Norwich Western Link

Environmental Statement Chapter 10: Biodiversity Appendix 10.16: Great Crested Newt Report 2021

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Contents

1 Introduction

1.1.1 WSP UK Ltd was commissioned by Norfolk County Council to complete GCN surveys, with the following objectives:

- Complete a Habitat Suitability Index (HSI) assessment of water bodies within and up to 500m from the Scheme boundary, which were not covered in the 2020 interim report, to assess their suitability as aquatic habitat for great crested newts.
- Complete a GCN eDNA survey to determine the presence or likely absence of this species from water bodies within and up to 500m from the Scheme boundary that were not subject to eDNA survey in 2020.
- Complete GCN population class size assessments (PCSAs) on ponds that had returned a positive eDNA result in 2020.
- Present the findings of the survey in a baseline report.

1.1.2 The survey findings will be used to inform the impact assessment and proposed mitigation for GCN and other amphibian species present across the Scheme. Details of the impact assessment and mitigation will be included within the Biodiversity Chapter of the Environmental Statement.

1.1.3 We have included a summary of key information shown in this document in an accessible format. However, some users may not be able to access all technical details. If you require this document in a more accessible format please contact norwichwesternlink@norfolk.gov.uk

Norfolk County Council

Norwich Western Link Road

Great Crested Newt 2021 Report

June 2022

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1 Introduction

1.1 Project background

1.1.1. The Norwich Western Link Road (NWL) is a highway scheme linking the A1270 Broadland Northway from its junction with the A1067 Fakenham Road to the A47 trunk road near Honingham.

1.1.2. The NWL, hereafter referred to as the Scheme, will comprise the following listed below.

- Dualling the A1067 Fakenham Road westwards from its existing junction with the A1270 to a new roundabout located approximately 400m to the north west.
- Construction of a new roundabout.
- Constructing a dual carriageway link from the new roundabout to a new junction with the A47 near Honingham.

1.1.3. As part of a separate planned scheme, National Highways proposes to realign and dual the A47 from the existing roundabout at Easton to join the existing dual carriageway section at North Tuddenham. If that scheme proceeds, it is expected that National Highways will construct the Honingham junction and the Norwich Western Link will connect to the north-eastern side of that junction.

1.1.4. The Scheme will cross the River Wensum and its flood plain by means of a viaduct. In addition, six other structures are proposed to cross minor roads and to provide habitat connectivity. The Scheme will include ancillary works such as provision for non-motorised users, necessary realignment of the local road network, including the stopping up of some minor roads, and the provision of environmental mitigation measures.

1.2 Ecological background

1.2.1. A Phase 1 Habitat Survey (WSP UK Ltd., 2020), undertaken in 2020, identified suitable aquatic and terrestrial habitat which could support Great Crested Newt *Triturus cristatus* (GCN). Habitats included numerous water bodies and terrestrial habitat such as tussocky grassland, woodland, scrub, wetland, field margins and other boundary features such as ditches and hedgerows. It was therefore recommended that a GCN environmental DNA (eDNA) survey be undertaken to establish a sufficient baseline to inform impact assessment.

1.2.2. In 2020 an interim GCN eDNA Survey Report (WSP UK Ltd., 2020) was produced. This report presented eDNA results from 24 ponds, two of which returned a positive result for great crested newt eDNA. Further survey work recommended for 2021 comprised further population size class assessment (PSCA) surveys on water bodies which returned positive results for GCN eDNA, as well as further eDNA surveys of water bodies which could not be surveyed in 2020 due to access or being dry, or where the result was classed as inconclusive/indeterminate.

1.3 Brief and objectives

1.3.1. WSP UK Ltd was commissioned by Norfolk County Council to complete GCN surveys, with the following objectives:

- Complete a Habitat Suitability Index (HSI) assessment of water bodies within and up to 500m from the Scheme boundary, which were not covered in the 2020 interim report, to assess their suitability as aquatic habitat for great crested newts.
- Complete a GCN eDNA survey to determine the presence or likely absence of this species from water bodies within and up to 500m from the Scheme boundary that were not subject to eDNA survey in 2020.
- Complete GCN population class size assessments (PCSAs) on ponds that had returned a positive eDNA result in 2020.
- Present the findings of the survey in a baseline report.

1.3.2. The survey findings will be used to inform the impact assessment and proposed mitigation for GCN and other amphibian species present across the Scheme. Details of the impact assessment and mitigation will be included within the Biodiversity Chapter of the Environmental Statement.

1.4 Study and survey area

1.4.1. An ecological Desk Study was completed in March 2020 to include recent data relevant to the Route. The Desk Study Area for this was defined as a 2km radius of the Scheme boundary (drawing 70061370-09-07-0001, see separate document Appendix A).

1.4.2. The Survey Area in relation to GCN comprised a 500m buffer from the Scheme boundary. All suitable water bodies, where access was permitted and no significant barriers to preventing GCN dispersal into the Scheme, identified as having potential to support GCN populations were surveyed. The Scheme and Survey Area are also shown on drawing 70061370-09-07-0001, see separate document Appendix A.

2 Relevant legislation

2.1 Legal compliance

2.1.1. GCN are afforded a high level of protection under the Conservation of Habitats and Species Regulations 2017 (the 'Habitats Regulations'), the legislation means that it is an offence to;

- Deliberately capture, injure or kill a wild great crested newt;
- Deliberately disturb wild great crested newts; '*disturbance of animals includes in particular any disturbance which is likely:*
  - *(a) to impair their ability -*
    - *(i) to survive, to breed or reproduce, or to rear or nurture their young; or*
    - *(ii) in the case of animals of a hibernating or migratory species, to hibernate or migrate; or*
  - *(b) to affect significantly the local distribution or abundance of the species to which they belong.'*
- Damage or destroy a breeding site or resting place used by this species.

2.1.2. Protection is also afforded under the Wildlife and Countryside Act 1981 (as amended) with respect to disturbance of animals when using places of shelter, and obstruction of access to places of shelter.

2.1.3. Due to the high level of protection afforded to GCN and their habitat, mitigation for this species is governed by a strict licensing procedure administered by Natural England (normally, planning permission must be obtained before a licence can be sought. However, works which do not require planning permission must still adhere to licensing requirements).

2.1.4. Licencing is subject to three tests, as defined under the Habitats Regulations, these must also be applied by a planning authority before granting permission for activities affecting GCN. For permission to be granted the following criteria must be satisfied;

- The proposal is necessary '*to preserve public health or public safety or other imperative reasons of overriding public interest including those of a social or economic nature and beneficial consequences of primary importance for the environment*';
- '*there is no satisfactory alternative'*; and
- The proposals '*will not be detrimental to the maintenance of the population of the species concerned at a favourable conservation status in their natural range'*.

2.1.5. GCN are also listed as a Species of Principal Importance (SPI) for the Conservation of Biodiversity in England in accordance with Section 41 of the Natural Environment and Rural Communities (NERC) Act 2006. Under Section 40 of the NERC Act (2006) public bodies (including local planning authorities) have a duty to have regard for the conservation of SPI when carrying out their functions, including determining planning applications.

3 Methods

3.1 Overview

3.1.1. A total of 23 water bodies were visited in 2021 following the limitations and recommendations highlighted in the 2020 interim report. Of these water bodies, 12 were subject to HSI and eDNA surveys, three were subject to population size class assessments (PSCA) and eight were found to be dry and could therefore not be subject to further survey.

3.1.2. The HSI and eDNA surveys took place on 15th April 2021, while PSCAs were undertaken over six visits between 17th March 2021 and 18th May 2021.

3.2 Habitat Suitability Index (HSI) assessment

3.2.1. All water bodies subject to eDNA surveys that required surveying following the limitations and recommendations highlighted in the 2020 interim report were assessed for their suitability to support great crested newts using the standard HSI assessment method (ARG UK, 2010) and (Oldham, Keeble, Swan, & Jeffcote, 2000).

3.2.2. Water bodies were assessed and scored on ten key variables which are known to influence breeding populations of great crested newts, in accordance with standard methods (ARG UK, 2010). These variables are;

- Geographic location;
- Water body area;
- Water body permanence;
- Water quality;
- Water body shading;
- Impact of waterfowl;
- Fish stocks;
- Number of water bodies within 1km;
- Terrestrial habitat around the water body; and
- Macrophyte cover of the water body.

