Oxford Flood Alleviation Scheme

Great Crested Newt Survey Report

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5 February 2021

Environment Agency

Document history and status

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Oxford Flood Alleviation Scheme

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Contents

Executive Summary

This report presents the results of a great crested newt *Triturus cristatus* Habitat Suitability Index (HSI) survey for the Oxford Flood Alleviation Scheme undertaken in April 2020 and recommendations for further survey. The purpose of the survey was to update previous information about ponds located within 500m of the Scheme and their suitability for great crested newts (GCN).

Previous GCN surveys were undertaken in 2016 and 2017 which identified a low population of GCN to the west of the scheme. Since these surveys were conducted there have been design changes and the scheme boundary has been updated. This 2020 GCN survey was conducted to ensure the Scheme can be implemented without negative impacts on the local GCN population.

The desk-based assessment and survey identified 29 waterbodies within 500m of the Scheme boundary. Of these, two newly created ponds were discovered during the survey. An HSI was not carried out of these ponds. The remaining water bodies were briefly assessed to determine if they would be suitable for an HSI survey. This process discounted 15 ponds. HSI surveys were then carried out on 13 ponds.

The HSI surveys were undertaken on 20th and 21st April 2020, using standard HSI methodology (Oldham *et al.* 2000), by ecologists experienced in surveying for great crested newts.

The great crested newt is a European Protected Species, protected under the Wildlife and Countryside Act 1981 (as amended) and along with its habitat under the Conservation of Habitats and Species Regulations 2010 (as amended).

The HSI results can be summarised as:

- 6 ponds received an HSI score of <0.5 and a pond suitability of **'Poor'**;
- 4 ponds received an HSI score of between 0.5 and 0.59 and a pond suitability of **'Below Average'**; and
- 3 ponds received an HSI score of between 0.6 – 0.69 and a pond suitability of '**Average**'.

Based on the results of the HSI survey along with other factors such as location and previous survey results, it was recommended for three ponds to be surveyed for presence/absence of GCN by eDNA analysis during the 2020 season. These ponds were found to be dry before eDNA analysis was conducted and the survey could not be undertaken.

GCN cannot be confirmed absent from the Scheme area so it is concluded that these ponds, along with the two newly created ponds discovered during the surveys, undergo eDNA analysis during the 2021 survey season from April to June to ensure they are not utilised by breeding GCN. Until those surveys are complete it should be assumed that GCN are present within these three ponds.

1. Introduction

1.1 Background

Jacobs was commissioned by the Environment Agency to undertake an updated Habitat Suitability Index (HSI) survey and presence/absence survey of great crested newt (GCN) *Triturus cristatus*, to identify potential constraints to the proposed alignments of the Oxford Flood Alleviation Scheme (FAS). The Oxford FAS is critical in reducing the long-term risk of flooding to residential and commercial properties in the floodplains. The principal component of the FAS is a new channel to the west of the city centre, accompanied by modifications to the Seacourt, Hinksey and Bulstake streams, designed to reduce water levels in the river during flood events without increasing levels further downstream. GCN surveys were carried out in 2016 and 2017 which now require updating.

The need for GCN surveys was recommended following an updated Ecological Walkover survey (Jacobs, 2020) which identified suitable GCN habitat and the need for targeted surveys to inform an Environmental Impact Assessment for the Scheme.

The aim of this survey is to provide updated information for all ponds within 500m of the updated Scheme boundary and their suitability for breeding GCN and to confirm presence/absence of GCN in any suitable ponds identified through eDNA analysis.

The main objectives of this report are to:

- Present the results of the GCN HSI assessments of ponds within 500m of the Scheme boundary;
- Plan for eDNA presence/absence surveys of any suitable ponds identified through the HSI assessment;
- Provide an assessment of the potential impacts of the Proposed Scheme on the local GCN population; and
- Recommend appropriate mitigation measures and opportunities for enhancement.

1.2 Report Structure

The report is structured as follows:

- Section 2 – *Methodology.* This section summarises the methodology used for undertaking the desk study and field surveys. In addition, it describes the basis for the evaluation of ecological features;
- Section 3 – *Legislation.* This section sets out the relevant legislation which underpins the recommendations set out in section 5.
- Section 4 – *Baseline Conditions.* This section describes the findings and context of the site with respect to GCN, including any other noted features; and
- Section 5 – *Evaluation and Recommendations.* This section sets out the conclusions and recommendations in relation to GCN and recommendations for further survey in relation to relevant legislation and nature conservation strategies.

2. Methodology

2.1 Desk Study

Records of protected species, including great crested newts, were provided by the Thames Valley Environmental Records Centre (TVERC) in 2020 and the Oxfordshire Amphibian and Reptile Group (OxARG) as part of the Ecological Appraisal (CH2M, 2015[a] & 2016[b]).

These records, along with Multi-Agency Geographic Information for the Countryside website (MAGIC) and Ordnance survey mapping, were consulted to locate waterbodies within 500 metres (m) of the proposed scheme.

Identifying past great crested newt records and understanding nature conservation issues within the wider area helps in the assessment of the ecological value of a site and the habitats and the species that a site supports.

2.2 Habitat Suitability Assessment (HSI)

The desk-based assessment and survey identified 29 waterbodies within 500m of the Scheme boundary, which were given a reference number (i.e. Waterbody 1). Of these, two newly created ponds were discovered during the survey. An HSI was not carried out of these ponds as they were clearly unsuitable for the presence of GCNs. The remaining water bodies were then assessed to determine if they would be suitable for an HSI survey. This process discounted a further 15 ponds (1-6, 13, 15, 16, 18, 19, 20, 25, 26 and 27) based on the following criteria:

- Any major barriers to dispersion, such as main roads or rivers, between the waterbody and the Scheme boundary;
- Regular flooding of the waterbody; and
- The waterbody being clearly unsuitable for the presence of great crested newts, for example not being present, or forming part of a flowing waterbody.

The location of waterbodies within 500m of the Scheme boundary is shown in Figure 1 (sheets 1-8).

The survey was undertaken on 20th and 21st April 2020, using standard HSI methodology (Oldham *et al*. 2000), by ecologists experienced in surveying for great crested newts.

The HSI survey comprised an assessment of the features of the waterbodies and the adjacent landscape to enable an evaluation to be made on waterbody habitat quality for breeding great crested newts. Standard methodology incorporates ten Suitability Indices (SI) comprising factors that influence great crested newt habitat, namely:

- SI^1 Location;
- SI^2 Pond area;
- SI^3 Pond drying;
- SI^4 Water quality;
- SI^5 Shade;
- SI^6 Fowl;
- SI^7 Fish;
- SI^8 Ponds (i.e. number of ponds within 1 km of survey pond);

- SI[9] Terrestrial habitat; and
- SI[10] Macrophytes (i.e. percentage of pond surface occupied by macrophyte [aquatic plant] cover).

A value for each of these indices is calculated between '0.01' and '1.0'. Unsuitable habitat is indicated with '0.01' and optimal habitat represented by '1.0'. The geometric mean of the index values is subsequently calculated to provide an individual pond HSI score between 0 and 1 as presented in Table 1

Table 1 - Categorisation of HSI Scores

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

The HSI guidance notes that a high scoring pond is more likely to support great crested newts than those with a low score, but it also states that *"the* [HSI scoring] *system is not sufficiently precise to allow the conclusion that any particular pond with a high score will support newts, or that any pond with a low score will not do so".*

Although the HSI provides useful guidance when assessing the suitability of ponds for great crested newts, it is not a substitute for undertaking a detailed presence/absence survey during the optimal survey months.

