



Hallgates to Elms Farm Pipeline Route

Leicester

Protected Species Report: Great Crested Newt

Prepared For: Severn Trent Water

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Executive Summary

Introduction	- Amphibian surveys have been undertaken by TEP to inform proposals for the construction of a new water pipeline to the north-west of Leicester. - The site comprises the linear pipeline route and a 50 m buffer, where potential habitat impacts could occur. 48 ponds were identified within 500 m of the site following a review of Ordnance Survey mapping. Five ponds are located within the site, 28 ponds are located within 250 m of the site and 15 are situated between 250 m and 500 m of the site. An additional two ponds were identified within 250 m of the site during the amphibian surveys. - Given the site is a long linear scheme and as GCN do not regularly range beyond 250m from a breeding pond, only ponds within 250m of the site were subject to survey. - The site contains suitable terrestrial habitat for amphibians including woodland, scrub, tall forb, hedgerow, arable field margins and grassland.
Surveys Undertaken	- A total of 35 ponds (this includes the two additional ponds identified during the survey) were scoped in for survey. Of these, three ponds were found to no longer exist and access was refused to a further two. As such, a GCN Habitat Suitability Index (HSI) Assessment was undertaken on 30 ponds. - Of these 30 ponds, two were found to be dry and vegetation restricted access to the water's edge on three of the ponds. As such, eDNA sampling was undertaken on 25 ponds. - The HSI and eDNA surveys were undertaken by a suitably licenced ecologist in June 2024.
Results	- HSI scores ranged from Poor to Excellent for the 30 ponds surveyed. - GCN were confirmed to be present in pond P31 on-site and pond P5 within 250 m of the site. GCN may also be present in the five other waterbodies that could not be accessed for survey (P25, P28, P29, P35, P38). - GCN were confirmed as absent in all other ponds. - All waterbodies subject to survey are considered to be suitable for supporting breeding common amphibian species. Evidence of smooth newt breeding was recorded on pond P12.
Conclusions/ Recommendations	- It is recommended that eDNA surveys of the five ponds that could not be surveyed are re-attempted during the 2025 survey season (15th April to 30th June inclusive) to confirm whether GCN are present within these ponds. - A licence from Natural England will be required in order for development works to proceed. Owing to the nature of the proposals, it is recommended that District Level Licencing (DLL) is used.

This Executive Summary is not a substitute for the full report. Refer to the full text of this report for further detail.

1.0 Introduction

Project Context

- 1.1 TEP was commissioned by Severn Trent Water (STW) (hereafter referred to as 'the Client') in May 2024 to undertake amphibian surveys to support proposals for the construction of a new water pipeline to the north-west of Leicester.
- 1.2 The proposed pipeline route is approximately 13 km in length and is located between Bradgate Park, near Cropston (approximate grid reference SK 53423 11450) and fields to the west of the A46 road near Groby (approximate grid reference SK 52651 05979) (Figure 1).

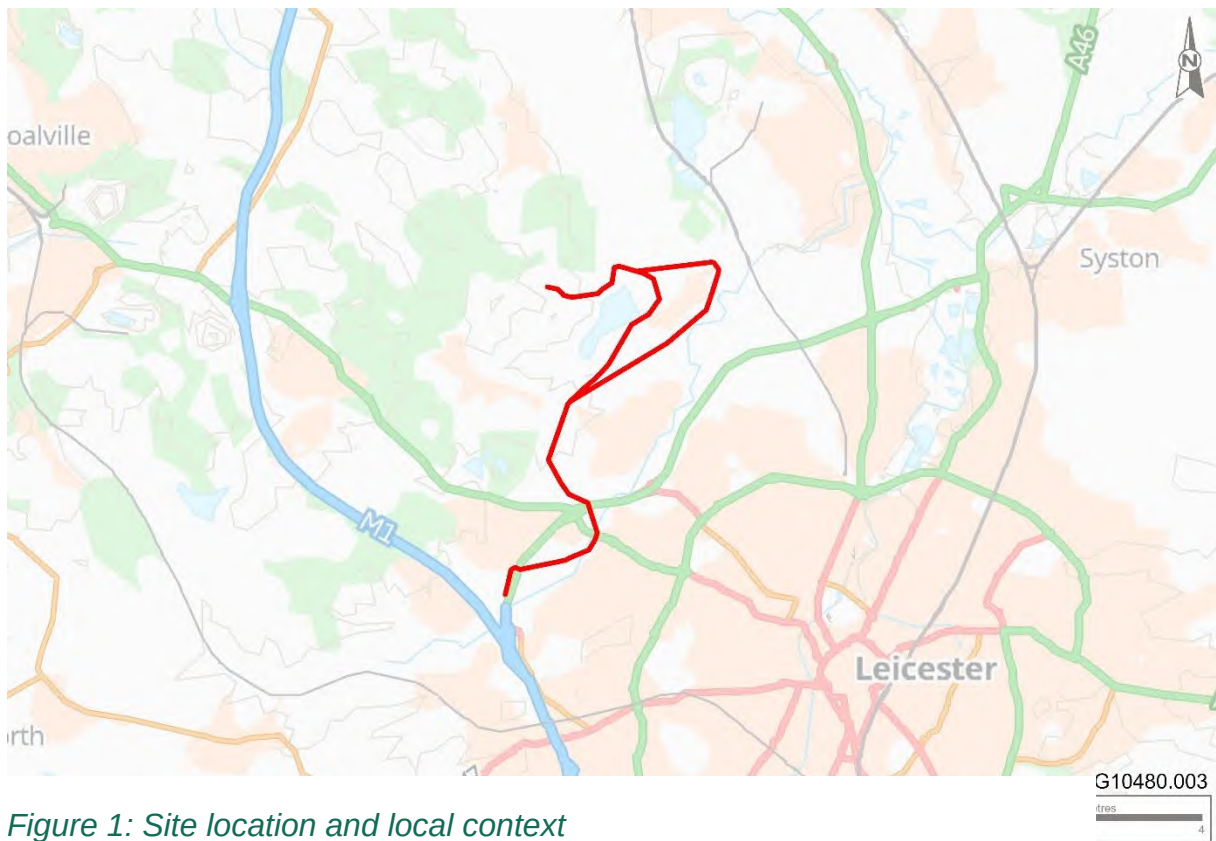


Figure 1: Site location and local context

- 1.3 TEP has previously undertaken an extended UK Habitat Survey and Habitat Condition Assessment of the proposed pipeline route and a 50 m buffer (hereafter referred to as 'the site') to inform the proposals. The results of this survey are presented in the Preliminary Ecological Assessment (PEA) report (TEP Report Ref: 10480.001, June 2024).

Ecological Context

- 1.4 A review of Ordnance Survey mapping identified that there are 48 ponds within 500 m of the site. Five ponds are present within the site, 28 ponds are located within 250 m of the site and 15 are situated between 250 m and 500 m of the site. An additional two ponds were identified within 250 m of the site during the amphibian surveys.
- 1.5 The extended UK Habitat Survey identified suitable terrestrial amphibian foraging, commuting and sheltering habitat within the site including woodland, scrub, tall forb, hedgerow, arable field margins and grassland. Large rubble piles were also noted within one part of the route which may also provide additional sheltering and hibernation opportunities for amphibians.