3.2.3. Scores for each of the above variables were used to calculate an overall HSI value for each water body. This was then cross referenced with the guidelines (ARG UK, 2010) to assign the pond to one of five categories, poor, below average, average, good or excellent, as shown in Table 3-1. Index calculation is not a failsafe method of identifying whether a water body supports GCN or not; therefore, professional judgement and availability of records of GCN in the locality has also been used to inform the requirement for further survey.

Table 3-1 – Pond Suitability Categorisation Based upon HSI Score

HSI Score	Pond Suitability
<0.5	Poor
0.5 – 0.59	Below average
0.6 – 0.69	Average
0.7 – 0.79	Good
>0.8	Excellent

3.3 eDNA water sampling

3.3.1. All water bodies found to provide suitable habitat for GCN e.g. those ranging from poor to excellent suitability (see Table 3.1 above), to which access was possible, were subject to further survey to determine the presence or likely absence of this species. A small number of water bodies were excluded from the eDNA survey effort. Their exclusion was based on professional judgement and where the habitat was considered completely unsuitable for GCN due to the size, depth and nature of the water body (for example, a shallow pond with a complete lack of aquatic vegetation and a water depth of <1.5 inches which was considered, likely to fully dry within a matter of days following the survey which coincided with the peak breeding season). In total, eight ponds were excluded for this reason.

3.3.2. Sampling of eDNA was undertaken concurrently with the HSI survey. Professional judgement gained from previous experience and knowledge of GCN ecology, was exercised in selecting water bodies appropriate for sampling.

3.3.3. Research published in 2013 established a technique for reliably detecting newt eDNA in water bodies, and Natural England subsequently approved a protocol for this to become a survey method. The surveys were undertaken following survey techniques described in Biggs et al (Biggs, et al., 2014):

- A single visit to each target water body was made between mid-April and late-June, during the newt breeding season.
- Twenty sub-samples of water were taken from each water body using sterile sampling equipment provided by the laboratory (NatureMetrics).
- The locations of the 20 sub-samples were spaced as evenly as possible around the water body margin, and where possible targeted areas of vegetation which could be used as egg laying substrate and open water areas which newts could use for displaying.
- The sub-samples were mixed and pipetted into six sample tubes containing an alcohol and pH buffer solution.

- The samples were sent to NatureMetrics for laboratory testing using real time polymerase chain reaction (PCR) to amplify part of the cytochrome 1 gene found in mitochondrial DNA.
- The water samples from each water body were assigned a positive or negative result as well as a score between 0 and 12 representing the number of positive replicates from a series of 12.

3.3.4. A positive eDNA result concludes that GCN DNA is present in the water sample, whilst a negative result concludes that the presence of GCN is considered unlikely within that water body. Negative eDNA results cannot conclusively say that GCN are not present within the water body, rather that DNA from the species was not detected. GCN expel DNA into the ponds in which they live when they deposit; skin cells, faeces, mucus, sperm or eggs into the water. The DNA in this material can persist, and be detected, in the water for several weeks. A negative eDNA result for the purposes of this report provides a conclusion of 'likely absent'.

3.4 Population size class assessment

3.4.1. PSCA surveys were completed on water bodies where GCN presence were confirmed following laboratory analysis of eDNA samples. PSCA involved completing six survey visits to each waterbody, spread across the recommended survey period (Gent & Gibson, 2003) (English Nature, 2001) (mid-March to mid-June, with at least three of the visits falling between mid-April and mid-May). Survey visits were completed in suitable weather conditions, when overnight temperatures were above 5°C and wind and rain were not sufficient to affect the torch survey results (through disturbance to the water surface).

3.4.2. Surveys were carried out with reference to good practice guidance (English Nature, 2001) with two survey methods used during each survey visit to count relative abundance of GCN. The methods consisted of:

- Torch survey - the waterbody was searched systematically for amphibians after dark using a bright torch; all amphibians observed were recorded, with the number of male, female and juvenile newts of each species noted. The duration of the torchlight survey was determined by the time taken to walk slowly around the water body perimeter; and
- Bottle-trapping – each water body was trapped using bottle traps constructed and set in accordance with standard guidance (JNCC, 1998). Traps were set at a ratio of approximately one for every 2m of water body perimeter, where access allowed. The traps were set prior to dusk and checked and removed the following morning.

3.4.3. The resultant peak counts of GCN, (the maximum number of adult GCN counted at a waterbody on any one visit through either torch survey or bottle trapping) were then cross referenced with standard guidelines (English Nature, 2001) to establish the population size class. The population size class categories within the guidelines are reproduced below for information:

- Small – maximum peak adult counts of up to 10;

- Medium – maximum peak adult counts of between 11 and 100; and
- Large – maximum peak adult counts over 100.

3.5 Dates of survey and personnel

3.5.1. Lead surveyors were competent and experienced in conducting these surveys and each hold a Natural England survey licence for this species (licence numbers can be made available on request).

3.5.2. The dates for each survey visit are displayed in Table 3-2 below.

Table 3-2 – Survey Dates

Water Body Ref.	Date of HSI	Date of eDNA	Dates of Population Survey
1	15/04/2021	N/A	N/A
8	15/04/2021	15/04/2021	N/A
15	See 2020 Interim Report	See 2020 Interim Report	17/03/2021 23/03/2021 20/04/2021 28/04/2021 11/05/2021 18/05/2021
16	See 2020 Interim Report	See 2020 Interim Report	17/03/2021 23/03/2021 20/04/2021 28/04/2021 11/05/2021 18/05/2021
17	See 2020 Interim Report	See 2020 Interim Report	17/03/2021 23/03/2021 20/04/2021 28/04/2021 11/05/2021 18/05/2021
18	15/04/2021	N/A	N/A
20	15/04/2021	15/04/2021	N/A
21	15/04/2021	15/04/2021	N/A
22	15/04/2021	15/04/2021	N/A
23	15/04/2021	15/04/2021	N/A
33	15/04/2021	N/A	N/A

Water Body Ref.	Date of HSI	Date of eDNA	Dates of Population Survey
36	15/04/2021	N/A	N/A
37	15/04/2021	15/04/2021	N/A
38	15/04/2021	15/04/2021	N/A
39	15/04/2021	15/04/2021	N/A
41	15/04/2021	15/04/2021	N/A
42	15/04/2021	15/04/2021	N/A
43	15/04/2021	15/04/2021	N/A
44	15/04/2021	15/04/2021	N/A
45	15/04/2021	N/A	N/A
46	15/04/2021	N/A	N/A
47	15/04/2021	N/A	N/A
49	15/04/2021	N/A	N/A

3.6 Notes and limitations

3.6.1. Although water body 17 did not return a positive eDNA result in 2020, it was still subject to PSCA in 2021. This was due to water body 16, situated immediately adjacent to water body 17 with no significant barriers to dispersal, testing positive for GCN eDNA, indicating a high likelihood of GCN presence (despite the negative eDNA result). It was therefore considered appropriate to include this water body in the PSCA survey effort.

3.6.2. The 2020 GCN report (WSP UK Ltd., 2020) highlighted several dry or inaccessible water bodies or water bodies where eDNA sampling returned an inconclusive result. Where the requirement for these water bodies to be re-visited in 2020 was highlighted, this was completed as part of the 2021 survey effort and those water body results are included within this report. At the time of the 2021 surveys, a total of eight water bodies were found to be dry. Sampling took place within the recommended period (mid-April – late June), however as these water bodies were dry or unsuitable in both 2020 and 2021, combined with the fact that GCN were not confirmed present in any other surveyed water bodies within 500m of these water bodies, it is considered highly unlikely these dry water bodies support viable populations of GCN, even if they hold water from time to time.

3.6.3. Survey data is considered to be out of date after three years, however, after 18 months a suitably qualified ecologist will be required to undertake a site visit and assess the validity of the report (CIEEM, 2019). As such, if this requirement is met the results of these surveys can be considered valid until 2024, after which time an ecologist should be consulted as to the requirement for updated GCN surveys.

4 Results

4.1 Overview

4.1.1. In total, 20 water bodies were visited as part of the HSI assessment. Of these eight were dry while the other 12 attained results ranging from 'below average' to 'good'. These 12 water bodies were then subject to eDNA testing and all returned negative result indicating the likely absence of GCN.