2.3 Environmental (e)DNA Survey

Following the results of the HSI survey and based on other factors such as location and previous results of GCN surveys for the Scheme (2016 and 2017), ponds considered likely to support breeding populations of GCN were recommended eDNA presence/absence GCN survey. Generally, ponds with a score of Below Average or above were recommended for eDNA analysis.

eDNA is DNA found within an environment, for example a pond or a lake, that has been released from an organism in the form of faeces, saliva, urine, skins cells or carcasses. In aquatic environments, the eDNA is diluted and distributed in the water where it persists for 7 to 21 days, depending on environmental conditions (Briggs *et al.* 2014).

eDNA sampling to confirm the presence or absence of GCN in ponds is a method that has been accepted by Natural England (NE). The procedure involves taking a minimum of 20 water samples from a single waterbody, then combining these samples in a vessel to allow six composite samples per waterbody to be taken for laboratory analysis. Full methodology for the eDNA survey can be found in Appendix A.

2.4 Survey Limitations

Three ponds (10, 11 and 12) within Network Rail land were HSI surveyed from adjacent land which was publicly accessible as there was no access to these ponds for survey.

The results of an HSI can only be based on the findings at the time the survey was undertaken. Weather conditions before the survey (i.e. heavy rain) were taken into consideration whilst carrying out the waterbody assessment.

All waterbodies recommended for eDNA survey following the results of the HSI were found to be dry before the eDNA survey could be conducted. Therefore, no water samples could be taken from the ponds and the eDNA surveys were cancelled for the 2020 season, instead being planned to be conducted prior to vegetation clearance for construction.

3. Legislation

3.1 Legislative Framework

Great crested newts and their habitats in water and on land are protected under the Wildlife and Countryside Act 1981 (as amended) and under the Conservation of Habitats and Species Regulations 2017 (as amended). Taken together, these make it an offence to:

- Deliberately capture, injure or kill a great crested newt;
- Deliberately disturb any great crested newt, in particular any disturbance which is likely to: (i) impair their ability to survive, breed, reproduce or to rear or nurture their young; or in the case of hibernating or migratory species, to hibernate or migrate; or (ii) to affect significantly the local distribution or abundance of the species to which they belong.
- To be in possession or control of any live or dead great crested newt or any part of, or anything derived from a great crested newt;
- Damage or destroy a breeding site or resting place of a great crested newt;
- Intentionally or recklessly obstruct access to any place that a great crested newt uses for shelter or protection;
- Intentionally or recklessly disturb a great crested newt while it is occupying a structure or place that it uses for shelter or protection.

The legislation covers all newt life stages such that eggs, tadpoles and adult newts are all equally protected. Actions that are prohibited can be made lawful by a licence issued by the appropriate Statutory Nature Conservation Organisation. The GCN is a Priority Species under the UK Biodiversity Action Plan and has been adopted as a Species of Principal Importance in England under section 41 of the NERC Act 2006.

4. Baseline Conditions

4.1 Context

Desk study information received by TVERC and OxARG included numerous records for amphibians including great crested newt in the wider Oxford area. However, there were no records of great crested within the Scheme boundary. The nearest records are within 225m to the west of the scheme boundary in Hinksey Heights. Previous surveys for the Scheme (CH2M 2016[d] & 2017) confirmed the presence of a low population of GCN in this area. This population is located to the west of the scheme, on the opposite side of the A34, which is currently considered a barrier to dispersal and this population was not considered further in the assessment. Other records were to the very east of the Scheme, on the other side of the River Thames which is a major barrier to dispersal.

Desk-based assessment discounted 15 ponds from HSI. The justification for the discounted pond is shown in Table 2.

Table 2 – Discounted pond justification

Waterbody Reference Number	Justification during desk-based assessment
1-4	Ponds connected to flowing watercourse and subject to frequent flooding
5	Road barrier to dispersal
6	Drained man-made pond and no suitable surrounding habitat
13	Isolated pond with no suitable surrounding habitat
15	Chlorinated outdoor pool
16	Disused swimming pool now drained
18-20	Road barrier to dispersal
25-27	Large watercourse as barrier to dispersal

4.2 HSI Survey Results

Figures showing waterbody locations and Habitat Suitability Index Results are shown in Figure 1. Great crested newt ecology is discussed in Appendix B. The HSI results are shown in Table 3.

Table 3 - HSI Survey Results

Waterbody Reference Number	HSI Score	Pond Suitability
7	0.61	Average
8	0.55	Below Average
9	0.47	Poor
10	0.60	Average
11	0.60	Average
12	0.53	Below Average
14	0.38	Poor
17	0.59	Below Average
21a	0.48	Poor
21b	0.46	Poor
22	0.41	Poor

23	0.55	Below Average
24	0.37	Poor

The HSI survey results can be summarised as follows:

- Ponds 9, 14, 21a, 21b, 22 and 23 received an HSI score of <0.5 and a pond suitability of '**Poor**';
- Ponds 8, 12, 17 and 23 received an HSI score of between 0.5 – 0.59 and a pond suitability of '**Below Average'**;
- Ponds 7, 10 and 11 received an HSI score of between 0.6 – 0.69 and a pond suitability of '**Average**'.

During the survey, two newly dug ponds were discovered in North Hinksey Golf course. These were given reference numbers 28 and 29 and are located at NGR SP 50336 04417 and SP 50096 04617 respectively. These ponds were visited but not given HSI assessment as they are recently created, unestablished ponds that were unsuitable for GCN. These ponds are not included in Table 2 above. Their location is noted in Figure 1.

4.3 eDNA Surveys

Ponds 9, 14, 21a, 21b, 22 and 23 had a pond suitability of '**Poor**' so were scoped out from further survey.

Ponds 12 and 23 had a pond suitability of '**Below Average'**. Ponds with a below average suitability would generally be considered for eDNA analysis but these two ponds were scoped out from further survey due to their overall conditions. Pond 12 is a small pond in Network Rail land which was not accessible for HSI survey but could be viewed from a distance, with no submerged and marginal vegetation and was heavily shaded by willow. This pond is bordered by Bulstake Stream to the north and the railway and River Thames to the east which act as barriers to dispersal from known populations of GCN, of which the closest known population is 1.4 km. Pond 23 is one of three landscaped ponds in the grounds of Oxford University, which is surrounded by buildings and amenity grassland. The water quality appeared poor and the terrestrial habitat was generally unsuitable for GCN. Again, this pond is bordered by dual carriageways to the north and west, and the railway and Hinksey Stream to the east which act as barriers to dispersal from known populations of GCN, of which the closest known population is 1.5 km. Both ponds were negative for GCN eDNA in 2016 (CH2M, 2016[d]) and not considered for eDNA analysis in 2020 due to their isolation from GCN colonisation by local populations.

Ponds 10 and 11 had a pond suitability of '**Average.'** Ponds with an average suitability for GCN would generally be given eDNA analysis but these ponds were scoped out from further survey as they have little submerged or marginal vegetation and are heavily shaded by willow. In the same location as pond 12 above, these ponds are bordered by Bulstake Stream to the north and the railway and River Thames to the east which act as barriers to dispersal from known populations of GCN which are located on the opposite sides of both the River Thames and A34 dual carriageway. These ponds received a negative eDNA result in 2016.