Legal Protection

- 1.6 All British amphibian species receive legal protection in the United Kingdom though the degree to which different species are protected varies. Great crested newts (GCN) *Triturus cristatus*, natterjack toad *Epidalea calamita* and pool frog *Pelophylax lessonae* and their habitats (places used for breeding and shelter) receive the highest level of protection in the UK under the combination of the Wildlife and Countryside Act 1981 as amended and the Conservation of Habitats and Species Regulations 2017 as amended. While natterjacks and pool frogs have restricted distributions within the UK, GCN are relatively widespread.
- 1.7 Licences to allow derogation from the protection afforded to GCN may be granted under the provisions of the 2017 Regulations.
- 1.8 Further information relating to legal protection afforded to native amphibian species is presented in Annex B.
- 1.9 GCN, natterjack and common toads and the pool frog are listed as Species of Principle Importance (SPI). Section 40 of the Natural Environment and Rural Communities Act 2006 imposes a Duty upon public authorities to conserve biodiversity. Commonly referred to as the 'biodiversity duty', this includes local planning authorities in their decision making for planning applications or other plans or projects which may affect SPI.

Survey Rationale

Survey Guidance

- 1.10 Amphibian survey follows best practice and published guidance, including:

- Habitat Suitability Index (HSI) Assessment (ARGUK, 2010)¹
- Great crested newt mitigation guidelines (English Nature, 2001)²
- Great crested newt conservation habitat (Froglife, 2001)³

Survey Design and Scope

- 1.11 The surveys were designed to determine whether or not GCN are present within ponds within ranging distance of the site.
- 1.12 It is generally accepted that GCN can range up to 500m from a breeding pond, however they do not regularly range beyond 250m. Given the site is a long linear scheme, surveying all ponds within 500m was considered to be disproportionate. Therefore, a 250m survey buffer was applied.
- 1.13 The site comprises the linear pipeline route and a 50 m buffer, where potential habitat impacts could occur. The 250 m GCN survey buffer was therefore applied to this 50 m buffer. As such, all ponds within 300m of the linear pipeline route were subject to survey.

Surveyor Qualifications and Competency

- 1.14 Amphibian surveys were undertaken by ecologists in possession of a Natural England GCN survey licence, with relevant training and experience. Further confirmation of survey competency can be provided upon request.

¹ ARG UK Advice Note 5 (May 2010) Great Crested Newt Habitat Suitability Index

² English Nature (2001) Great Crested Newt Mitigation Guidelines (Version August 2001), English Nature, Peterborough

³ Langton, T.E.S., Beckett, C.L., and Foster, J.P. (2001), Great Crested Newt Conservation Handbook, Froglife, Halesworth

2.0 Methods

Data Search

- 2.1 As part of the PEA report, species records for the last 10 years within 1 km of the site were requested from Leicestershire and Rutland Environmental Records Centre (LRERC).
- 2.2 A review of Natural England's open datasets held on MAGIC Map was also undertaken.

Aquatic Habitat Suitability Index (HSI) Assessments

- 2.3 Aquatic HSI assessments were completed at a total of 31 ponds within 250m of the site, where access had been granted.
- 2.4 HSI is a standard measure of calculating the suitability of a pond to support breeding GCN, based on an assessment of 10 characteristics (indices), including size, shading, depth, and vegetation profile. The assessment generates a number between 0 and 1 for each of the indices which are combined to provide an overall assessment of a pond's suitability to support GCN on a categorical scale, as presented in HSI is a standard measure of calculating the suitability of a pond to support breeding great crested newts, based on an assessment of 10 characteristics (indices), including size, shading, depth, and vegetation profile. The assessment generates a number between 0 and 1 for each of the indices which are combined to provide an overall assessment of a pond's suitability to support GCN on a categorical scale, as presented in **Error! Not a valid bookmark self-reference..** The assessment has not been designed for or tested on other waterbodies such as ditches.
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Table 1: HSI Suitability Scores

HSI Score	Suitability	Predicted GCN Occupancy of Ponds in each Category
<0.5	Poor	3%
0.5 to 0.59	Below Average	20%
0.6 to 0.69	Average	55%
0.7 to 0.79	Good	79%
>0.8	Excellent	93%

Environmental DNA (eDNA) Sampling

- 2.7 Environmental DNA (eDNA) sampling was undertaken at a total of 26 ponds within 250m of the site.
- 2.8 Sample collection was undertaken by licensed surveyors. Sample kits and analysis were provided by ADAS. Both organisations followed the relevant sections of the method set out in the DEFRA funded study endorsed by Natural England. In summary the sampling protocol is as follows:
- 20 samples were taken from around the entire perimeter of the waterbody.
 - The surveyor stayed out of the water while taking the samples (extension poles were used in situations where open/sufficiently deep water was at a distance from the dry banks.
 - Survey locations were distributed around the pond perimeter, but micro-siting was used to select locations most likely to be used by GCN.
 - At each sample location the water column was stirred prior to taking the sample but care was taken to avoid disturbing the sediment on the base of the pond.
 - Once all 20 samples were taken, 15ml of the total sample were pipetted into each of the 6 sampling tubes, whilst ensuring that the water in the sample bag was mixed before taking each 15ml sample and that only one sample tube was opened at any one time.
 - At all times the surveyor ensured that the risk of contaminating the sampling equipment was minimised by avoiding the placement of the ladle or pipette on the ground or on any otherwise potentially contaminated surfaces and by changing gloves between the initial sampling stage and the pipetting stages of the method.

Chain of Custody

- 2.9 On receipt from ADAS the sampling kits were registered on a central database using the unique bar codes. Immediately prior to survey, sampling kits were issued to surveyors with individual Sample Forms using the unique bar code as identification. The site name and date of issue was also recorded on this form (and on the central database). Once in the field and at the ponds, the surveyor confirmed that the appropriate field survey sheet was being completed by checking the bar code on the box and double checking the corresponding bar codes on the sample tubes. The surveyor then filled in the date of survey and the pond ID number (as well as other information relating to survey conditions) on the Sample Form.
- 2.10 On returning to the office the Sample Forms were signed to confirm for each sample who received the samples and checked them into the fridge and the temperature of the fridge. The pond IDs on each form were checked against a site map confirming which ponds had been sampled and this map was stored with the Sample Forms. All this information was also recorded on the central database. The sample preserving tubes were stored in a fridge until the morning of collection by the courier. The Sample Forms and the central database were updated to confirm the date of collection by the courier.
- 2.11 The unique bar codes were used by ADAS to report results. All results were recorded in the central database by one member of staff and cross checked by a second member of staff before issuing to the project leader for review.

Survey Limitations

- 2.12 Thirty-three ponds were originally scoped in for the HSI assessment and eDNA survey. During the surveys, two additional ponds were identified within 250 m of the site (P23a and P34a) and these were subsequently incorporated within the survey scope. One further pond identified during the UK Habitat Survey (P13a) was excluded from the amphibian survey scope, however.
- 2.13 During the surveys three ponds were found to no longer exist (P6, P18 and P42) and access was refused to a further two (P35, P38). These ponds were therefore not subject to survey.
- 2.14 HSI surveys were subsequently undertaken on the remaining 30 ponds. However, it was not possible to complete eDNA surveys of all of these waterbodies.
- 2.15 eDNA sampling could not be undertaken on three ponds (P25, P28 and P29) due to the presence of dense scrub and woodland restricting access to the water to allow samples to be taken. In addition, ponds P8 and P14 were found to be dry at the time of survey and

therefore could not be surveyed. As such, eDNA surveys were only undertaken on 25 ponds.

- 2.16 Although Cropston Reservoir (P48) falls within 250 m of the site, given the large size of this waterbody it was considered appropriate to only survey the section of reservoir that falls within the site boundary (P48a).

Survey Method Summary

- 2.17 An overview of the survey methods employed at each pond and dates of surveys is presented in Table 2.