4.1.2. Water bodies 15, 16 and 17 were subject to PSCA surveys. A single adult male GCN was captured during the bottle trapping survey on the fourth survey visit in water body 15. No other GCN were recorded. This constitutes a small population being present in water body 15.

4.2 Habitat Suitability Index (HSI) assessment

4.2.1. A summary of the HSI results and location information for the water bodies is included on drawing 70061370-09-23-0002, see separate document Appendix B. The HSI calculation is included in Table C-1 in Appendix C and the water body numbers correspond to those on drawings 70061370-09-23-0003, in separate document Appendix D and 70061370-23-09-0004 in separate document Appendix E. Photographs of each water body where eDNA surveys took place are included in Appendix F.

4.2.2. A total of 20 water bodies required an HSI survey following the limitations and recommendations highlighted in the 2020 interim report and each of these was visited as part of an HSI assessment. Of these water bodies, a total of eight were found to be dry (1, 18, 33, 36, 45, 46, 47 and 49) and therefore only 12 water bodies were able to be subject to HSI survey. The water bodies in each category is as follows;

- **Poor** – zero water bodies;
- **Below average** – one water body (20);
- **Average** – seven water bodies (37, 38, 39, 41, 42, 43 and 44);
- **Good** – four water bodies (8, 21, 22 and 23); and
- **Excellent** – zero water bodies.

4.3 eDNA water sampling

4.3.1. A summary of the eDNA results is provided alongside the HSI scores in Table 4-1 and shown on drawing 70061370-09-07-0003 in separate document Appendix D. Full laboratory results are available in separate document Appendix G.

4.3.2. Water sampling for eDNA analysis was undertaken immediately following the HSI assessment and of the 20 water bodies visited, 12 were able to be subject to eDNA sampling during the optimal period (mid-April – late-June). The remaining eight water bodies could not be sampled due to being dry or having very low water levels insufficient for sampling (assessed as dry in the eDNA result).

4.3.3. Of the 12 water bodies sampled, all returned a negative result indicating the likely absence of GCN in all these water bodies. Therefore, none of the water bodies subject to eDNA survey in 2021 required follow-up PSCA surveys.

Table 4-1 – Summary of HSI and eDNA Results

Water body Ref.	Grid Reference	Proximity to Scheme (m)	Connectivity to Scheme	HSI Score	HSI Category	eDNA Result
1	TG1037712012	377	Over 250m from Scheme	Dry	Dry	Not suitable for eDNA testing - dry
8	TG0997612905	12	Good	0.71	Good	Negative
18	TG1135814249	162	Isolated within arable field	Dry	Dry	Not suitable for eDNA testing - dry
20	TG1139514861	317	Over 250m from Scheme	0.58	Below Average	Negative
21	TG1176915218	356	Over 250m from Scheme	0.73	Good	Negative
22	TG1139215764	408	Over 250m from Scheme	0.77	Good	Negative
23	TG1163616380	150	Good	0.80	Good	Negative
33	TG1445716195	480	Over 250m from Scheme	Dry	Dry	Not suitable for eDNA testing - dry
36	TG1409715079	326	Over 250m from Scheme	Dry	Dry	Not suitable for eDNA testing - dry
37	TG1427615335	187	Good	0.66	Average	Negative
38	TG1436715263	212	Good	0.64	Average	Negative

Water body Ref.	Grid Reference	Proximity to Scheme (m)	Connectivity to Scheme	HSI Score	HSI Category	eDNA Result
39	TG1435315237	249	Good	0.66	Average	Negative
41	TG1453514973	425	Over 250m from Scheme	0.68	Average	Negative
42	TG1457014927	466	Over 250m from Scheme	0.66	Average	Negative
43	TG1458515014	412	Over 250m from Scheme	0.62	Average	Negative
44	TG1457014927	485	Over 250m from Scheme	0.65	Average	Negative
45	TG1481915393	0	Within Scheme	Dry	Dry	Not suitable for eDNA testing - dry
46	TG1481915393	0	Within Scheme	Dry	Dry	Not suitable for eDNA testing - dry
47	TG1481915393	0	Within Scheme	Dry	Dry	Not suitable for eDNA testing - dry
49	TG1147814649	87	Good	Dry	Dry	Not suitable for eDNA testing - dry

4.4 Population size class assessment

4.4.1. Water bodies 15, 16 and 17 were subject to the two survey methodologies as outlined in section 3.4. No GCN were identified during the torchlight surveys in any of the water bodies and no GCN were recorded during the bottle trapping surveys in either water body 16 or water body 17.

4.4.2. A peak count of one adult GCN was recorded in water body 15, indicating the presence of a 'small' population in this water body in accordance with standard guidelines (English Nature, 2001). This consisted of a single adult male caught in a bottle trap on the fourth survey visit only, with no other GCN recorded at this water body.

4.4.3. As no GCN were recorded in either water body 16 or water body 17 during any of the six survey visits, it is considered that the positive eDNA result for water body 16 returned in 2020 was a false positive and GCN are not present.

4.4.4. All surveys were completed under appropriate conditions, with overnight minimum temperatures ranging between 5ºC and 8ºC and pond conditions suitable for methods used to be effective. Full details of the surveys, including weather and pond conditions on each survey visit, are included in Appendix H.

5 References

5.1 Project references

WSP UK Ltd. (2020). *Phase 1 Habitat Survey*. Cambridge. Report Number: 70061370-09-08

WSP UK Ltd. (2020). *Great Crested Newt eDNA Survey Report.* Cambridge. Report Number: 70061370-09-07

5.2 Technical references

ARG UK. (2010). *ARG UK Advice Note 5. Great Crested Newt Habitat Suitability Indiex. UK Amphibian and Reptile Groups of the United Kingdom.*

Biggs, J., Ewald, N., Valentini, A., Gabouriaud, C., Griffiths, R., Foster, J., … Dunn, F. (2014) *Analytical and methodical development for improved surveillance of great crested newt. Appendix 5. Technical advice note for field and laboratory sampling of great crested newt (Triturus cristatus) environment DNA.* Oxford. Freshwater Habitats Trust.

CIEEM. (2019). Advice Note on the lifespan of Ecological Reports and Surveys. Winchester CIEEM.