Based upon the results of the HSI survey along with other factors such as location and previous survey information, **three** ponds were identified as requiring presence/absence surveys for great crested newts during the 2020 season as they obtained HSI scores in the 'Below Average' category or better. These ponds were 7, 8 and 17. However, these three ponds recommended for eDNA analysis were found to have dried before the eDNA survey could be conducted. Water samples could not be taken from these ponds and the eDNA survey was cancelled for the 2020 Season.

5. Evaluations and Recommendations

5.1 Evaluation

Ponds 8 and 17 had a pond suitability of '**Below Average**'. Both ponds were put forward for eDNA survey. Pond 8 is a pooled culvert with well-established emergent vegetation, located within the Scheme boundary, along Willow Walk which has not been surveyed for GCN previously. Pond 17 is a small pond in horse grazed pasture located within 20m of the Scheme boundary.

Pond 7 had a pond suitability of '**Average'** and was put forward for eDNA survey. Pond 7 is a pooled culvert with well-established submerged and emergent vegetation, located within the Scheme boundary along Willow Walk which has not been surveyed for GCN previously.

Ponds 28 and 29 are newly dug ponds thought to have been constructed in winter 2019. As they were newly created, these ponds were not given an HSI survey during the 2020 season as they are unestablished and unsuitable for GCN at present. However, these ponds are located in an area with a confirmed population of GCN and will therefore need to be considered for survey in the future. While Ponds 28 and 29 are positioned west of the Scheme, on the opposite side of the A34, there are culverts and drainage ditches beneath the road which may eventually provide a means of GCN dispersal into the Scheme area.

Though Ponds 7, 8 and 17 dried up during the 2020 survey season, this does not guarantee GCN are absent. These surveys should be put forward for eDNA survey more than 12 months prior to the start of construction as they are the ponds most likely to be impacted by the works.

The great crested newt is a European Protected Species. In the absence of more detailed survey data, there is a potential for the proposed works to have a negative impact on the great crested newt population.

Terrestrial habitat suitable for GCN foraging/hibernating will be lost as a result of the Scheme. As great crested newts spend much of their lives out of water within terrestrial habitats, there may be direct negative impacts (such as mortality) on individual great crested newts during the removal of hedgerows and scrub vegetation; and indirect negative impacts from the removal of suitable habitat resulting in a reduction of habitat utilised by newts for shelter, foraging and hibernation.

5.2 Mitigation Measures

GCN cannot be confirmed absent from the Scheme boundary from the 2020 surveys, as presence/absence surveys could not be completed and a known low population of GCN is present to the west of the Proposed Scheme.

Detailed mitigation measures will be developed following completion of the eDNA surveys. Provided that no waterbodies containing GCN will be lost or impacted by the construction works, a Precautionary Working Method Statement during construction should include the following:

- Any refugia (e.g. piles of wood or rubble) within the working area will be dismantled by hand and removed from the working area, this should be done under direct supervision of a suitably qualified ecologist and in advance of the works. This should only be done during the GCN active season (March – October, weather dependent).

- Where vegetation clearance is required, in areas identified as suitable terrestrial habitat for GCN, it should be done during the GCN active season (March – October, weather dependent) using a staged cut approach. A first cut of the vegetation down to approximately 15cm should be undertaken followed 24 hours later by a cut to ground level. This will encourage any animals present to leave the works footprint. This should be done under direct supervision of the supervising ecologist.

- **If a GCN is found all works will cease immediately and Natural England will be consulted, and a mitigation licence may be required.**

5.3 Recommendations

An eDNA survey is recommended for Ponds 7, 8 and 17 between 15 April and 30 June, more than one full year before the start of vegetation clearance. If pre-construction vegetation clearance is programmed to start in the autumn/winter of 2023/24, this would mean conducting surveys in 2022. This will determine if GCN are likely to be utilising the Scheme area and inform any further mitigation which may be required, while leaving enough time for mitigation to be planned and licences obtained.

Depending on the results of the eDNA survey, further population assessment surveys may be required. Should the Scheme boundary change significantly, additional surveys will be required.

6. References

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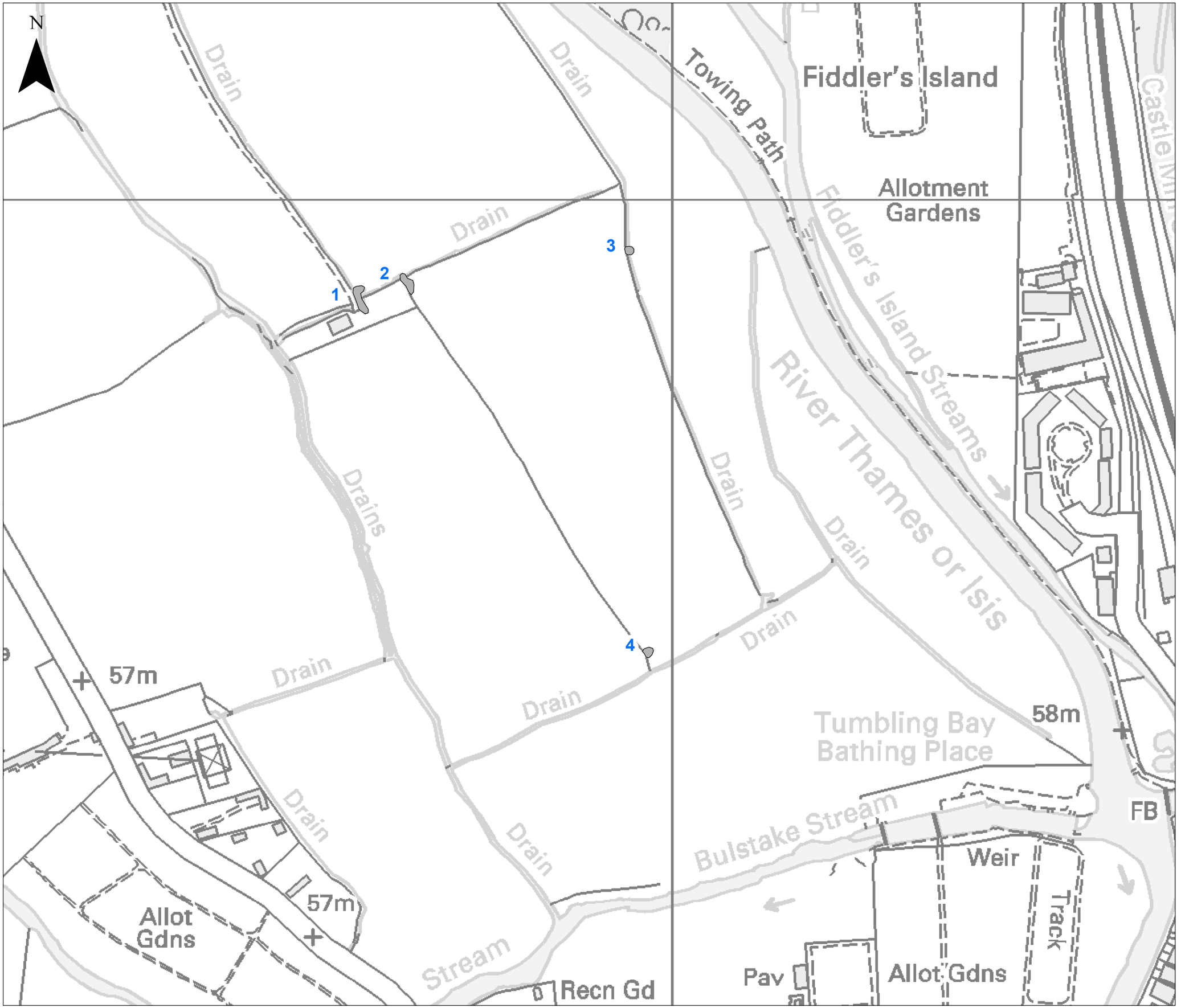
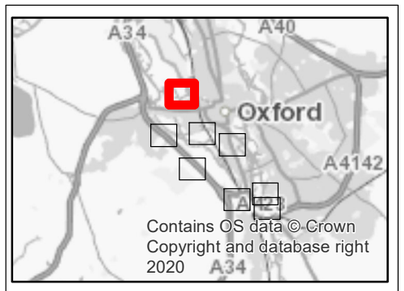
Websites