Table 2: Survey Method Summary

Waterbody ID	Zone (relative to the site)	HSI Date	eDNA Date
P5	<250m	05/06/24	05/06/24
P6	N/A – does not exist		
P8	<250m	05/06/24	N/A – dry
P10	<250m	05/06/24	05/06/24
P11	<250m	03/06/24	03/06/24
P12	<250m	05/06/24	05/06/24
P13	Within the site	05/06/24	05/06/24
P14	<250m	05/06/24	N/A – dry
P16	<250m	06/06/24	06/06/24
P18	N/A – does not exist		
P19	<250m	07/06/24	07/06/24
P20	<250m	07/06/24	07/06/24
P21	<250m	07/06/24	07/06/24
P22	<250m	06/06/24	06/06/24
P23	<250m	06/06/24	06/06/24
P23a	<250m	06/06/24	06/06/24

Waterbody ID	Zone (relative to the site)	HSI Date	eDNA Date
P25	<250m	03/06/24	N/A – inaccessible
P28	<250m	04/06/24	N/A – inaccessible
P29	<250m	04/06/24	N/A – inaccessible
P30	<250m	04/06/24	04/06/24
P31	Within the site	04/06/24	04/06/24
P33	Within the site	03/06/24	03/06/24
P34	<250m	03/06/24	03/06/24
P34a	<250m	03/06/24	03/06/24
P35	<250m	N/A – no access	
P36	<250m	03/06/24	03/06/24
P37	<250m	06/06/24	06/06/24
P38	<250m	N/A – no access	
P39	<250m	04/06/24	04/06/24
P41	Within the site	03/06/24	03/06/24
P42	N/A – does not exist		
P43	<250m	04/06/24	04/06/24
P44	<250m	04/06/24	04/06/24
P45	<250m	04/06/24	04/06/24
P48a	Within the site	04/06/24	04/06/24

3.0 Results

Data Search

- 3.1 Several records of amphibians were returned by LRERC for within 1 km of the site, including:
- GCN
 - Common toad *Bufo bufo*
 - Common frog *Rana temporaria*
 - Smooth newt *Lissotriton vulgaris*
- 3.2 None of these records were recorded within the site. The closest record of GCN was located approximately 0.2 km to the north of the site, between the A46 and A50 roads in Groby. The closest record of common toad was approximately 0.05 km to the south of the site, within Bradgate Park near the start of the route.
- 3.3 Two active and two historic EPS licences for GCN were identified on MAGIC Map within 1km of the site. The active licences relate to damage of a resting place and destruction of a resting place.
- 3.4 Seven GCN class licence returns were also identified within 1km of the site between 2017 and 2019.

HSI Assessment

- 3.5 Detailed pond descriptions and HSI Assessment results are presented in Annex B. Pond locations are shown on the Pond Location Plan (TEP Drawing Ref: G10480.001) and the HSI Results are displayed on the HSI results plan (TEP Drawing Ref: G10480.012).
- 3.6 The five ponds within the site were shown to have Poor to Excellent suitability to support breeding GCN.
- 3.7 The 25 ponds within 250 m of the site also ranged from having Poor to Excellent suitability to support breeding GCN.

eDNA Survey

- 3.8 The eDNA survey results are illustrated on the Great Crested Newt Survey Results map (Drawing Ref: G10480.014). Full results are provided in Annex C.

- 3.9 GCN were confirmed to be present within one of the five on-site ponds (P31).
- 3.10 GCN were confirmed to be present within one of the 25 ponds within 250 m of the site that were subject to survey (P5).

Results Summary

- 3.11 Table 3 summarises the results of the HSI and eDNA surveys.

Table 3: Survey Results Summary

Waterbody ID	Zone (relative to the site)	HSI Assessment	GCN eDNA Score	GCN Present?
P5	<250m	Average	Positive (2/12)	Yes
P6	<250m	<i>Not surveyed – does not exist</i>		
P8	<250m	Poor	<i>Not surveyed - dry</i>	
P10	<250m	Good	Negative (0/12)	No
P11	<250m	Good	Negative (0/12)	No
P12	<250m	Average	Negative (0/12)	No
P13	Within the site	Poor	Negative (0/12)	No
P14	<250m	Below Average	<i>Not surveyed – dry</i>	
P16	<250m	Good	Negative (0/12)	No
P18	<250m	<i>Not surveyed – does not exist</i>		
P19	<250m	Average	Negative (0/12)	No
P20	<250m	Average	Negative (0/12)	No
P21	<250m	Poor	Negative (0/12)	No
P22	<250m	Good	Negative (0/12)	No
P23	<250m	Poor	Negative (0/12)	No
P23a	<250m	Poor	Negative (0/12)	No

Waterbody ID	Zone (relative to the site)	HSI Assessment	GCN eDNA Score	GCN Present?
P25	<250m	Good	<i>Not surveyed – could not access</i>	
P28	<250m	Average	<i>Not surveyed – could not access</i>	
P29	<250m	Below Average	<i>Not surveyed – could not access</i>	
P30	<250m	Poor	Negative (0/12)	No
P31	Within the site	Excellent	Positive (12/12)	Yes
P33	Within the site	Average	Negative (0/12)	No
P34	<250m	Good	Negative (0/12)	No
P34a	<250m	Excellent	Negative (0/12)	No
P35	<250m	<i>Not surveyed – no access</i>		
P36	<250m	Good	Negative (0/12)	No
P37	<250m	Average	Negative (0/12)	No
P38	<250m	<i>Not surveyed – no access</i>		
P39	<250m	Excellent	Negative (0/12)	No
P41	Within the site	Excellent	Negative (0/12)	No
P42	<250m	<i>Not surveyed – does not exist</i>		
P43	<250m	Average	Negative (0/12)	No
P44	<250m	Below Average	Negative (0/12)	No
P45	<250m	Good	Negative (0/12)	No
P48a	Within the site	Good	Negative (0/12)	No

- 3.12 No amphibians were observed within any of the surveyed ponds during the HSI assessment and eDNA survey. However, smooth newt eggs were observed within Pond P12.

4.0 Evaluation

Habitat Suitability Index (HSI) Assessments

- 4.1 Aquatic habitat suitability was assessed applying the HSI method. Across all ponds assessed, HSI categories ranged from Poor to Excellent.
- 4.2 Of the 30 ponds accessed within 250m of the site, 25 ponds were found to be suitable for GCN breeding (Below Average HSI category or higher). This broadly is 81% of the available aquatic habitat within the survey area.
- 4.3 Six of the surveyed ponds (P8, P13, P21, P23, P23a and P30) were assessed as having Poor suitability to support GCN. While it is unlikely GCN breeding would be supported in ponds with poor suitability, this does not preclude GCN presence, or presence of other amphibians or aquatic species of note.
- 4.4 All ponds subject to survey have potential to support breeding common amphibian species. Evidence of breeding smooth newt was identified at pond P12.

Environmental DNA (eDNA) Sampling

- 4.5 GCN were confirmed to be present within two of the surveyed ponds (P5 and P31) following completion of the eDNA surveys.
- 4.6 eDNA sampling of Ponds P8 and P14 were not possible as they were dry at time of survey. Given these ponds dry out, it is considered unlikely that GCN would utilise these waterbodies for breeding.
- 4.7 Ponds P25, P28 and P29 could not be sampled due to the presence of dense scrub preventing safe access. It is possible that GCN could be present within these waterbodies.
- 4.8 GCN were confirmed to be absent in all other ponds surveyed.

5.0 Conclusions and Further Requirements

- 5.1 The eDNA surveys undertaken confirmed the presence of GCN in two of the 25 waterbodies subject to survey (P5 and P31). It is therefore likely a licence from Natural England will be required to facilitate the development proposals.
- 5.2 Three ponds (P25, P28 and P29) could not be safely accessed for survey, whilst access was refused for two ponds (P35 and P38) and an additional pond (P13a) was not included in the 2024 surveys. It is possible that GCN are present in these waterbodies.