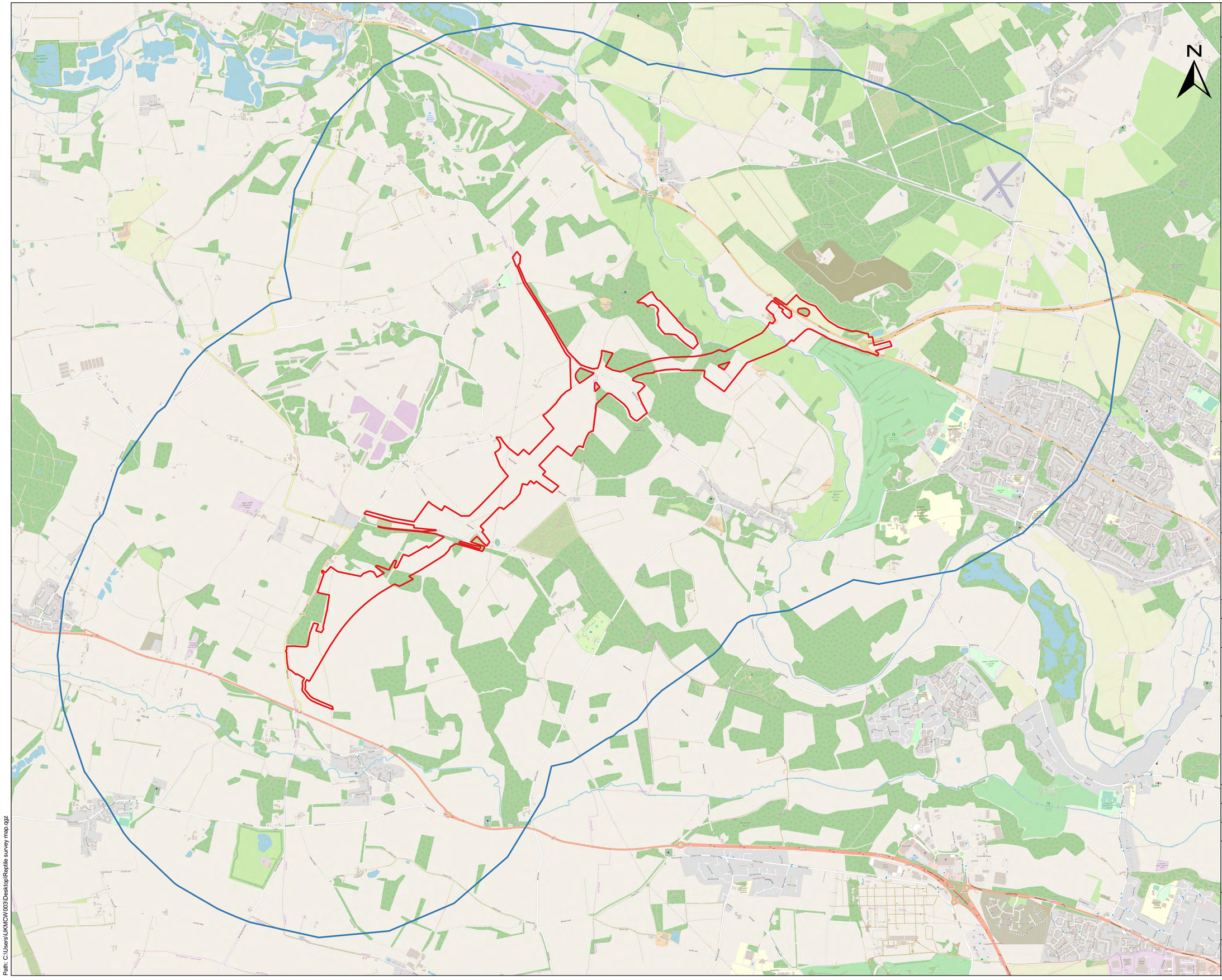
English Nature. (2001). *Great Crested Newt Mitigation Guidelines.* Peterborough: English Nature.

Gent, A., & Gibson, S. (2003). *Herpetodauna Workers Manual.* Peterborough. Joing Nature Conservation Committee.

Oldham, R., Keeble, J., Swan, M., & Jeffcote, M. (2000). Evaluating the suitability of habitat for the great crested newt. *Herpotological Journal*(10), 143-155.

Appendix A

Desk Study Results



THIS DRAWING MAY BE USED ONLY FOR THE PURPOSE INTENDED AND ONLY WRITTEN DIMENSIONS SHALL BE USED

Legend:

- 2km Study Area
- March 2020 Scheme Boundary

Revision Details	By / Check	Date	Suffix

Drawing Status: FINAL

Job Title: Norfolk County Council Norwich Western Link

Drawing Title: March 2020 Desk Study

Scale at A1: 1:15,000

Drawn	Stage 1 Check	Stage 2 Check	Originated	Date
UKMCW003	OP	AH	MW	07/10/2020

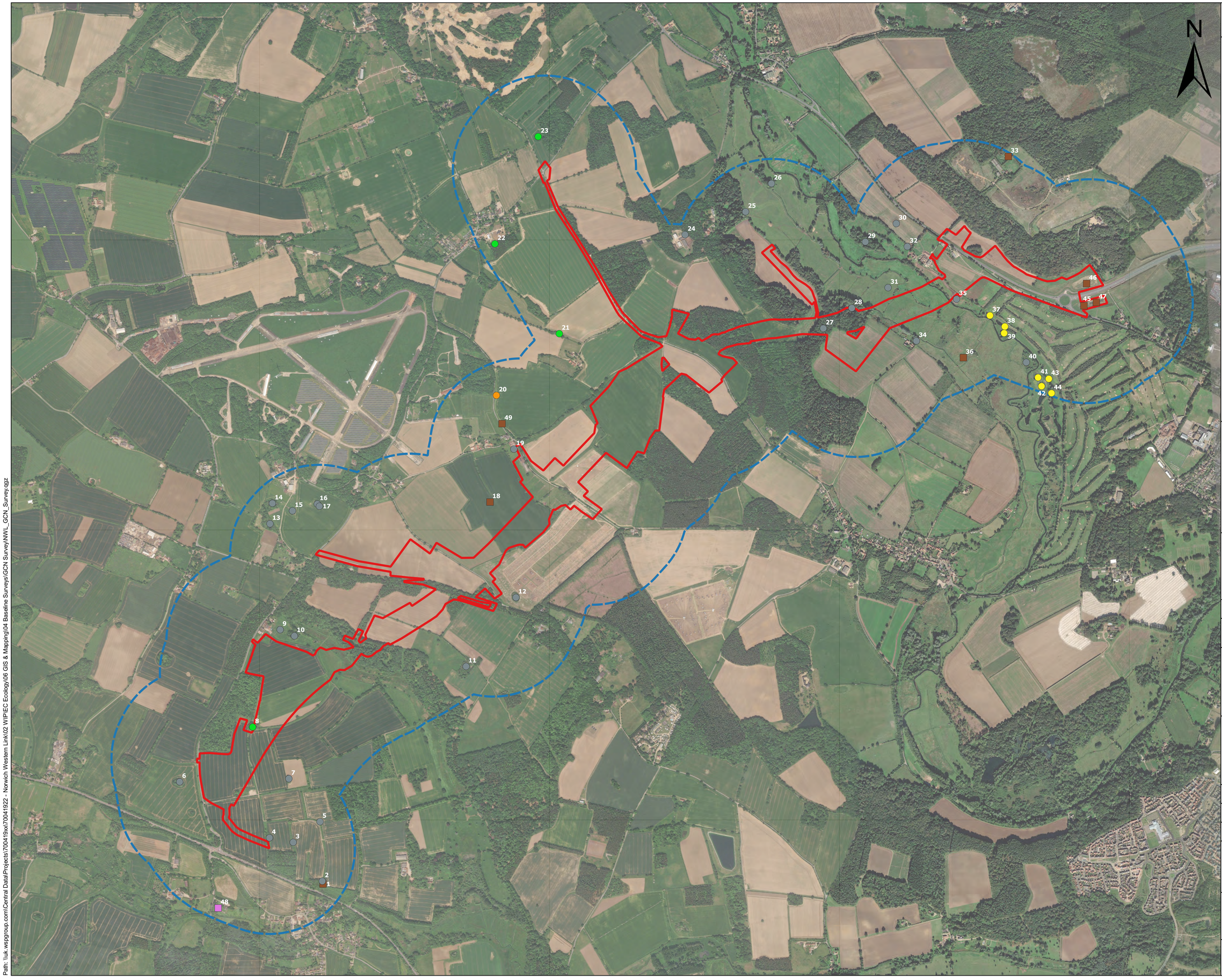
0 200 400 600 800 1,000 m

Drawing Number: 70061370-09-07-0001

Path: C:\Users\UKMCW003\Desktop\Reptile survey map.qgz

Appendix B

2021 HSI Results Map



Path: \\uk.wspgroup.com\Central Data\Projects\70041922 - Norwich Western Link\02 WIP\EC Ecology\06 GIS & Mapping\04 Baseline Surveys\GCN Survey\NWL_GCN_Survey.qgz

THIS DRAWING MAY BE USED ONLY FOR THE PURPOSE INTENDED AND ONLY WRITTEN DIMENSIONS SHALL BE USED

Legend

- Scheme Boundary
- Survey Area

HSI Result

- Good
- Average
- Below Average
- Surveyed 2020
- Not suitable for survey - Dry
- Surveys not required (A47 significant barrier to dispersal)