MAGIC: https://magic.defra.gv.uk

Figure 1 Pond Location Details and HSI Results





Legend

Habitat Suitability Index Results

- Excellent
- Good
- Average
- Below Average
- Poor
- Ponds unsuitable for an HSI survey
- N/A - new pond

Recommendations

- Further surveys are not recommended
- A Great Crested Newt presence/absence survey is recommended

1 Waterbody Reference Number

0 30 60 120
Metres

Rev	By	Chkd	Apprvd	Date	Description

Client

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Drawing :
Great Crested Newt - Habitat Suitability Index Results
Figure 1 (Sheet 1 of 8)

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Checked By :	RM	Date: 14/12/2020
Approved By :	DM	Date: 14/12/2020

Drawing No. :	Revision
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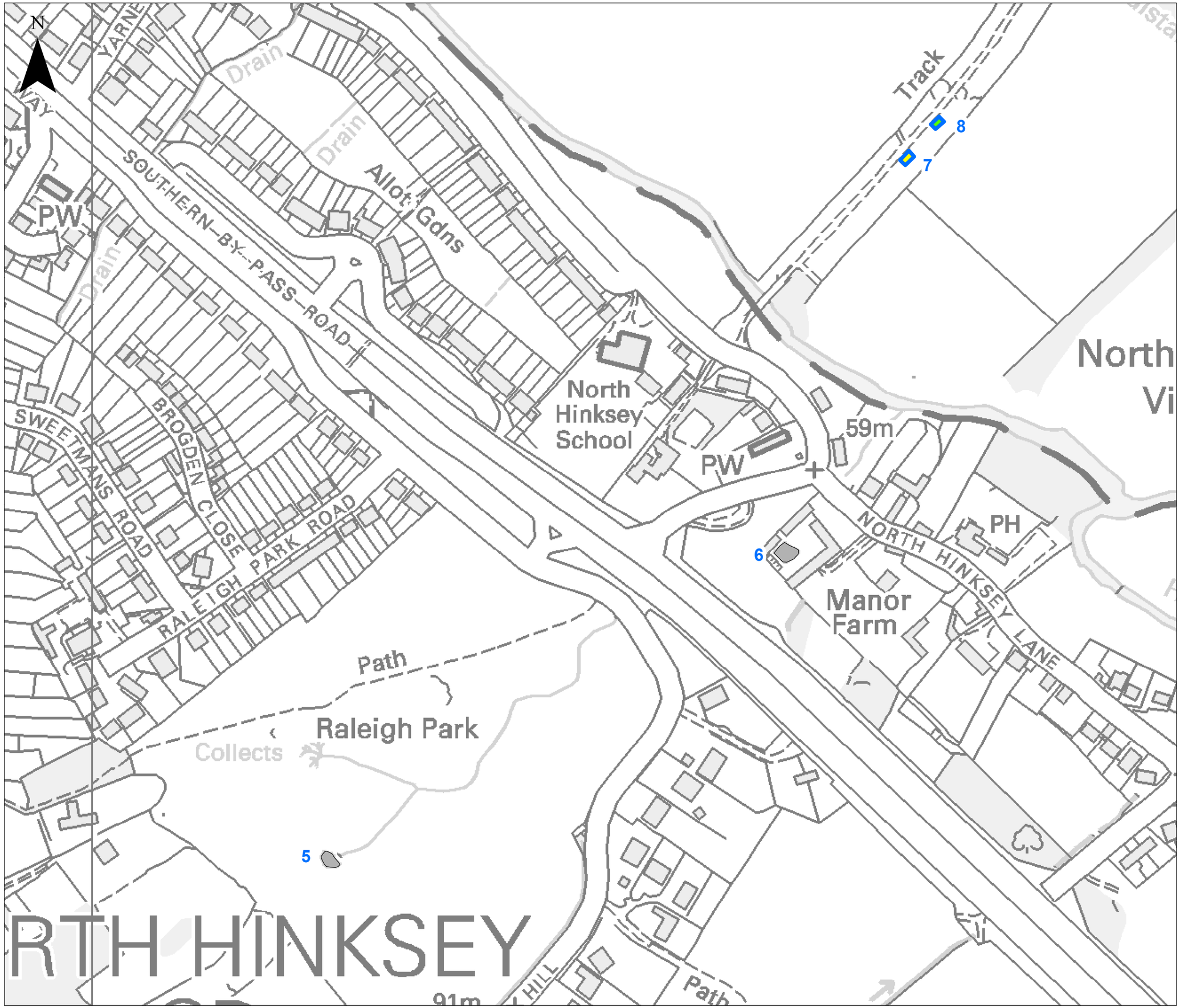

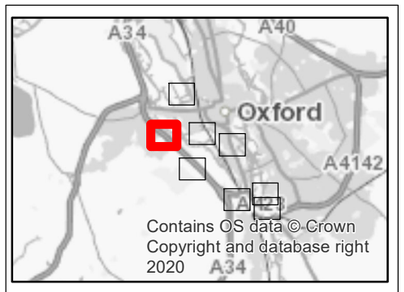

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Drawing :
Great Crested Newt - Habitat Suitability Index Results
Figure 1 (Sheet 2 of 8)

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Approved By :	DM	Date: 14/12/2020