Further Surveys

- 5.3 It is recommended that eDNA surveys of Ponds P25, P28, P29, P35 and P38 are re-attempted during the 2025 survey season (15th April to 30th June inclusive) to confirm whether GCN are present within these ponds. Surveys of Pond P13a should also be attempted.
- 5.4 Vegetation clearance is likely to be required to facilitate safe access to some of these ponds. A Precautionary Working Method Statement (PWMS) for nesting birds and amphibians may be required to facilitate these clearance works.
- 5.5 GCN survey data are valid for a minimum of two seasons, potentially up to four seasons depending on the specific use of the data, local conditions and the potential impact predicted on GCN. When survey data becomes greater than two seasons old, advice should be sought from an appropriately experienced ecologist as to whether repeat surveys may be needed.

Potential Impacts

- 5.6 GCN have been confirmed to be present within P31 within the site boundary. This pond is located within Section 1-C of the route, to the south of Cropston Reservoir.
- 5.7 Detailed proposals are not currently available. However, there is potential for direct impacts to occur to this waterbody if this pond is to be lost to facilitate the proposals and to individual GCN as a result of construction activities and as a result of loss of terrestrial habitat. Furthermore, there is potential for indirect impacts to arise as a result of pollution run-off during construction.
- 5.8 GCN were also confirmed to be present within P5 within 250 m of the site. This waterbody is located to the west of Section 2-A of the route, between Bradgate Road and Anstey Lane. No direct or indirect impacts to pond P5 are anticipated to arise, however.

- 5.9 All ponds subject to survey are also considered to be suitable for supporting breeding common amphibians. Evidence of smooth newt breeding was recorded on pond P12 within 250 m of the site. There is potential for ponds within the site (P13, P31, P33, P41 and P48a) to be directly or indirectly impacted by the proposals, and for indirect impacts to occur to ponds within 250 m of the site.
- 5.10 There is also potential for individual amphibians, including GCN, ranging between these ponds and the site to be killed or injured during construction.

Mitigation Approach

- 5.11 To ensure there is no net loss of GCN breeding habitat on site, it is strongly recommended that the on-site pond with confirmed GCN presence (P31) is retained within the proposals. A buffer around this pond, within which no works take place, should also be applied.
- 5.12 All other waterbodies within the site should also be retained where possible, to avoid the loss of common amphibian breeding habitat and potential direct impacts on breeding common amphibians.
- 5.13 A GCN licence from Natural England will be required to facilitate the proposed works. Owing to the nature of the proposals, it is recommended that District Level Licencing (DLL) is used.
- 5.14 There may be opportunities to join the Leicestershire DLL scheme. A development licence can only be granted once planning permission has been obtained. The results of the eDNA surveys undertaken to inform the planning application would be sufficient to inform the DLL application.
- 5.15 Where DLL is to be used, an 'agreement in principle' must be obtained from Natural England through an Impact Assessment and Conservation Payment Certificate⁴ with associated enquiry fee. The Certificate will need to be submitted to the local planning authority as evidence of joining the licencing scheme.
- 5.16 A DLL application can then be made to Natural England once the planning application has been approved. A conservation payment will need to be made as outlined in the agreement enquiry and a licence fee payment will also need to be made. Works can commence once the District Level Licence has been issued.

⁴ www.gov.uk/government/publications/great-crested-newts-district-level-licensing-schemes/developers-how-to-join-the-district-level-licensing-scheme-for-gcns

- 5.17 An amphibian Precautionary Working Method Statement (PWMS) will also be required to ensure that killing or injury of common amphibians including common toad that may be present within the site are avoided during site clearance. The PWMS will need to include methods such as staged strimming, hand searching and removal to a safe location of any amphibians found.

- 5.18 Best Practice Pollution Prevention Measures must be implemented to prevent indirect impacts occurring to retained aquatic habitats and their associated species. The appointed contractors' Construction Environment Management Plan (CEMP) should include industry standard, best-practice methods on how site run-off will be controlled, how site waste will be managed, how fuel and other spillages will be prevented and will include emergency procedures in the event of a pollution incident.

- 5.19 If the DLL option is not available, traditional EPS licencing and mitigation will be required. This would require a suite of six traditional GCN surveys on waterbodies with a positive eDNA result to confirm the GCN population size. These surveys should be undertaken between mid-March and mid-June inclusive.

- 5.20 Once the traditional licence application is granted, a trapping scheme will also need to be established for a minimum of 25 days and a maximum of 30 days for a small population. Replacement of suitable terrestrial habitat being lost will also be required, either on-site or within land adjacent to the site that is under the same control. A GCN Mitigation Strategy would also need to be prepared and submitted with the planning application.

Annex A: Legal and National Policy Context

Annex A: Legal and National Planning Context

Disclaimer: This is a guide to legislation and procedure relating to biodiversity in England. It is general guidance, and it does not give specific advice in relation to any site, species, or project. It represents an interpretation of legislation and procedure as of June 2024. Readers should note that legislation and procedure changes continually and is interpreted on a case-specific basis. Nothing in this Annex should be construed as an offer of advice or legal opinion.

All British amphibian species receive legal protection in the United Kingdom though the degree to which different species are protected varies.

Great crested newts (GCN) *Triturus cristatus* and natterjack toads *Epidalea calamita* and their habitats (aquatic and terrestrial) are afforded full protection by a combination of national legislation.

They are listed under Schedule 5 of the Wildlife and Countryside Act 1981 (as amended) and under Schedule 2 of The Conservation of Habitats and Species Regulations 2017 (as amended). The Wildlife and Countryside Act 1981 is the domestic implementation of the Convention on the Conservation of European Wildlife and Natural Habitats (the Bern Convention) which was amended by the Countryside and Rights of Way Act 2000.

In brief, this legislation makes it an offence to:

- deliberately capture, kill, disturb, or injure these species;
- damage or destroy a breeding or resting place of these species;
- deliberately or recklessly obstruct access to resting or sheltering places of these species (deliberately or by not taking enough care);
- possessing, selling, controlling, or transporting live or dead specimens of these species, or parts of them; or
- take eggs of these species.

This Act is the domestic implementation of the Convention on the Conservation of European Wildlife and Natural Habitats (the Bern Convention). The Act has been amended by the Countryside and Rights of Way Act 2000 and by the Conservation of Habitats and Species Regulations 2017 (the 2017 Regulations), as amended.

The other more widespread British amphibians - common frog *Rana temporaria*, common toad *Bufo bufo*, smooth newt *Triturus vulgaris* and palmate newt *Triturus helveticus* - are not subject to such strict legal protection. These species are protected only against sale (Section 9(5) of the Wildlife and Countryside Act 1981).

In all cases, the legislation applies to all life stages including spawn, eggs, juveniles, and adults.

Section 40 of the Natural Environment and Rural Communities (NERC) Act 2006 imposes a Duty upon public authorities to conserve biodiversity. Specifically, this Act states that *'Every public authority must, in exercising its functions, have regard, so far as is consistent with the proper exercise of those functions, to the purpose of conserving biodiversity.'* Section 41 (S41) of the Act requires the Secretary of State to publish a list of habitats and species which are of principal importance for the conservation of biodiversity in England. The S41 list is used to guide decision-makers such as public bodies, including local and regional planning authorities, in implementing their 'biodiversity duty' imposed under Section 40.

Current amphibian species recorded on the list include GCN and common toad. Presence of S41 listed species, referred to as Species of Principal Importance (SPI) within influence of the site may be considered by the planning authority as a material consideration, irrespective of the level of legal protection afforded to the SPI.