Revision Details	By / Check	Date	Suffix

Drawing Status: FINAL

Job Title: Norfolk County Council
Norwich Western Link

Drawing Title: 2021 Habitat Suitability Index (HSI) Assessment Results

Scale at A1: 1:10,000

Drawn: UKMCW003

Stage 1 Check	Stage 2 Check	Originated	Date
SM	IE	MW	05/10/2021

0 100 200 300 400 500 m

Drawing Number: 70061370-09-23-0002

Appendix C

HSI Calculations

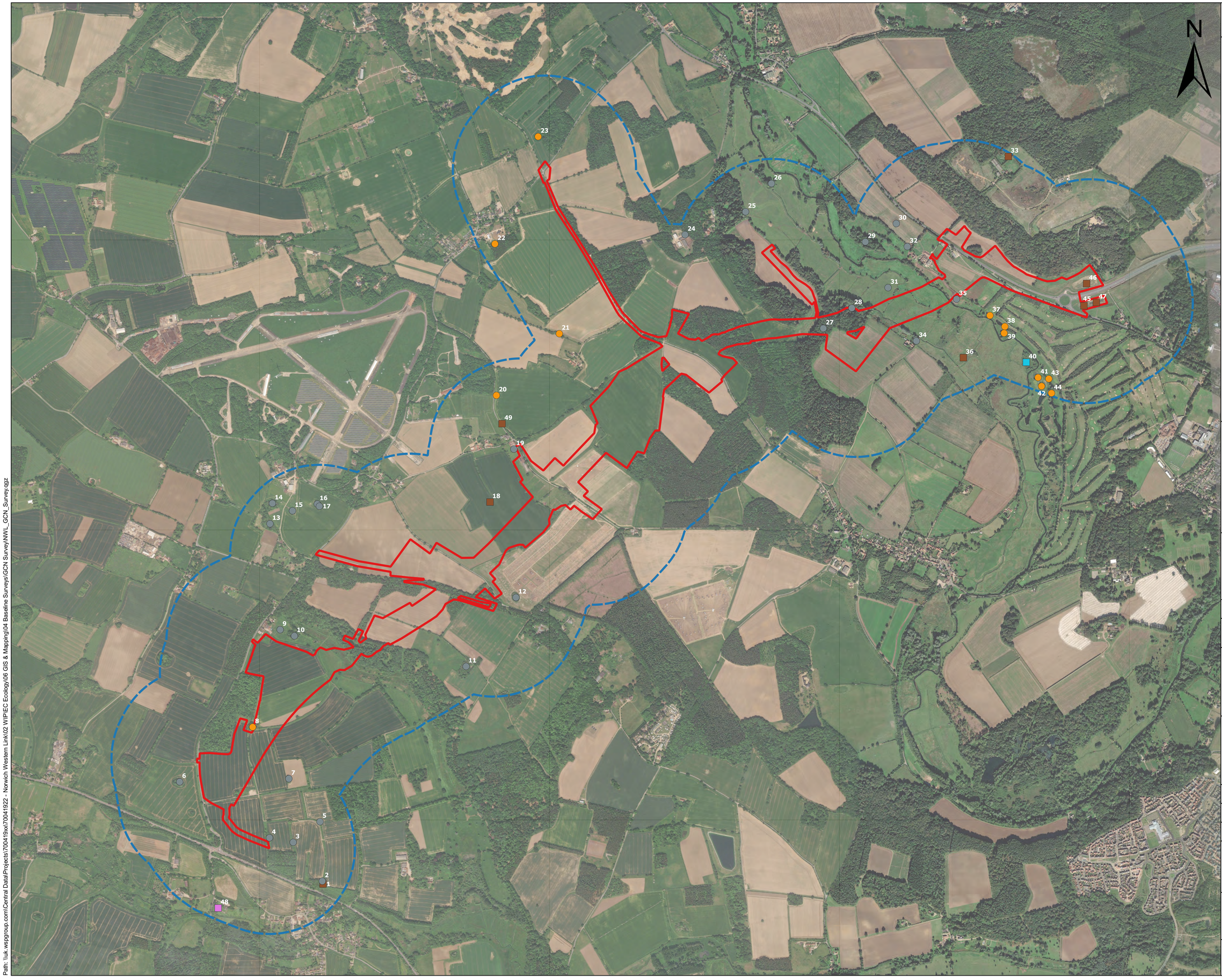
Table C-1 – HSI Calculations

Pond Reference	Grid Reference	Date of Visit	Geographic Location	Pond Area	Permanence	Water Quality	Shade	Fowl	Fish	Pond Count	Terrestrial	Macrophytes	HSI Score	HSI Category
1	TG1037712012	15/04/2021	Dry – HSI not undertaken	Dry – HSI not undertaken	Dry – HSI not undertaken	Dry – HSI not undertaken	Dry – HSI not undertaken	Dry – HSI not undertaken	Dry – HSI not undertaken	Dry – HSI not undertaken	Dry – HSI not undertaken	Dry – HSI not undertaken	N/A - Dry	N/A - Dry
8	TG0997612905	15/04/2021	1	0.91	0.50	0.33	0.40	1	1	1	1	0.51	0.71	Good
18	TG1135814249	20/04/2021	Dry – HSI not undertaken	Dry – HSI not undertaken	Dry – HSI not undertaken	Dry – HSI not undertaken	Dry – HSI not undertaken	Dry – HSI not undertaken	Dry – HSI not undertaken	Dry – HSI not undertaken	Dry – HSI not undertaken	Dry – HSI not undertaken	N/A - Dry	N/A - Dry
20	TG1139514861	15/04/2021	1	0.32	0.50	0.33	0.40	1	1	1	0.67	0.31	0.58	Below Average
21	TG1176915218	15/04/2021	1	0.32	0.50	0.67	1	1	1	1	0.67	0.61	0.73	Good
22	TG1139215764	15/04/2021	1	0.80	0.90	1	1	0.67	0.33	1	0.67	0.71	0.77	Good
23	TG1163616380	15/04/2021	1	1	0.90	0.67	1	0.67	0.33	0.95	1	0.81	0.80	Good
33	TG1445716195	15/04/2021	Dry – HSI not undertaken	Dry – HSI not undertaken	Dry – HSI not undertaken	Dry – HSI not undertaken	Dry – HSI not undertaken	Dry – HSI not undertaken	Dry – HSI not undertaken	Dry – HSI not undertaken	Dry – HSI not undertaken	Dry – HSI not undertaken	N/A - Dry	N/A - Dry
36	TG1409715079	20/04/2021	Dry – HSI not undertaken	Dry – HSI not undertaken	Dry – HSI not undertaken	Dry – HSI not undertaken	Dry – HSI not undertaken	Dry – HSI not undertaken	Dry – HSI not undertaken	Dry – HSI not undertaken	Dry – HSI not undertaken	Dry – HSI not undertaken	N/A - Dry	N/A - Dry
37	TG1427615335	15/04/2021	1	1	0.90	0.67	1	0.67	0.33	1	0.33	0.36	0.66	Average
38	TG1436715263	15/04/2021	1	0.74	0.90	0.67	1	0.67	0.33	1	0.33	0.36	0.64	Average
39	TG1435315237	15/04/2021	1	1	0.90	0.67	1	0.67	0.33	1	0.33	0.36	0.66	Average
41	TG1453514973	15/04/2021	1	0.92	0.90	0.67	1	0.67	0.33	1	0.33	0.51	0.68	Average
42	TG1457014927	15/04/2021	1	1	0.90	0.67	1	0.67	0.33	1	0.33	0.36	0.66	Average
43	TG1458515014	15/04/2021	1	0.47	0.90	0.67	1	0.67	0.33	1	0.33	0.41	0.62	Average
44	TG1457014927	15/04/2021	1	0.71	0.90	0.67	1	0.67	0.33	1	0.33	0.41	0.65	Average

Pond Reference	Grid Reference	Date of Visit	Geographic Location	Pond Area	Permanence	Water Quality	Shade	Fowl	Fish	Pond Count	Terrestrial	Macrophytes	HSI Score	HSI Category
45	TG1481915393	15/04/2021	Dry – HSI not undertaken	Dry – HSI not undertaken	Dry – HSI not undertaken	Dry – HSI not undertaken	Dry – HSI not undertaken	Dry – HSI not undertaken	Dry – HSI not undertaken	Dry – HSI not undertaken	Dry – HSI not undertaken	Dry – HSI not undertaken	N/A - Dry	N/A - Dry
46	TG1481915393	15/04/2021	Dry – HSI not undertaken	Dry – HSI not undertaken	Dry – HSI not undertaken	Dry – HSI not undertaken	Dry – HSI not undertaken	Dry – HSI not undertaken	Dry – HSI not undertaken	Dry – HSI not undertaken	Dry – HSI not undertaken	Dry – HSI not undertaken	N/A - Dry	N/A - Dry
47	TG1481915393	15/04/2021	Dry – HSI not undertaken	Dry – HSI not undertaken	Dry – HSI not undertaken	Dry – HSI not undertaken	Dry – HSI not undertaken	Dry – HSI not undertaken	Dry – HSI not undertaken	Dry – HSI not undertaken	Dry – HSI not undertaken	Dry – HSI not undertaken	N/A - Dry	N/A - Dry
49	TG1147814649	15/04/2021	1	0.18	0.10	0.01	0.30	1	1	1	0.33	0.31	0.30	Poor