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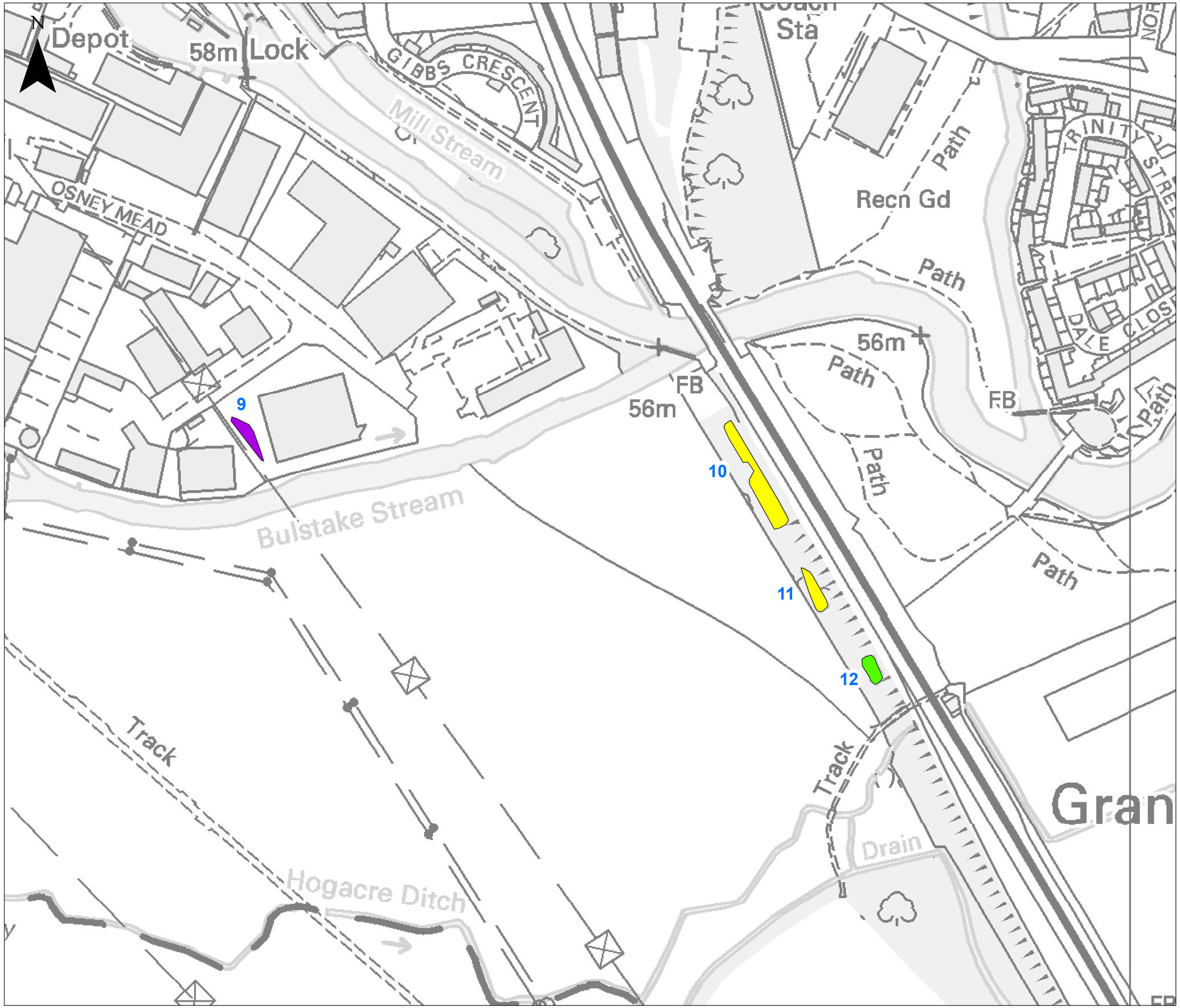

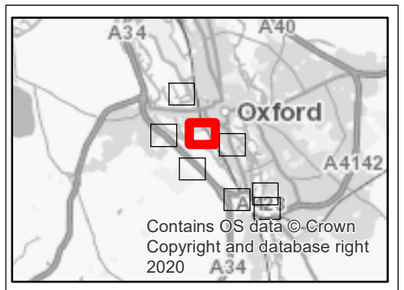

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Figure 1 (Sheet 3 of 8)

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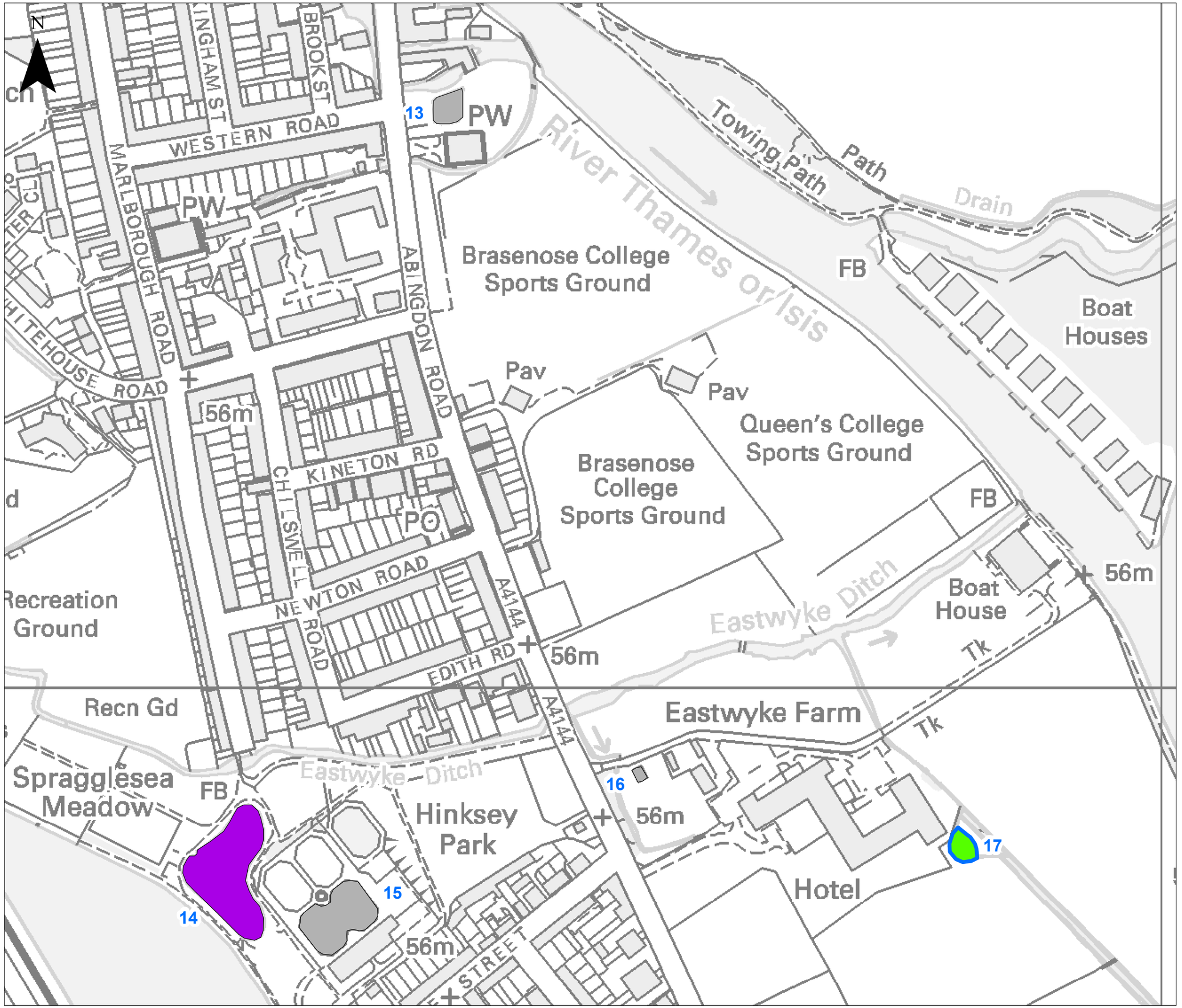

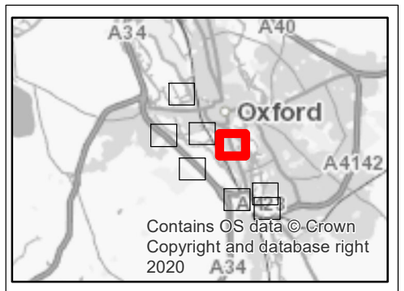

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- Further surveys are not recommended
- A Great Crested Newt presence/absence survey is recommended

1 Waterbody Reference Number

0 30 60 120
Metres

Rev	By	Chkd	Apprvd	Date	Description

Client
Environment Agency
Kings Meadow House
Kings Meadow Road
Reading
RG1 8DQ

Jacobs
1180 Eskdale Road, Winnersh,
Wokingham, RG41 5TU
Tel: +44 (0)1189 467000
Fax: +44 (0)1189 467001
www.jacobs.com