Annex B: Detailed Survey Tables

Detailed Survey Tables

Table A.1: Pond Descriptions

Waterbody ID	Grid ref (NGR)	Photo	Description
P5	SK53270863		Hourglass shaped pond, northern part dry. Mature willows surround pond edge. Pond located amongst grassland fields with woodland approximately 70m west. Turbid water and signs of pollution runoff from fields. Rubble hibernacula present by pond.
P8	SK53460925		Very small pond in corner of field against hedgerows. Almost dry at time of survey. 100% shaded by trees and hedgerows.
P10	SK53580834		Small pond within arable field. Abundant algae present. Amphibious bistort/floating water plantain, willowherb, pondweed sp. Narrow 1m band of tall ruderal around pond edge, with one willow and one hawthorn tree on banks. Some grass encroachment into water. Very turbid. Depth approx. 1-2m.

Waterbody ID	Grid ref (NGR)	Photo	Description
P11	SK53731119		Square pond connected to field drain so constant flow of water. Abundant rush in middle and pond edge. Water depth average 5cm at time of survey. Evidence of deer using pond. Mud/stone substrate.
P12	SK53780969		Small pond (fenced off) in arable field. Mature and young trees around edge providing shade. Approx. 1m deep, shallow banks. Minimal vegetation for egg laying, some algae. Clear water. Smooth newt egg found.
P13	SK53790929		Small woodland copse pond by road and field. Clearly polluted, film on top of water and extremely turbid. Duckweed on 50% of surface, two patches of yellow iris. Pond surrounded by small area of scrub/woodland. Some egg laying vegetation in form of grasses.
P14	SK53880908		Very small pond on edge of grassland field. Southern bank adjacent to hedgerow and small willow trees providing shade. Grass species and willowherb abundant, almost 100% pond coverage. Dry at time of survey.

Waterbody ID	Grid ref (NGR)	Photo	Description
P16	SK54151006		Pond located across access path from reservoir (P48). Reservoir overflowing into P16. Northern bank inaccessible. Duckweed present. Common reed abundant at east and west ends of pond. Swan and mallard present nearby.
P19	SK54420769		Large SUDs pond surrounded by scrub and grassland, likely connected to A46 run-off. Evidence of pollution (film on water). Dense common reed and willowherb. Would hold a lot of water during peak wet weather/season. Evidence of otter (paw print in mud). Two inlets on north bank.
P20	SK54530740		Large woodland pond, 100% duckweed cover, a lot of leaf litter and fallen branches in water. Good terrestrial habitat including nearby hibernacula. No emergent or floating vegetation.
P21	SK54510735		Only most southwest body holding water, remainder of pond inaccessible due to vegetation. 100% duckweed cover. No egg laying vegetation. Lots of leaves and fallen branches. Low water level and turbid. Small woodland pond with likely overflow stream.

Waterbody ID	Grid ref (NGR)	Photo	Description
P22	SK54611198		Overflow pond for P23 on edge of woodland. Fish present in all ponds in garden so probable fish presence. P22 overflows into stream to south. Shallow depth approximately 0.5m, clear water with minimal signs of pollution. Yellow iris, rushes and grass vegetated banks. Watermint present. Pond has very slow flow leading to overflow.
P23	SK54651204		Large ornamental garden pond, stocked with fish: trout, carp. Mallard, swan, heron, moorhen, geese, coot seen. Large vegetated island with mature willows. Banks vegetated: grasses, iris, willowherb, reed, sweetgrass. Minimal floating vegetation, some submerged pondweed. Tadpoles, water boatmen observed.
P23a	SK54611208		One of two small ornamental ponds connected by a narrow channel (the second pond is located beyond the 250 m survey buffer). A single sturgeon is present. No floating vegetation. Manmade stone/concrete banks. Shallow water depth but clear. Some iris and sedge on one bank.

Waterbody ID	Grid ref (NGR)	Photo	Description
P25	SK54711164		Large pond surrounded by dense scrub. Access/view only possible from northern section. Pond had overflowed covering public footpath. No egg laying vegetation. Dense leaf litter at bottom.
P28	SK54821040		Woodland pond, inaccessible pond edge. Almost 100% shaded by mature trees and scrub. Likely good water depth - was turbid. Good terrestrial habitat.
P29	SK54941044		Pond with small copse of woodland. Surrounded by mature willows. Northern half of pond had dried. Dense scrub around pond edge. Dense duckweed in areas. Turbid water with dense leaf litter and fallen branches in water.
P30	SK55001049		Similar to P28 & P29. Within block of woodland. Dense scrub and trees creating almost 100% shade. Low water level, turbid. No egg laying vegetation.

Waterbody ID	Grid ref (NGR)	Photo	Description
P31	SK55011089		Small pond within woodland. Clear evidence of management/residential wildlife pond. Abundant floating and emergent vegetation. Mature tree canopy cover shading parts of pond.
P33	SK55161179		Woodland pond, heavily shaded. Approximate water depth 1m maximum. Some duckweed present but minimal egg laying vegetation. Dense leaf litter substrate.
P34	SK55191133		Large pond, likely overflows into P36. Large mature trees around pond edge create shade. Almost no duckweed or floating vegetation. Two islands with trees in the middle of pond. Varying levels beneath water (deep, shallow etc). Water depth 2m maximum. Minimal egg laying vegetation.
P34a	SK55141130		Once formed part of P34, with a 2-3m wide dam separating the two ponds. Abundant floating and submerged vegetation - pondweeds, willowherbs, rushes etc. Water depth approximately 2m at deepest.

Waterbody ID	Grid ref (NGR)	Photo	Description
P36	SK55261139		Large kidney shaped pond. 100% covered in duckweed. Moorhen, mallard and heron present. Vegetation includes yellow flag iris, rushes, bulrush, common reed.
P37	SK55411105		Large circular pond on edge of grassland field and garden. Fenced off to stop cattle ingress. Small vegetated island in middle. Some rushes on pond bank. Banks almost vertical. Fish seen during survey. Water depth likely quite deep. Very turbid water.
P39	SK55441036		Large ornamental pond at front of small industrial park. Surrounded by mown grassland, block of woodland to the south. Yellow iris dominates almost all pond edge (up to 1m). Small vegetated island in the middle.
P41	SK55931186		Rectangular pond, heavily shaded edge. Most of pond inaccessible. Willowherb and bramble dominating pond edge and willow. Minimal egg laying vegetation. Water depth assumed to be deep.

Waterbody ID	Grid ref (NGR)	Photo	Description
P43	SK56281097		Large pond on edge of arable field. Two young willow trees on pond edge. Abundant rush and emergent vegetation. Water depth approximately 20cm at time of survey, would hold more water at peak times. Abundant algae present. Pond has dried out a lot.
P44	SK56331099		Small pond close to pylon, possibly connected to field drain. Dense scrub surrounding almost entire pond edge. Abundant vegetation (sedges, rushes) providing suitable egg laying opportunities. Water depth approximately 50cm.
P45	SK56371107		Rectangular, man-made pond within fenced off area of wildflower meadow. Small island with tree. Bulrush and Common Reed dominate pond edge up to 1m from edge. Dense bramble dominates western bank.
P48a	SK55041092		Edge of reservoir with stone/rubble banks and some vegetation suitable for egg laying. Some sheltered areas with reduced water movement.