Appendix D

2021 eDNA Results Map



THIS DRAWING MAY BE USED ONLY FOR THE PURPOSE INTENDED AND ONLY WRITTEN DIMENSIONS SHALL BE USED

Legend

- Scheme Boundary
- Survey Area

eDNA Result

- Negative
- Surveyed 2020
- Not suitable for eDNA testing - Dry
- Not suitable for eDNA testing - Inaccessible
- Surveys nor required (A47 significant barrier to dispersal)

Revision Details	By / Check	Date	Suffix

Drawing Status: FINAL

Job Title: Norfolk County Council
Norwich Western Link

Drawing Title: 2021 GCN eDNA Results

Scale at A1: 1:10,000

Drawn: UKMCW003

Stage 1 Check	Stage 2 Check	Originated	Date
SM	IE	MW	05/10/2021

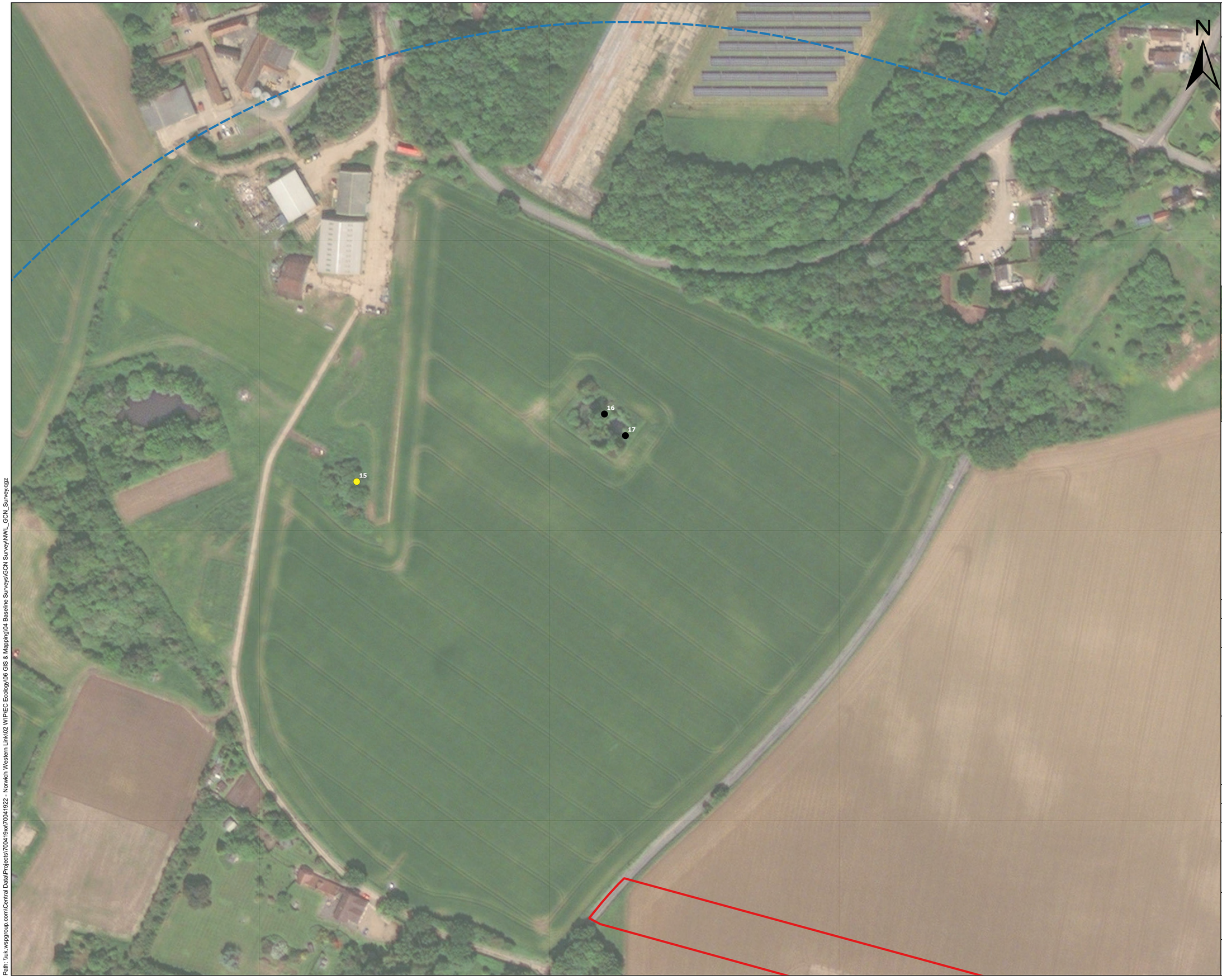
0 100 200 300 400 500 m

Drawing Number: 70061370-09-23-0003

Path: \\uk.wspgroup.com\Central Data\Projects\70041922 - Norwich Western Link\02 WIP\EC Ecology\06 GIS & Mapping\04 Baseline Surveys\GCN Survey\NWL_GCN_Survey.qgz

Appendix E

Population Size Class Assessment Results Map



THIS DRAWING MAY BE USED ONLY FOR THE PURPOSE INTENDED AND ONLY WRITTEN DIMENSIONS SHALL BE USED

Legend

- Scheme Boundary
- Survey Area

PSCA Result

- Small population
- No population

Revision Details	By / Check	Date	Suffix

Drawing Status: FINAL

Job Title: Norfolk County Council
Norwich Western Link

Drawing Title: 2021 Population Size Class Assessment Results

Scale at A1: 1:1,000

Drawn			
UKMCW003			
Stage 1 Check	Stage 2 Check	Originated	Date
SM	IE	MW	05/10/2021

0 10 20 30 40 50 m

Drawing Number: 70061370-09-23-0004

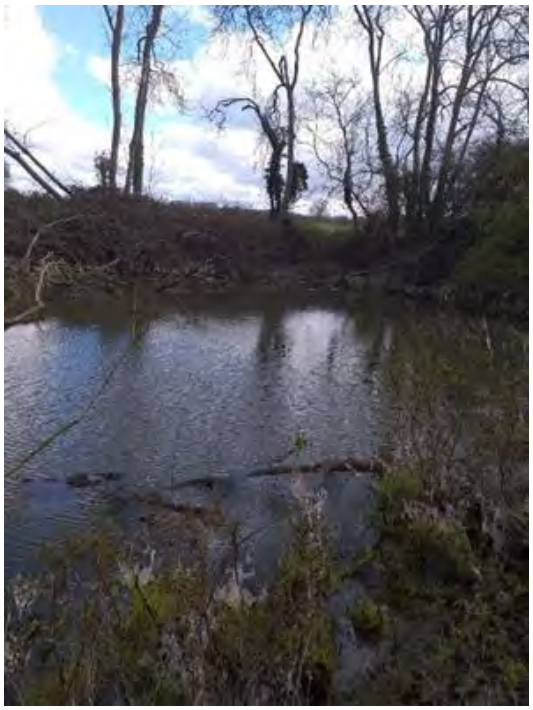
Path: \\uk.wspgroup.com\Central Data\Projects\70041922xx\70041922 - Norwich Western Link\02 WIP\EC Ecology\06 GIS & Mapping\04 Baseline Surveys\GCN Survey\NWL_GCN_Survey.qgz