Project :
Oxford Flood Alleviation Scheme

Drawing :
Great Crested Newt - Habitat Suitability Index Results
Figure 1 (Sheet 4 of 8)

Drawn By :	JG	Date: 14/12/2020
Checked By :	RM	Date: 14/12/2020
Approved By :	DM	Date: 14/12/2020

Drawing No. :
Revision -

Drawing Scale : 1:2,500

Document Path: \\uksfpp02\Water\wh\tec\Oxford FAS\Environment\GIS\MXDs\Ecology_Plans\Oxford_FAS_GCN_HSI_2020_v2.mxd

N

59m

29

28

Drain

Track

Tk

Hinksey Hill Farm

Golf Course

Kestrel Lodge

Drain

South Hinksey Bridge

64m

SOUTHERN BY-PASS ROAD

18

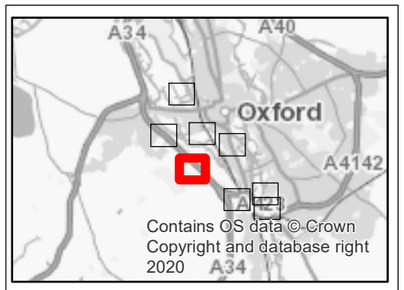
19

20

Hinksey Hill Cottages

Track

Track



Legend

Habitat Suitability Index Results

- Excellent
- Good
- Average
- Below Average
- Poor
- Ponds unsuitable for an HSI survey
- N/A - new pond

Recommendations

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Figure 1 (Sheet 5 of 8)

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Approved By :	DM	Date: 14/12/2020

Drawing No. : | Revision -

Drawing Scale : 1:2,500

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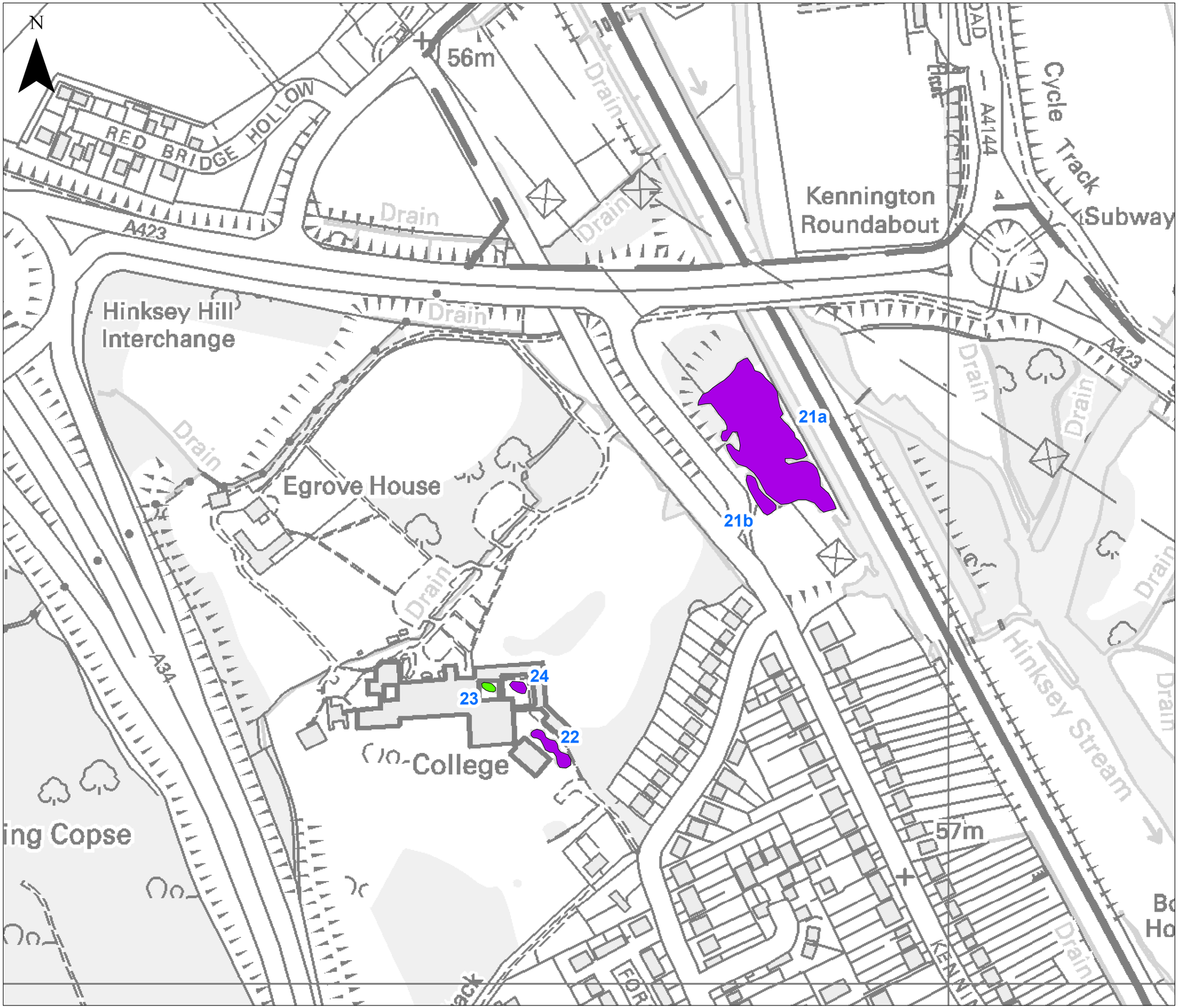

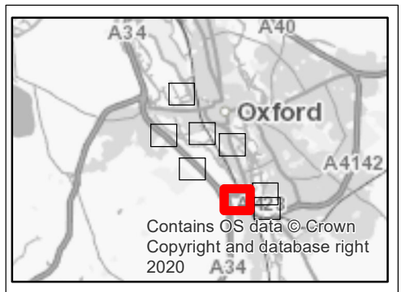

Legend

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Project :
Oxford Flood Alleviation Scheme

Drawing :
Great Crested Newt - Habitat Suitability Index Results
Figure 1 (Sheet 6 of 8)

Drawn By :	JG	Date: 14/12/2020
Checked By :	RM	Date: 14/12/2020
Approved By :	DM	Date: 14/12/2020