Table A.2: HSI Assessment Results

Waterbody ID	SI1 Location	SI2 Area	SI3 Permanence	SI4 Water Quality	SI5 Shade	SI6 Waterfowl	SI7 Fish	SI8 Density	SI9 Terrestrial Habitats	SI10 Macrophyte Cover	HSI Overall Score	Suitability Category
P5	1	0.5	0.5	0.33	0.6	1	1	1	0.33	0.4	0.60	Average
P6	Not surveyed – pond does not exist											
P8	1	0.05	0.1	0.33	0.2	1	1	0.9	0.33	0.4	0.36	Poor
P10	1	0.5	0.5	1	1	1	1	1	0.33	1	0.78	Good
P11	1	0.1	1	0.67	1	1	1	0.77	1	1	0.74	Good
P12	1	0.2	0.5	0.67	1	1	1	0.65	0.67	0.4	0.64	Average
P13	1	0.5	0.1	0.01	0.2	1	1	0.91	0.67	0.5	0.00	Poor
P14	1	0.2	0.1	0.33	1	1	1	0.975	0.33	0.8	0.53	Below Average
P16	1	0.95	1	0.33	0.8	0.67	0.67	0.82	1	0.8	0.77	Good
P18	Not surveyed – pond does not exist											
P19	1	0.9	0.1	0.33	1	0.67	1	0.38	1	1	0.61	Average
P20	1	0.98	1	0.33	0.2	1	1	0.55	1	0.35	0.64	Average



Waterbody ID	SI1 Location	SI2 Area	SI3 Permanence	SI4 Water Quality	SI5 Shade	SI6 Waterfowl	SI7 Fish	SI8 Density	SI9 Terrestrial Habitats	SI10 Macrophyte Cover	HSI Overall Score	Suitability Category
P21	1	0.3	0.1	0.33	0.2	1	1	0.55	1	0.35	0.46	Poor
P22	1	0.8	0.9	0.67	1	0.67	0.67	0.82	1	0.5	0.78	Good
P23	1	Null	0.9	1	1	0.67	0.01	0.72	1	0.4	0.49	Poor
P23a	1	0.05	0.9	0.33	1	0.67	0.33	0.77	0.67	0.4	0.48	Poor
P25	1	Null	1	0.67	0.2	1	1	0.77	1	0.4	0.70	Good
P28	1	0.7	0.5	0.33	0.3	1	1	0.82	1	0.4	0.64	Average
P29	1	0.5	0.5	0.33	0.2	1	1	0.82	1	0.4	0.59	Below Average
P30	1	0.3	0.1	0.33	0.2	1	1	0.82	1	0.35	0.47	Poor
P31	1	0.4	1	1	1	1	1	0.77	1	0.7	0.86	Excellent
P33	1	0.3	0.5	1	0.4	1	1	0.72	1	0.5	0.68	Average
P34	1	0.88	0.9	1	1	0.67	0.67	0.55	1	0.4	0.78	Good
P34a	1	0.98	0.9	1	1	0.67	0.67	0.55	1	0.7	0.83	Excellent
P35	Not surveyed – no access											



Waterbody ID	SI1 Location	SI2 Area	SI3 Permanence	SI4 Water Quality	SI5 Shade	SI6 Waterfowl	SI7 Fish	SI8 Density	SI9 Terrestrial Habitats	SI10 Macrophyte Cover	HSI Overall Score	Suitability Category
P36	1	0.95	0.9	0.67	1	0.67	0.67	0.55	1	0.5	0.77	Good
P37	1	0.99	0.9	0.67	1	1	0.33	0.38	0.67	0.4	0.68	Average
P38	Not surveyed – no access											
P39	1	1	0.9	0.67	1	1	1	0.89	0.67	0.5	0.84	Excellent
P41	1	0.99	0.9	1	0.6	1	0.67	0.9	1	0.4	0.81	Excellent
P42	Not surveyed – pond does not exist											
P43	1	0.95	0.1	0.67	1	0.67	1	0.65	0.67	0.8	0.66	Average
P44	1	0.1	0.1	0.67	0.5	1	1	0.65	0.67	1	0.52	Below Average
P45	1	0.4	0.9	0.67	1	1	1	0.72	1	0.6	0.80	Good
P48a	1	Null	0.9	0.33	1	0.67	0.67	0.77	0.67	0.8	0.7	Good

Annex C: ADAS eDNA Survey Results

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Sample ID: ADAS-5655 Condition on Receipt: Low Sediment Volume: Passed
Client Identifier: P20 Hallgates Description: pond water samples in preservative
Date of Receipt: 14/06/2024 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	19/06/2024
Degradation Control [§]	Within Limits	Real Time PCR	19/06/2024
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	19/06/2024
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by: Dr Helen Rees Report Issued by: Dr Ben Maddison

Signed:

Signed:

Position: Director: Biotechnology Position: MD: Biotechnology

Date of preparation: 21/06/2024 Date of issue: 21/06/2024

eDNA analysis was carried out in accordance with the stipulated methodology found in the Technical Advice Note (WC1067 Appendix 5 Technical Advice Note) published by DEFRA and adopted by Natural England.

** If all PCR controls and extraction blanks give the expected results a sample is considered: negative for great crested newt if all of the replicates are negative; positive for great crested newt if one or more of the replicates are positive.*

[†] Recorded as the number of positive replicate reactions at expected C_t value. If the expected C_t value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.

[§] No degradation is expected within time frame of kit preparation, sample collection and analysis.

[#] Additional positive controls (10⁻¹, 10⁻², 10⁻³ ng/μL) are also routinely run, results not shown here.

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Sample ID: ADAS-5657 Condition on Receipt: Low Sediment Volume: Passed
Client Identifier: P21 Hallgates Description: pond water samples in preservative
Date of Receipt: 14/06/2024 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	19/06/2024
Degradation Control [§]	Within Limits	Real Time PCR	19/06/2024
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	19/06/2024
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by: Dr Helen Rees Report Issued by: Dr Ben Maddison

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Position: Director: Biotechnology Position: MD: Biotechnology

Date of preparation: 21/06/2024 Date of issue: 21/06/2024

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[†] Recorded as the number of positive replicate reactions at expected C_t value. If the expected C_t value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.

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[#] Additional positive controls (10⁻¹, 10⁻², 10⁻³ ng/μL) are also routinely run, results not shown here.

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Sample ID: ADAS-5658 Condition on Receipt: High Sediment Volume: Passed
Client Identifier: P19 Hallgates Description: pond water samples in preservative
Date of Receipt: 14/06/2024 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	19/06/2024
Degradation Control [§]	Within Limits	Real Time PCR	19/06/2024
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	19/06/2024
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by: Dr Helen Rees Report Issued by: Dr Ben Maddison

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Date of preparation: 21/06/2024 Date of issue: 21/06/2024

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[#] Additional positive controls (10⁻¹, 10⁻², 10⁻³ ng/μL) are also routinely run, results not shown here.

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Sample ID: ADAS-5661 Condition on Receipt: Low Sediment Volume: Passed
Client Identifier: P5 Hallgates Description: pond water samples in preservative
Date of Receipt: 14/06/2024 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	19/06/2024
Degradation Control [§]	Within Limits	Real Time PCR	19/06/2024
Great Crested Newt*	2 of 12 (GCN positive)	Real Time PCR	19/06/2024
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by: Dr Helen Rees Report Issued by: Dr Ben Maddison

Signed:

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Signed:

A handwritten signature in black ink, appearing to read "B. Maddison".

Position: Director: Biotechnology Position: MD: Biotechnology

Date of preparation: 21/06/2024 Date of issue: 21/06/2024

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[†] Recorded as the number of positive replicate reactions at expected C_t value. If the expected C_t value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.

[§] No degradation is expected within time frame of kit preparation, sample collection and analysis.

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Sample ID: ADAS-5662 Condition on Receipt: Good Volume: Passed
Client Identifier: P23 Hallgates Description: pond water samples in preservative
Date of Receipt: 14/06/2024 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	19/06/2024
Degradation Control [§]	Within Limits	Real Time PCR	19/06/2024
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	19/06/2024
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [#]	4 of 4	Real Time PCR	As above for GCN

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Date of preparation: 21/06/2024 Date of issue: 21/06/2024

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[†] Recorded as the number of positive replicate reactions at expected C_t value. If the expected C_t value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.

[§] No degradation is expected within time frame of kit preparation, sample collection and analysis.

