensicEcology@surescreen.com](mailto:ForensicEcology@surescreen.com)

**Reported by:** Chris Troth

**Approved by:** Chris Troth

#### **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.



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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.
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# Annex 1

## eDNA Laboratory Results



Folio No: E17585  
Report No: 1  
Purchase Order: AESS-23-013  
Client: AVIAN ECOLOGY LTD  
Contact: Dan Foy

### 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: 23/05/2023  
Date Reported: 30/05/2023  
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
R405	Drax - P29	-	Pass	Pass	Pass	Negative	0
R406	Drax - P22	-	Pass	Pass	Pass	Negative	0
R408	Drax - P36	-	Pass	Pass	Pass	Negative	0
R409	Drax - P4	-	Pass	Pass	Pass	Negative	0
R410	Drax - P9	-	Pass	Pass	Pass	Negative	0
R414	Drax - P6	-	Pass	Pass	Pass	Negative	0
R417	Drax - P37	-	Pass	Pass	Pass	Negative	0
R420	Drax - P24	-	Pass	Pass	Pass	Negative	0

If you have any questions regarding results, please contact us: [ForensicEcology@surescreen.com](mailto:ForensicEcology@surescreen.com)

Reported by: Chris Troth

Approved by: Chelsea Warner



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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.

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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.
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## Annex 2

### Pond Description and Photograph Panel

Photograph	Description
	<p><b>Photo 1: Pond 1 –</b> Large, heavily vegetated pond, featuring reed cover for approximately 60% of the pond’s surface area.</p> <p>Water clarity appeared to be clear, although the pond featured a muddy base. Observed to be drying at the time of survey.</p> <p>The pond further featured a small island at its centre, which included a feeder (the pond was noted to act as a potential duck decoy pond).</p> <p>Terrestrial vegetation comprised mainly grassland, in addition to woodland and scattered tree parcels.</p>
	<p><b>Photo 2: Pond 2 –</b> Large pond enclosed within woodland habitat, although lacking emergent vegetation.</p> <p>Water clarity appeared clear, although containing a high amount of organic matter. However, associated invertebrates appeared to be limited.</p> <p>Pond also featured a central island and a feeder, suggesting use as a duck decoy pond.</p> <p>Terrestrial habitat consisted of immediate woodland found in association with pond’s margins, with arable land found further out. Himalayan balsam was noted to be present.</p>
	<p><b>Photo 3: Pond 3 –</b> Relatively large pond, featuring wide margins populated by flag iris, but heavily shaded by bankside trees and scrub.</p> <p>Water clarity appeared clear, although containing a high amount of organic matter, with associated invertebrates also observed (e.g., mayfly larvae). Pond is confirmed to occasionally dry out, as confirmed by landowner.</p> <p>Again, pond featured a central island dominated by willow.</p> <p>Besides marginal trees and scrub, local terrestrial habitat is predominantly comprised of grassland, intermixed with a relative diversity of herbs.</p>
	<p><b>Photo 4: Pond 3a –</b> Small, artificial garden ponds, consisting of four adjacent sections, noted to feature a lined base and pump fish.</p> <p>Water clarity noted to be clear, although the pond’s surface was covered by duckweed and algae. However, relatively high invertebrate activity also observed.</p> <p>Surrounding terrestrial environment comprised of allotments and gardens, with areas of grassland found beyond.</p>


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### Pond Description and Photograph Panel

	<p><b>Photo 5: Pond 4 –</b> Narrow drying pond, enclosed within a small woodland parcel and featuring earth banks of varying steepness (30-60°). Some pondweed observed to be present within pond.</p> <p>Water clarity noted to be somewhat turbid, though to offer poor water quality, and likely low invertebrate diversity.</p> <p>Pond features vegetated banksides, with some areas of exposed earth; vegetation includes nettle, dock, willowherb, and bramble. Surrounding woodland includes horse chestnut, elder and ash.</p>
	<p><b>Photo 6: Pond 5 –</b> Drying pond found at woodland edge, featuring limited aquatic vegetation, although flag iris was noted to be present at pond margin, in addition to Himalayan balsam.</p> <p>Water clarity was noted to be relatively clear, although a high amount of organic matter was observable on pond bed.</p> <p>Associated terrestrial environment included woodland stands, shading the pond. Grassland featuring long swards are also present to the north, and arable land to the south.</p>
	<p><b>Photo 7: Pond 6 –</b> Small, drying pond enclosed within woodland parcel, featuring relatively gentle earth banks, and lacking emergent vegetation.</p> <p>Water quality noted to be bad, likely supporting limited invertebrate species.</p> <p>Banks noted to feature areas of exposed earth and dense vegetation, including bramble, fennel and bluebell. Surrounding woodland predominantly includes beech and sycamore. Immediate vicinity includes woodland habitats, in addition to an adjacent hedgerow bordering a road.</p>
	<p><b>Photo 8: Pond 9 -</b> Relatively large pond, featuring limited macrophyte cover and shading from bankside vegetation.</p> <p>Observations of water quality suggested the pond as being of moderate quality. Fish were thought to be absent, and the impact of wildfowl upon the pond's suitability considered to be minor.</p> <p>Terrestrial habitat found in immediate association was comprised woodland, enclosed by arable land and pasture, thought to be of relatively poor suitability for GCN.</p>