Appendix F

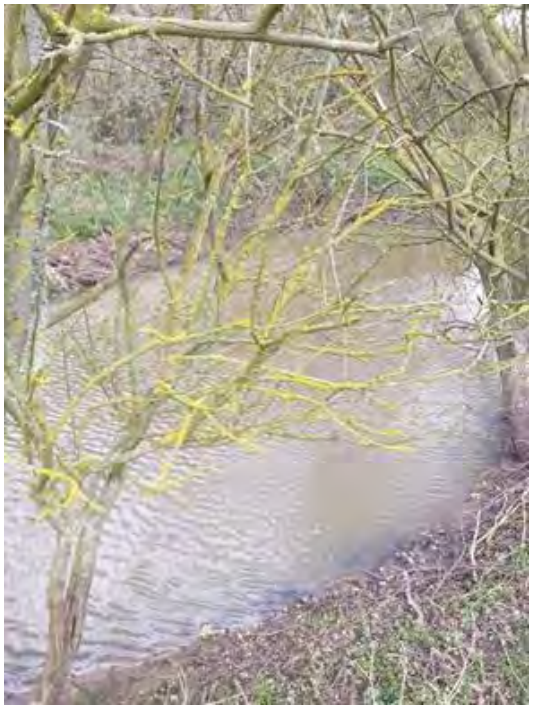
Photographs

Table F-1 – Water body Photographs

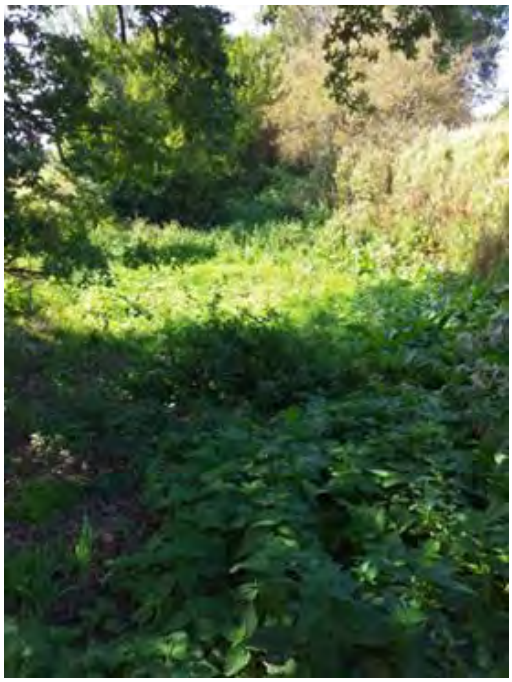
Water body Number: 8



Water body Number: 20



Water body Number: 21



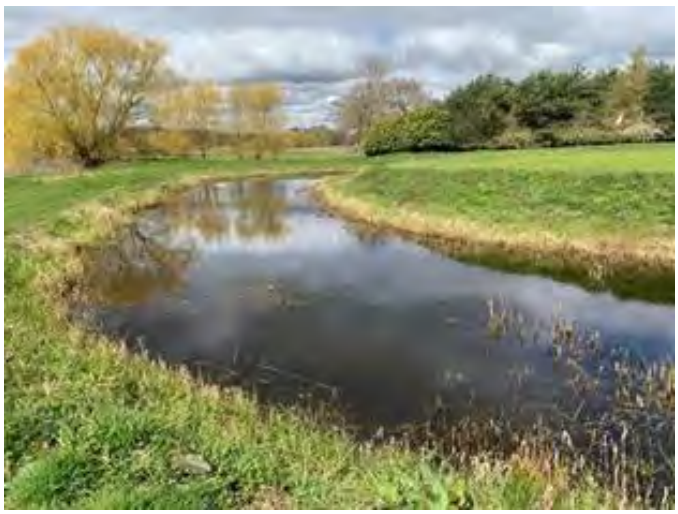
Water body Number: 22

No picture available

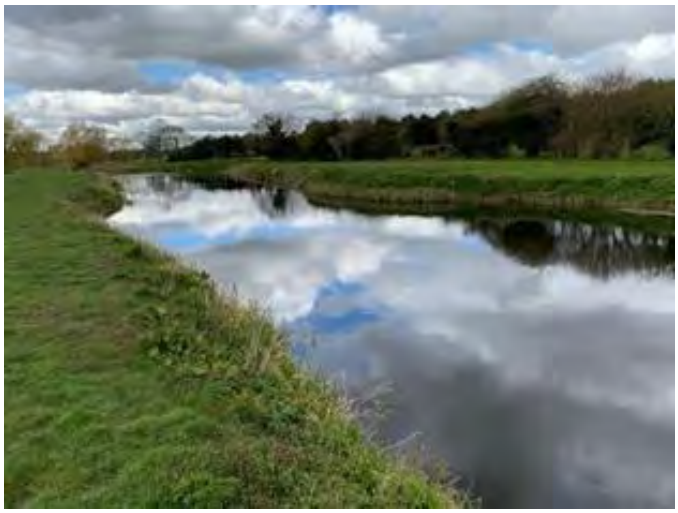
Water body Number: 23

No picture available

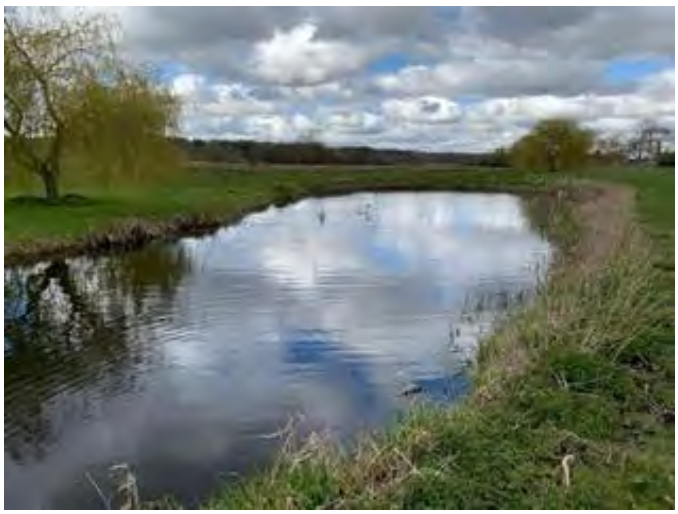
Water body Number: 37



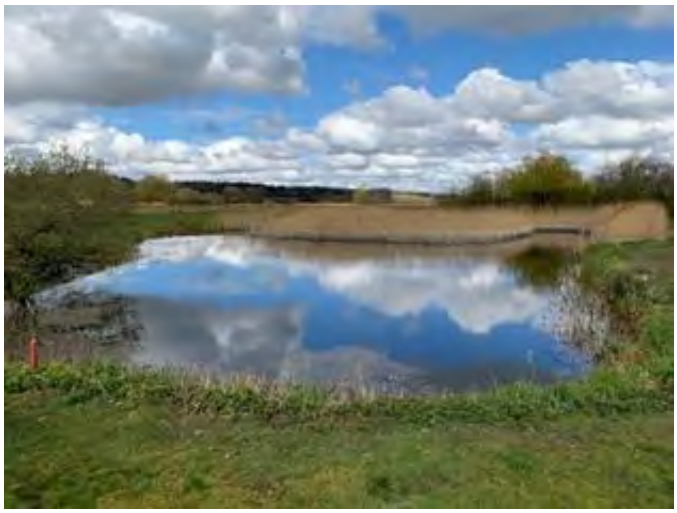
Water body Number: 38



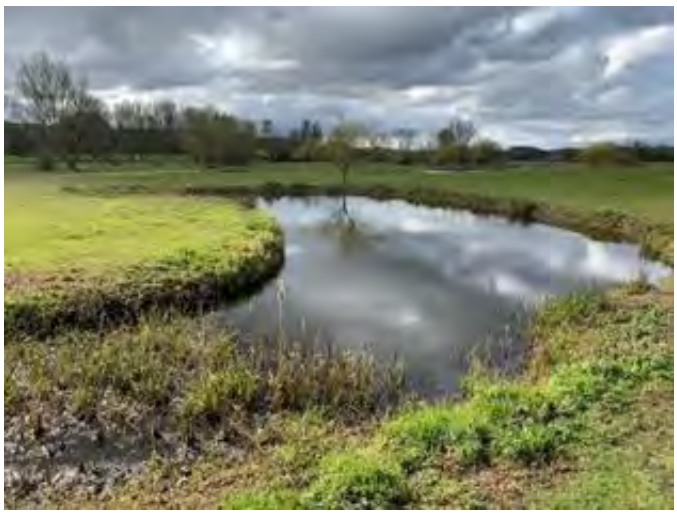
Water body Number: 39



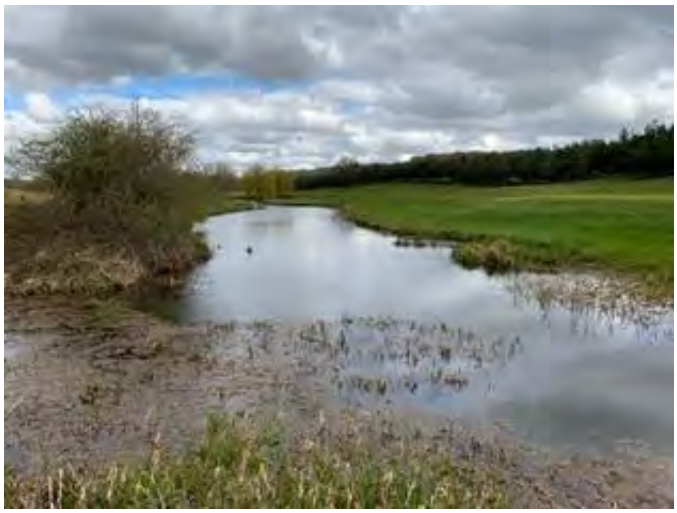
Water body Number: 41



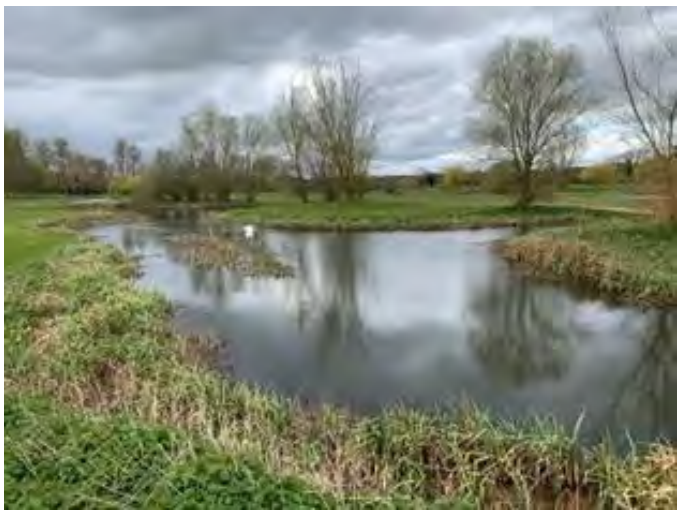
Water body Number: 42



Water body Number: 43



Water body Number: 44



Appendix G

Laboratory Results

GREAT CRESTED NEWT **DETECTION RESULTS**

Company:	WSP [redacted]
Order number:	101978
Project code:	NWL eDNA
Date of Report:	13 September 2021
Number of samples:	12

Thank you for sending your samples for analysis by NatureMetrics. Your samples have been processed in accordance with the protocol set out in Appendix 5 of Biggs et al. (2014).

Summary of the results

Results indicate GCN absence in '8', '20', '21', '22', '23', '37', '38', '39', '41', '42', '43' and '44'.

The negative controls were blank, the extraction blank control was negative, and the positive controls and their replicates were standard.

Results are based on the samples as supplied by the client to the laboratory. Incorrect sampling methodology may affect the results. Note that a negative result does not preclude the presence of Great Crested Newts at a level below the limits of detection.

Methods

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

The qPCR method follows the recommendations set out by NatureMetrics for Natural England in the qPCR validation project and helps improve the reliability of the interpretation of the data. Results from the assay are considered to have a high rating of confidence according to our Validation Scale (Harper et al. 2021).

The quality control methods exceed the requirements outlined in Biggs et al. (2014) Appendix 5. These consist of the use of kit blanks, additional extraction blanks and template negative controls, and positive controls standards of known concentration in triplicate to generate limits of detection and give confidence to the low and late amplifications.

Kit ID	Pond ID	Arrived	Inhibition	Degradation	Score	Status
501	'8'	20-Apr	No	No	0	Negative
508	'20'	20-Apr	No	No	0	Negative
499	'21'	20-Apr	No	No	0	Negative
483	'22'	18-May	No	No	0	Negative
509	'23'	20-Apr	No	No	0	Negative
507	'37'	20-Apr	No	No	0	Negative
504	'38'	20-Apr	No	No	0	Negative
510	'39'	20-Apr	No	No	0	Negative
506	'41'	20-Apr	No	No	0	Negative
505	'42'	20-Apr	No	No	0	Negative
502	'43'	20-Apr	No	No	0	Negative
503	'44'	20-Apr	No	No	0	Negative
501	'8'	20-Apr	No	No	0	Negative

END OF REPORT

Report issued by: **Thomas Shannon**

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

Understanding your results

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

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

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

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

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

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

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

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