Drawing No. :	Revision
	-

Drawing Scale : 1:2,500

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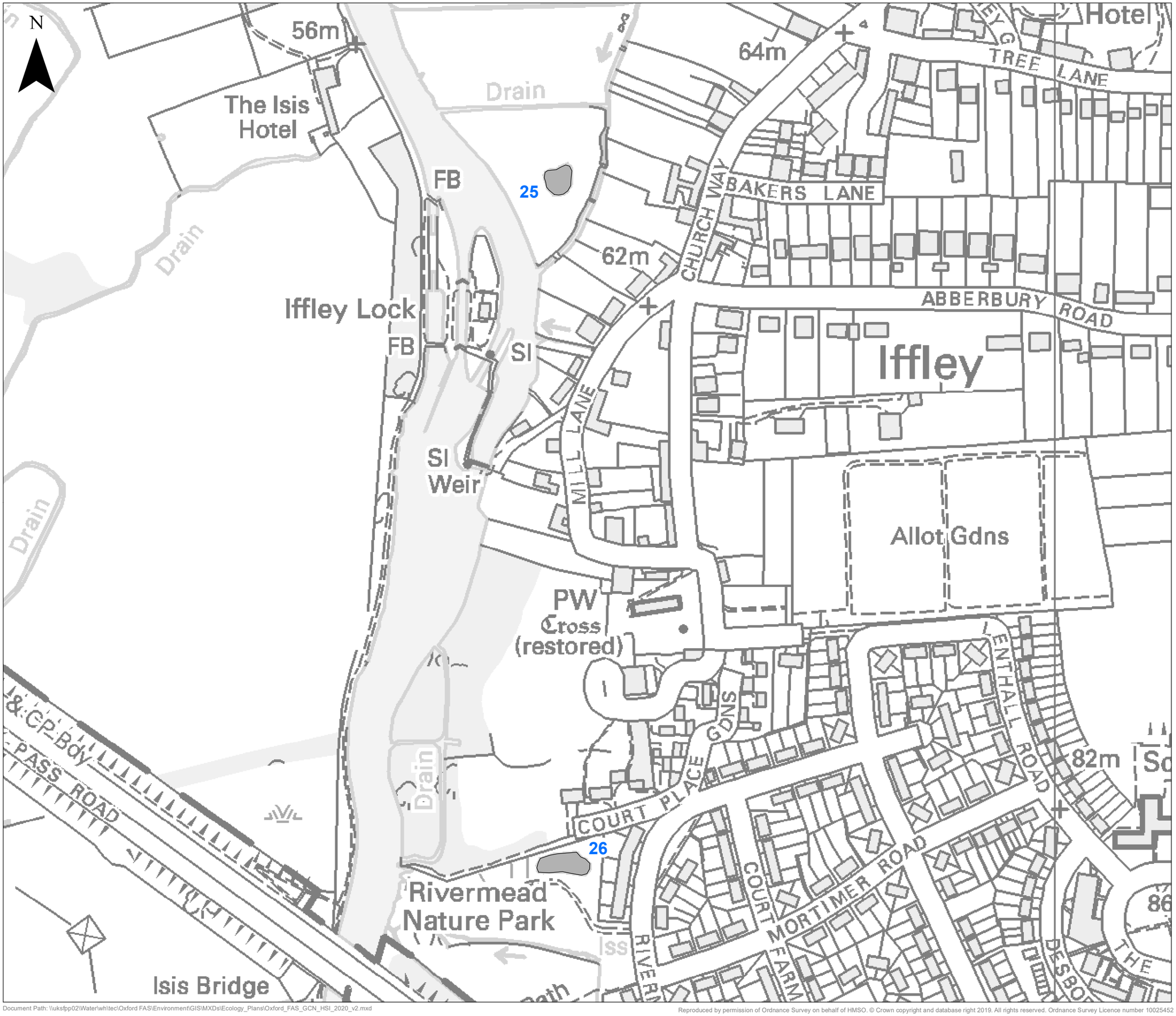

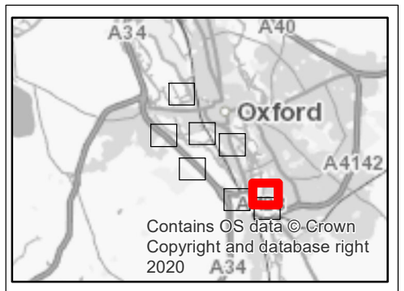

Legend

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Drawing :
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Figure 1 (Sheet 7 of 8)

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Checked By :	RM	Date: 14/12/2020
Approved By :	DM	Date: 14/12/2020

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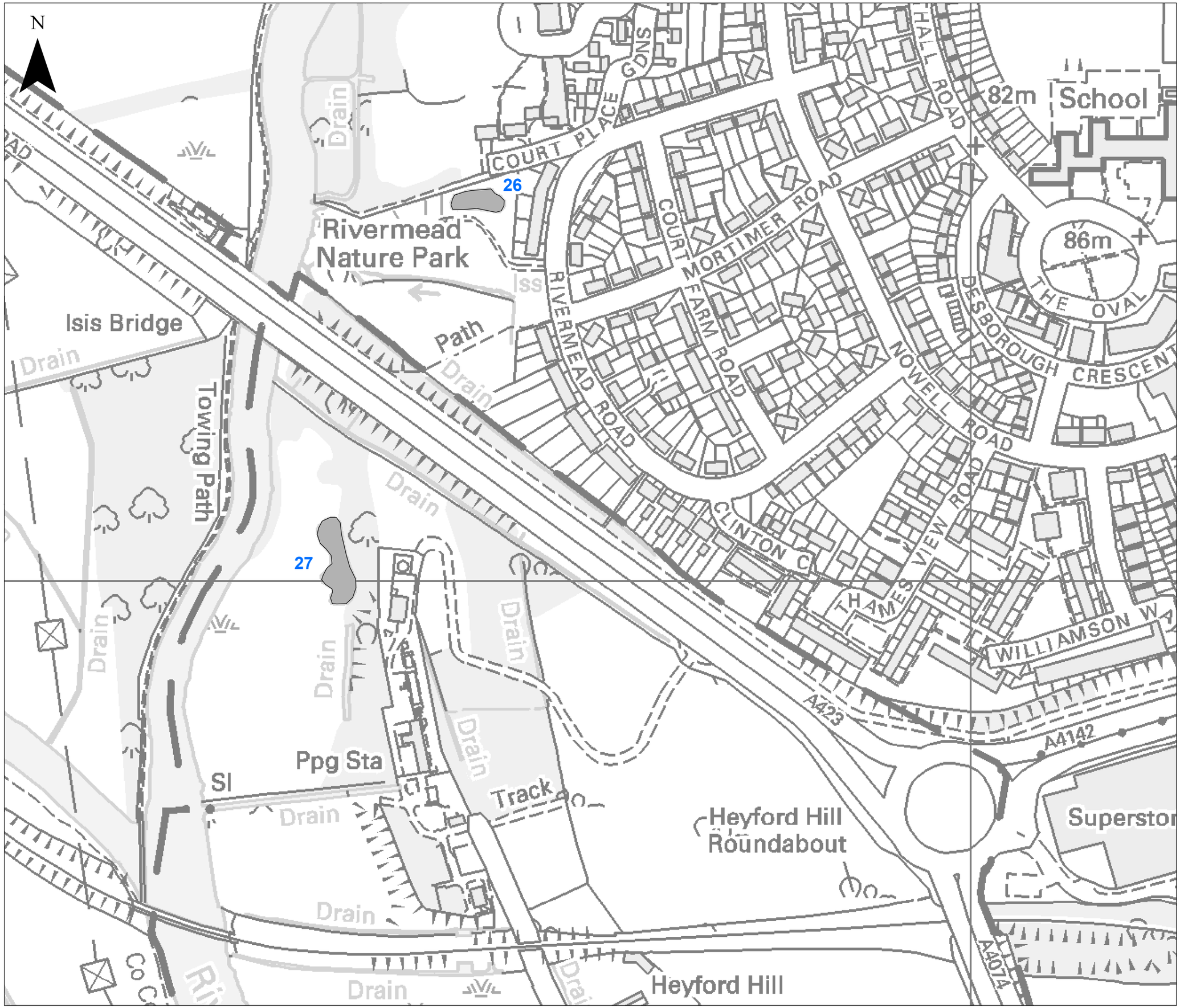
Drawing Scale : 1:2,500