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Sample ID: ADAS-5663 Condition on Receipt: Medium Sediment Volume: Passed
Client Identifier: P10 Hallgates Description: pond water samples in preservative
Date of Receipt: 14/06/2024 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	18/06/2024
Degradation Control [§]	Within Limits	Real Time PCR	18/06/2024
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	18/06/2024
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by: Dr Helen Rees Report Issued by: Dr Ben Maddison

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Date of preparation: 21/06/2024 Date of issue: 21/06/2024

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[†] Recorded as the number of positive replicate reactions at expected C_t value. If the expected C_t value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.

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[#] Additional positive controls (10⁻¹, 10⁻², 10⁻³ ng/μL) are also routinely run, results not shown here.

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Sample ID: ADAS-5664 Condition on Receipt: Low Sediment Volume: Passed
Client Identifier: P34A Hallgates Description: pond water samples in preservative
Date of Receipt: 14/06/2024 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	19/06/2024
Degradation Control [§]	Within Limits	Real Time PCR	19/06/2024
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	19/06/2024
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by: Dr Helen Rees Report Issued by: Dr Ben Maddison

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Date of preparation: 21/06/2024 Date of issue: 21/06/2024

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** If all PCR controls and extraction blanks give the expected results a sample is considered: negative for great crested newt if all of the replicates are negative; positive for great crested newt if one or more of the replicates are positive.*

[†] Recorded as the number of positive replicate reactions at expected C_t value. If the expected C_t value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.

[§] No degradation is expected within time frame of kit preparation, sample collection and analysis.

[#] Additional positive controls (10⁻¹, 10⁻², 10⁻³ ng/μL) are also routinely run, results not shown here.

Client: Nicola Morris,
The Environment Partnership



ADAS
Spring Lodge
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Tel: 01159 229249
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Sample ID: ADAS-5665 Condition on Receipt: Low Sediment Volume: Passed
Client Identifier: P37 Hallgates Description: pond water samples in preservative
Date of Receipt: 14/06/2024 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	19/06/2024
Degradation Control [§]	Within Limits	Real Time PCR	19/06/2024
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	19/06/2024
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by: Dr Helen Rees Report Issued by: Dr Ben Maddison

Signed:

Signed:

Position: Director: Biotechnology Position: MD: Biotechnology

Date of preparation: 21/06/2024 Date of issue: 21/06/2024

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[†] Recorded as the number of positive replicate reactions at expected C_t value. If the expected C_t value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.

[§] No degradation is expected within time frame of kit preparation, sample collection and analysis.

[#] Additional positive controls (10⁻¹, 10⁻², 10⁻³ ng/μL) are also routinely run, results not shown here.

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Sample ID: ADAS-5666 Condition on Receipt: Good Volume: Passed
Client Identifier: P23A Hallgates Description: pond water samples in preservative
Date of Receipt: 14/06/2024 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	20/06/2024
Degradation Control [§]	Within Limits	Real Time PCR	20/06/2024
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	20/06/2024
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by: Dr Helen Rees Report Issued by: Dr Ben Maddison

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Position: Director: Biotechnology Position: MD: Biotechnology

Date of preparation: 21/06/2024 Date of issue: 21/06/2024

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** If all PCR controls and extraction blanks give the expected results a sample is considered: negative for great crested newt if all of the replicates are negative; positive for great crested newt if one or more of the replicates are positive.*

[†] Recorded as the number of positive replicate reactions at expected C_t value. If the expected C_t value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.

[§] No degradation is expected within time frame of kit preparation, sample collection and analysis.

[#] Additional positive controls (10⁻¹, 10⁻², 10⁻³ ng/μL) are also routinely run, results not shown here.

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Sample ID: ADAS-5667 Condition on Receipt: Low Sediment Volume: Passed
Client Identifier: P16 Hallgates Description: pond water samples in preservative
Date of Receipt: 14/06/2024 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	19/06/2024
Degradation Control [§]	Within Limits	Real Time PCR	19/06/2024
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	19/06/2024
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by: Dr Helen Rees Report Issued by: Dr Ben Maddison

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Position: Director: Biotechnology Position: MD: Biotechnology

Date of preparation: 21/06/2024 Date of issue: 21/06/2024

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** If all PCR controls and extraction blanks give the expected results a sample is considered: negative for great crested newt if all of the replicates are negative; positive for great crested newt if one or more of the replicates are positive.*

[†] Recorded as the number of positive replicate reactions at expected C_t value. If the expected C_t value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.

[§] No degradation is expected within time frame of kit preparation, sample collection and analysis.

[#] Additional positive controls (10⁻¹, 10⁻², 10⁻³ ng/μL) are also routinely run, results not shown here.

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Sample ID: ADAS-5668 Condition on Receipt: Good Volume: Passed
Client Identifier: P23B Hallgates Description: pond water samples in preservative
Date of Receipt: 14/06/2024 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	19/06/2024
Degradation Control [§]	Within Limits	Real Time PCR	19/06/2024
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	19/06/2024
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by: Dr Helen Rees Report Issued by: Dr Ben Maddison

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Position: Director: Biotechnology Position: MD: Biotechnology

Date of preparation: 21/06/2024 Date of issue: 21/06/2024

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[†] Recorded as the number of positive replicate reactions at expected C_t value. If the expected C_t value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.

[§] No degradation is expected within time frame of kit preparation, sample collection and analysis.

[#] Additional positive controls (10⁻¹, 10⁻², 10⁻³ ng/μL) are also routinely run, results not shown here.

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Sample ID: ADAS-5673 Condition on Receipt: Low Sediment Volume: Passed
Client Identifier: P48A Hallgates Description: pond water samples in preservative
Date of Receipt: 14/06/2024 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	18/06/2024
Degradation Control [§]	Within Limits	Real Time PCR	18/06/2024
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	18/06/2024
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by: Dr Helen Rees Report Issued by: Dr Ben Maddison

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Position: Director: Biotechnology Position: MD: Biotechnology

Date of preparation: 21/06/2024 Date of issue: 21/06/2024

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[†] Recorded as the number of positive replicate reactions at expected C_t value. If the expected C_t value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.

[§] No degradation is expected within time frame of kit preparation, sample collection and analysis.

[#] Additional positive controls (10⁻¹, 10⁻², 10⁻³ ng/μL) are also routinely run, results not shown here.

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Sample ID: ADAS-5674 Condition on Receipt: Good Volume: Passed
Client Identifier: P30 Hallgates Description: pond water samples in preservative
Date of Receipt: 14/06/2024 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	18/06/2024
Degradation Control [§]	Within Limits	Real Time PCR	18/06/2024
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	18/06/2024
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by: Dr Helen Rees Report Issued by: Dr Ben Maddison

Signed:

A handwritten signature in black ink, appearing to read "H. Rees".

Signed:

A handwritten signature in black ink, appearing to read "B. Maddison".

Position: Director: Biotechnology Position: MD: Biotechnology

Date of preparation: 21/06/2024 Date of issue: 21/06/2024

eDNA analysis was carried out in accordance with the stipulated methodology found in the Technical Advice Note (WC1067 Appendix 5 Technical Advice Note) published by DEFRA and adopted by Natural England.

** If all PCR controls and extraction blanks give the expected results a sample is considered: negative for great crested newt if all of the replicates are negative; positive for great crested newt if one or more of the replicates are positive.*

[†] Recorded as the number of positive replicate reactions at expected C_t value. If the expected C_t value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.

[§] No degradation is expected within time frame of kit preparation, sample collection and analysis.

[#] Additional positive controls (10⁻¹, 10⁻², 10⁻³ ng/μL) are also routinely run, results not shown here.