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### Pond Description and Photograph Panel

<p style="text-align: center;"><b>No Photograph Available</b></p>	<p><b>Pond 10:</b> Man-made, lined pond featuring a relative abundance of aquatic vegetation (e.g., bull rush, pond weed).</p> <p>Water clarity was noted to be relatively clear, exhibiting a moderate level of invertebrate activity.</p> <p>Associated terrestrial habitat includes horse-grazed pasture, comprised of various sward heights.</p>
	<p><b>Photo 9: Pond 11 –</b> Large, man-made garden pond, featuring ornamental koi carp, in addition to a relatively diverse assemblage of aquatic vegetation (e.g., lily sp., bistort, reed mace, large sedge etc.).</p> <p>Water clarity was noted to be relatively clear, exhibiting a moderate level of invertebrate activity (e.g., mayfly larvae, pond skater etc.).</p> <p>Associated terrestrial habitat comprised of grassland featuring a common assemblage of species, although notable refugia opportunities were noted in close proximity (e.g., wood and rubble piles).</p>
	<p><b>Photo 10: Pond 14 –</b> Deep, man-made pond reaching a maximum depth of 20ft. Relative abundance of aquatic vegetation observed (e.g., bullrush, lily sp., moss, and algae).</p> <p>Water clarity was noted to be relatively clear, exhibiting a moderate level of invertebrate activity (e.g., mayfly larvae, water boatman, brown hawker, emperor dragonfly, blue damselfly pond skater etc.).</p> <p>Surrounding terrestrial habitat comprised of a mosaic of grassland, scrub and woodland, with well-grazed horse pasture found within the wider area.</p> <p>Notably, smooth newt was observed at the pond, in addition to various refugia opportunities found in close association (e.g., log piles).</p>
	<p><b>Photo 11: Pond 22 –</b> Small pond located within and fully shaded by a line of trees, found adjacent to footpath. Relatively gentle earth bank, with pond base noted to be comprised of sediment, featuring heavy leaf litter.</p> <p>Water noted to be murky and noticeably stagnant, with no emergent vegetation observed.</p> <p>Banks observed to be mainly bare, although scattered bramble and overhanging hawthorn and oak present close to bank. Wider habitat includes horse and cow grazed pastures predominantly comprised of long sward grass.</p>




## Annex 2

### Pond Description and Photograph Panel

	<p><b>Photo 12: Pond 24 –</b>            Dry at time of habitat survey, although water levels vary by year, as confirmed by eDNA sampling in 2023.</p> <p>Relatively small pond when holding water, thought to dry annually; very limited aquatic vegetation observed when full.</p> <p>Water quality when surveyed was thought to be of poor quality, although no impact from either fish or waterfowl was evident.</p> <p>The pond was enclosed in an area of woodland, and heavily shaded. Wider habitat included arable land, although the pond was potentially isolated by the presence of a main road directly east.</p>
	<p><b>Photo 13: Pond 29 –</b>            Large pond located within centre of local golf course. Banks cut, 70-80° and partially banked by wooden borders in some areas.</p> <p>Water quality relatively clear, likely to support moderate invertebrate assemblages. Emergent vegetation observed, including bullrush, pond lily, flag iris, celery buttercup, watercress and pond weed.</p> <p>Surrounding habitat includes some scattered bankside trees, with the pond enclosed within managed, short sward grassland. Moorhen were noted to be nesting on the pond, with young present.</p>
	<p><b>Photo 14: Pond 32 –</b>            Pond located within horse-grazed pasture, in a state of drying out during the survey. No aquatic vegetation present, and heavily poached by horses.</p> <p>Water clarity observed to be turbid, with the pond base being heavily silted. Frequently disturbed by horses.</p> <p>The pond's associated terrestrial habitat is comprised of horse-grazed pasture, with hedgerows found to the west, and a arable fields found in close proximity.</p>
	<p><b>Photo 15: Pond 33 –</b>            Mainly dry pond, long narrow section of pond dry at the time of survey, with only a small section along the eastern portion still wet. Marginal vegetation including flag iris, reed and soft rush observed.</p> <p>Water clarity was noted to be relatively clear, although the pond's base was heavily silted, and featured high amounts of organic matter. Limited invertebrate activity observed.</p> <p>Associated terrestrial habitat includes both grassland, scrub and woodland.</p>