Document Path: \\uksfpp02\Water\wh\tec\Oxford FAS\Environment\GIS\MXDs\Ecology_Plans\Oxford_FAS_GCN_HSI_2020_v2.mxd



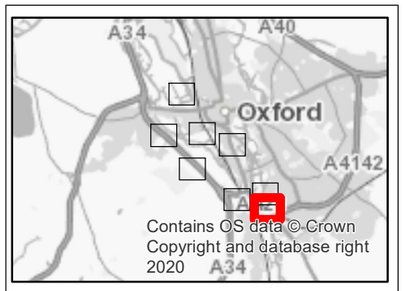


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Figure 1 (Sheet 8 of 8)

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Drawing No. :		Revision	-
Drawing Scale :	1:2,500		

Appendix A. eDNA Survey Methodology

eDNA is DNA found within a particular environment, for example a pond or lake, that has been released from an organism in the form of faeces, saliva, urine, skin cells or carcasses. In aquatic environments, the eDNA is diluted and distributed in the water where it persists for 7 to 21 days, depending on environmental conditions (Biggs, et al., 2014). eDNA sampling to confirm the presence or absence of GCN in ponds is a method that has been accepted by Natural England (NE). The procedure involves taking 20 water samples from a pond then combining these samples in a vessel to allow one composite sample per pond to be taken for laboratory analysis.

eDNA samples can be gathered between 15 April and 30 June. Only one visit to each pond is required however, if presence is confirmed at a pond, a population estimate using conventional bottle trapping, torching and egg-searching methods will likely be required for licensing purposes.

eDNA is nuclear or mitochondrial DNA that is released from an organism into the environment. Recent research has shown that the DNA from a range of aquatic organisms can be detected in water samples at very low concentrations using quantitative Polymerase Chain Reaction (qPCR) methods. Water samples are taken from each waterbody, which are then analysed for the presence of GCN mitochondrial DNA, confirming the presence or absence of GCN in the waterbody during that season. Jacobs used an accredited laboratory to provide sampling equipment and carry out laboratory analysis. The name of the laboratory is not provided in this document for reasons of commercial confidentiality. The following methodology was adhered to in accordance with Natural England's guidance note, WC1067 Technical Advice Note (Biggs, et al., 2014) with additional information taken from the accredited laboratory's survey protocol:

1) Samples are collected between 15 April and 30 June.

2) Gloves are worn throughout the sampling process and are changed between sample collection from the pond and pipetting into the sterile sub-sample tubes.

3) Samples are collected without entering the water by standing on the banks of the waterbody. This prevents disturbance of the substrate which could stir up historical GCN eDNA and contaminate the samples producing a potentially false positive result. This also prevents cross contamination of samples between ponds which could provide false results.

4) At the pond, surveyors identify where 20 samples are to be taken. The locations of sub-samples are spaced as evenly as possible around the pond margin and, where possible, targeted to areas where vegetation could be used by newts as egg laying substrate and open water areas, which could be used for displaying. Sample taking is avoided in areas of densely packed vegetation.

5) The surveyor collects 20 samples of 30 ml of pond water from around the pond using a ladle, and empties each sample into the Whirl-Pak bag.

6) Once 20 samples are taken, the bag is securely closed using the top tabs and shaken for 10 seconds. This mixes any DNA across the whole water sample.

7) A new pair of gloves is worn to keep the next stage uncontaminated.

8) A clear plastic pipette is used to take c15 ml of water from the Whirl-Pak bag and pipette it into a sterile tube containing 35 ml of ethanol to preserve the eDNA sample (so the tube to the 50 ml mark). The tube is then closed ensuring the cap is tight, but not over-tightened as this could cause the sample vessels to leak.

9) The tube is shaken vigorously for 10 seconds to mix the sample and preservative. This is essential to prevent DNA degradation. This is repeated for each of the six conical tubes in the kit. Before taking each sample, the water is stirred in the bag to homogenize the sample as the DNA constantly sinks to the bottom.

10) The samples are sealed in the box to prevent them being mixed up and to further reduce the risk of contamination.

11) The box of preserved sub-samples is stored in a cool box during the remainder of the day's survey and dispatched as soon as possible by courier to the laboratory for analysis.

Appendix B. Great Crested Newt Ecology

Lifecycle

Adult great crested newts migrate to their breeding ponds as early as late January, though this factor is dependent on air and soil temperatures (notably evening temperatures)1. However, most migrate later, reaching ponds by mid-March, with the peak courtship and egg laying period from mid-March to mid-May.

Great crested newts move mainly at night to avoid predation and help conserve moisture loss from their permeable skins, and most movement occurs when air temperature is above 5°C. Other breeding amphibians can have mass migrations in a short period of time to their ponds, but newt migration is considerably more gradual.

Great crested newts often return to the same breeding site and inhabit ponds that are part of a 'pond cluster', with individuals moving between ponds. They may move to new or regenerated ponds if their existing pond has become sub-optimal (e.g. through drought). It has been noted that small isolated populations based on a single pond are normally less likely to persist in the long term (English Nature, 2001).

The female newt lays individual eggs on the leaves of submerged plants, from which the larvae hatch out approximately three weeks later. These larvae develop over a period of two to three months into 'efts' (i.e. juveniles). The majority of adult newts leave the ponds between late May and July, spending most of their time within terrestrial habitats. By late September - early October, adults and immature newts are entering their hibernation phase and utilise refugia such as woodpiles, tree roots, mammal burrows, rubble piles or crevices in walls.

Habitat

Great crested newts use a combination of aquatic and terrestrial habitats for their lifecycles, occupying small home ranges approximately 500 m from their breeding ponds. The optimal habitat for breeding comprises natural/semi-natural ponds and other standing water habitats with neutral to slightly alkaline water, such as field ponds, clay pits, moats, large ditches and quarry ponds. They favour lowland ponds which are well vegetated and not too shaded, including those which occasionally dry up in late summer or autumn.

The Great Crested Newt Mitigation Guidelines (English Nature, 2001) identifies the following pond characteristics as being favourable for sustaining great crested newt populations:

- Small to medium sized breeding ponds (50-250 m^2);
- Variable depth, but preferably not so deep that aquatic and emergent vegetation is unable to take root. A maximum depth around 4 m is acceptable;
- Substantial cover of submerged and marginal vegetation;
- Open areas to facilitate courtship behaviour;
- Good populations of invertebrates and other amphibians as prey;
- Ponds in clusters rather than in isolation;
- Absence of shading on the south side of the pond;
- Absence of fish; and

[1] As great crested newts are 'ectotherms', i.e. they utilise external heat sources to raise their body temperature, which subsequently encourages physical movement.

- Absence of waterfowl.
- Conversely, the following characteristics are unfavourable for breeding great crested newts:
- Ponds with greater than 20% shading have reduced potential for the presence of great crested newt (Cooke *et al.,* 1994);
- Ponds that regularly dry out are unsuitable for this species as they do not provide viable breeding;
- Arable land within 100 m of breeding sites is associated with low populations of great crested newts (Franklin, 1993), so distances greater than this are likely to be devoid of newts and act as barriers to amphibian movement;
- Newly created ponds, although they can be colonised by newts as long as populations of newts exist within 350 m (Franklin, 1993). Ponds identified as 'new' are not likely to support great crested newts if no other viable waterbodies fall within this distance; and
- The presence of predatory fish and high density of waterfowl reduces the value of ponds often to the point where they may not be used for breeding. However, such ponds are still important to sustain a population (English Nature, 2001).