Glossary

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

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

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

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

- positive Used to determine whether the assay is working correctly. In addition to the 4 standard dilutions prescribed by the Biggs et al. 2014 protocol, we include an additional standard dilution and amplify all standards in triplicate. We can use this increased number of replicates and standards to generate standard curves that will allow us to calculate the limit of detection (LOD).

- LOD Limit of detection. The lowest concentration of positive control DNA that amplifies. LOD is determined for every single reaction performed. Target amplification below the LOD cannot automatically be considered as negative but should be further investigated as spurious amplifications are more prevalent at these low concentrations.

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

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

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

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

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

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

References

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

Harper KJ, Tang CQ, Bruce K, Ross-Gillespie A, Ross-Gillespie V, and Egeter B 2021. A framework for assessing confidence in environmental DNA qPCR assays and results. Natural England Report.

Thalinger B, Deiner K, Harper LR, Rees HC, Blackman RC, Sint D, Traugott M, Goldberg CS, and Bruce K (2021). A validation scale to determine the readiness of environmental DNA assays for routine species monitoring. bioRxiv 2020.04.27.063990; doi: https://doi.org/10.1101/2020.04.27.063990

Appendix H

Population Size Class Assessment Results

Table H-1 – Pond 15 Survey Results

Date	GCN Detected	Peak Adult Count Using One Survey Method	Minimum Overnight Air Temperature (ºC)	Vegetation Cover (0-5)	Turbidity (0-5)	Other Amphibians Recorded	GCN Population Size Class
17/03/2021	No	0	6		2	No	N/A
23/03/2021	No	0	7	3	3	Yes – 1 x smooth newt	N/A
20/04/2021	No	0	6	2	2	Yes – 1 x smooth newt	N/A
28/04/2021	Yes	1	5	4	2	Yes – 3 x smooth newt	Small
11/05/2021	No	0	8	4	1	Yes – 2 x smooth newt	N/A
18/05/2021	No	0	7	2	4	No	N/A

Table H-2 – Pond 16 Survey Results

Date	GCN Detected	Peak Adult Count Using One Survey Method	Minimum Overnight Air Temperature (ºC)	Vegetation Cover (0-5)	Turbidity (0-5)	Other Amphibians Recorded	GCN Population Size Class
17/03/2021	No	0	6	1	2	Yes - 1 x common frog & spawn	N/A
23/03/2021	No	0	7	2	2	Yes – 4 x smooth newt	N/A
20/04/2021	No	0	6	2	3	Yes – 1 x smooth newt	N/A
28/04/2021	No	0	5	2	2	Yes – 11 x smooth newt	N/A
11/05/2021	No	0	8	3	1	Yes – 18 x smooth newt	N/A
18/05/2021	No	0	7	2	2	Yes – 4 x smooth newt	N/A

Table H-3 – Pond 17 Survey Results

Date	GCN Detected	Peak Adult Count Using One Survey Method	Minimum Overnight Air Temperature (ºC)	Vegetation Cover (0-5)	Turbidity (0-5)	Other Amphibians Recorded	GCN Population Size Class
17/03/2021	No	0	6	1	2	Yes – 9 x smooth newts	N/A
23/03/2021	No	0	7	2	2	Yes – 5 x smooth newts	N/A
20/04/2021	No	0	6	2	2	Yes – 2 x smooth newts	N/A
28/04/2021	No	0	5	2	2	Yes – 2 x smooth newts	N/A
11/05/2021	No	0	8	3	3	Yes – 2 x smooth newts	N/A
18/05/2021	No	0	7	2	2	Yes – 4 x smooth newts	N/A

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