## Annex 2

### Pond Description and Photograph Panel

	<p><b>Photo 16: Pond 34 –</b> Pond apparently devoid of emergent vegetation; likely used as a duck decoy pond.</p> <p>Water clarity was noted to be relatively clear, although the pond’s base was heavily silted, and featured high amounts of organic matter. Invertebrate activity thought to be limited.</p> <p>Relative abundance of bankside vegetation present (e.g., bramble, willowherbs, wild angelica , red campion), in addition to marginal scrub and trees (e.g., willow, ash, oak, sycamore). Wider terrestrial habitat comprised of shorter sward grassland, woodland parcels and arable land.</p>
	<p><b>Photo 17: Pond 36 –</b> Irregular shaped pond, forming a thin ring of water around a central island, with relatively steep, vegetated banks.</p> <p>Water quality appeared clear, with a relatively high abundance of invertebrate species observed in association. Aquatic vegetation observed, including common reed, lily species, water crowsfoots.</p> <p>Terrestrial bankside vegetation includes forget-me-not, buttercup and common vetch, and a predominantly meadow grass and fescue grass sward of varying height. The pond is located within a wider area which includes mown golf course, with unmanaged, tall swards forming edge habitats in association with scattered scrub and woodland parcels.</p>
	<p><b>Photo 18: Pond 37 –</b> Large, deep and roughly square shaped pond, with earth banks of varying steepness.</p> <p>Noted to feature good water quality, and with indicative invertebrates observed in association (e.g., damselfly and dragonfly species). Aquatic vegetation present, which includes lily species and water crowsfoot.</p> <p>Bankside marginal vegetation includes celery leaved buttercup, and grass swards comprised of meadow grass and fescue species. The wider terrestrial habitat is shared with P36, although P37 is found directly adjacent to a woodland edge, located within a wider golf course complex.</p> <p>Mallard with young also noted to be present.</p>

## Annex 2

### Pond Description and Photograph Panel

	<p><b>Photo 19: Ditch 1 (Section 1)</b> Northern section of wet ditch, thought to consistently hold water. Featured vegetated banks of relatively steep gradient, with one margin bordered by a hedgerow which included scattered trees throughout.</p> <p>Ditch section was observed to contain a relatively high cover of macrophytes, and appeared to be of moderate water quality.</p> <p>Immediate ditch margins featured grass and tall ruderal vegetation, whilst the associated terrestrial habitat consisted of arable land and pasture.</p>
	<p><b>Photo 20: Ditch 1 (Section 2)</b> Extension of D1 extending south of D1.S1; identical in description, although featuring a reduced cover of macrophytes.</p>
	<p><b>Photo 21: Ditch 1 (Section 3)</b> Extension of D1, extending south of D1.S2; identical in description to D1.S2.</p>
	<p><b>Photo 22: Ditch 1 (Section 4)</b> Extension of D1, extending south of D1.S3; identical in description to D1.S1, featuring relatively greater cover of macrophytes than D1.S2 and D1.S3.</p>

## Annex 2

### Pond Description and Photograph Panel



**Photo 23: Ditch 2 (Section 1)**

Well maintained wet ditch, featuring steep banks generally vegetated by short swards of grass and scrub on north-west bank, with the south-east bank featuring higher amounts of bramble, scrub and trees.

Ditch section was observed to contain a relatively sparse coverage of macrophytes, and appeared to be of moderate water quality.

Associated terrestrial habitat consists of woodland to the south and arable land to the east and west.



**Photo 24: Ditch 2 (Section 2)**

Extension of D2 extending north of D2.S1; identical in description, although the immediate terrestrial habitat consists of arable land beyond immediate grass margins.



**Photo 25: Ditch 2 (Section 3)**

Extension of D2 extending north of D2.S2; identical in description, although the immediate terrestrial habitat consists of associated trees and scrub in some parts, with **P1** found in close proximity to the west.