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Sample ID: ADAS-5675 Condition on Receipt: Medium Sediment Volume: Passed
Client Identifier: P13 Hallgates Description: pond water samples in preservative
Date of Receipt: 14/06/2024 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	0 of 2	Real Time PCR	19/06/2024
Degradation Control [§]	Within Limits	Real Time PCR	19/06/2024
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	19/06/2024
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by: Dr Helen Rees Report Issued by: Dr Ben Maddison

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Position: Director: Biotechnology Position: MD: Biotechnology

Date of preparation: 21/06/2024 Date of issue: 21/06/2024

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** If all PCR controls and extraction blanks give the expected results a sample is considered: negative for great crested newt if all of the replicates are negative; positive for great crested newt if one or more of the replicates are positive.*

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[§] No degradation is expected within time frame of kit preparation, sample collection and analysis.

[#] Additional positive controls (10⁻¹, 10⁻², 10⁻³ ng/μL) are also routinely run, results not shown here.

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Sample ID: ADAS-5676 Condition on Receipt: Good Volume: Passed
Client Identifier: P22 Hallgates Description: pond water samples in preservative
Date of Receipt: 14/06/2024 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	19/06/2024
Degradation Control [§]	Within Limits	Real Time PCR	19/06/2024
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	19/06/2024
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by: Dr Helen Rees Report Issued by: Dr Ben Maddison

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Position: Director: Biotechnology Position: MD: Biotechnology

Date of preparation: 21/06/2024 Date of issue: 21/06/2024

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[#] Additional positive controls (10⁻¹, 10⁻², 10⁻³ ng/μL) are also routinely run, results not shown here.

Client: Nicola Morris,
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Sample ID: ADAS-5677 Condition on Receipt: Low Sediment Volume: Passed
Client Identifier: P31 Hallgates Description: pond water samples in preservative
Date of Receipt: 14/06/2024 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	18/06/2024
Degradation Control [§]	Within Limits	Real Time PCR	18/06/2024
Great Crested Newt*	12 of 12 (GCN positive)	Real Time PCR	18/06/2024
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by: Dr Helen Rees Report Issued by: Dr Ben Maddison

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Position: Director: Biotechnology Position: MD: Biotechnology

Date of preparation: 21/06/2024 Date of issue: 21/06/2024

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** If all PCR controls and extraction blanks give the expected results a sample is considered: negative for great crested newt if all of the replicates are negative; positive for great crested newt if one or more of the replicates are positive.*

[†] Recorded as the number of positive replicate reactions at expected C_t value. If the expected C_t value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.

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[#] Additional positive controls (10⁻¹, 10⁻², 10⁻³ ng/μL) are also routinely run, results not shown here.

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Sample ID: ADAS-5678 Condition on Receipt: Low Sediment Volume: Passed
Client Identifier: P39 Hallgates Description: pond water samples in preservative
Date of Receipt: 14/06/2024 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	1 of 2	Real Time PCR	19/06/2024
Degradation Control [§]	Within Limits	Real Time PCR	19/06/2024
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	19/06/2024
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by: Dr Helen Rees Report Issued by: Dr Ben Maddison

Signed:

A handwritten signature in black ink, appearing to read "H. Rees".

Signed:

A handwritten signature in black ink, appearing to read "B. Maddison".

Position: Director: Biotechnology Position: MD: Biotechnology

Date of preparation: 21/06/2024 Date of issue: 21/06/2024

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[†] Recorded as the number of positive replicate reactions at expected C_t value. If the expected C_t value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.

[§] No degradation is expected within time frame of kit preparation, sample collection and analysis.

[#] Additional positive controls (10⁻¹, 10⁻², 10⁻³ ng/μL) are also routinely run, results not shown here.

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Sample ID: ADAS-5679 Condition on Receipt: Good Volume: Passed
Client Identifier: P45 Hallgates Description: pond water samples in preservative
Date of Receipt: 14/06/2024 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	18/06/2024
Degradation Control [§]	Within Limits	Real Time PCR	18/06/2024
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	18/06/2024
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by: Dr Helen Rees Report Issued by: Dr Ben Maddison

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Position: Director: Biotechnology Position: MD: Biotechnology

Date of preparation: 21/06/2024 Date of issue: 21/06/2024

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** If all PCR controls and extraction blanks give the expected results a sample is considered: negative for great crested newt if all of the replicates are negative; positive for great crested newt if one or more of the replicates are positive.*

[†] Recorded as the number of positive replicate reactions at expected C_t value. If the expected C_t value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.

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Sample ID: ADAS-5680 Condition on Receipt: Algae Present Volume: Passed
Client Identifier: P43 Hallgates Description: pond water samples in preservative
Date of Receipt: 14/06/2024 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	18/06/2024
Degradation Control [§]	Within Limits	Real Time PCR	18/06/2024
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	18/06/2024
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by: Dr Helen Rees Report Issued by: Dr Ben Maddison

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Position: Director: Biotechnology Position: MD: Biotechnology

Date of preparation: 21/06/2024 Date of issue: 21/06/2024

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Sample ID: ADAS-5681 Condition on Receipt: Good Volume: Passed
Client Identifier: P41 Hallgates Description: pond water samples in preservative
Date of Receipt: 14/06/2024 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	19/06/2024
Degradation Control [§]	Within Limits	Real Time PCR	19/06/2024
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	19/06/2024
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by: Dr Helen Rees Report Issued by: Dr Ben Maddison

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Position: Director: Biotechnology Position: MD: Biotechnology

Date of preparation: 21/06/2024 Date of issue: 21/06/2024

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[#] Additional positive controls (10⁻¹, 10⁻², 10⁻³ ng/μL) are also routinely run, results not shown here.

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Sample ID: ADAS-5682 Condition on Receipt: Good Volume: Passed
Client Identifier: P44 Hallgates Description: pond water samples in preservative
Date of Receipt: 14/06/2024 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	18/06/2024
Degradation Control [§]	Within Limits	Real Time PCR	18/06/2024
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	18/06/2024
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by: Dr Helen Rees Report Issued by: Dr Ben Maddison

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Position: Director: Biotechnology Position: MD: Biotechnology

Date of preparation: 21/06/2024 Date of issue: 21/06/2024

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Sample ID: ADAS-5683 Condition on Receipt: Low Sediment Volume: Passed
Client Identifier: P12 Hallgates Description: pond water samples in preservative
Date of Receipt: 14/06/2024 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	19/06/2024
Degradation Control [§]	Within Limits	Real Time PCR	19/06/2024
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	19/06/2024
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by: Dr Helen Rees Report Issued by: Dr Ben Maddison

Signed:  Signed: 

Position: Director: Biotechnology Position: MD: Biotechnology

Date of preparation: 21/06/2024 Date of issue: 21/06/2024

eDNA analysis was carried out in accordance with the stipulated methodology found in the Technical Advice Note (WC1067 Appendix 5 Technical Advice Note) published by DEFRA and adopted by Natural England.

** If all PCR controls and extraction blanks give the expected results a sample is considered: negative for great crested newt if all of the replicates are negative; positive for great crested newt if one or more of the replicates are positive.*

[†] Recorded as the number of positive replicate reactions at expected C_t value. If the expected C_t value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.

[§] No degradation is expected within time frame of kit preparation, sample collection and analysis.

[#] Additional positive controls (10⁻¹, 10⁻², 10⁻³ ng/μL) are also routinely run, results not shown here.

Client: Nicola Morris,
The Environment Partnership



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Sample ID: ADAS-5684 Condition on Receipt: Good Volume: Passed
Client Identifier: P36 Hallgates Description: pond water samples in preservative
Date of Receipt: 14/06/2024 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	19/06/2024
Degradation Control [§]	Within Limits	Real Time PCR	19/06/2024
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	19/06/2024
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by: Dr Helen Rees Report Issued by: Dr Ben Maddison

Signed:

Signed:

Position: Director: Biotechnology Position: MD: Biotechnology

Date of preparation: 21/06/2024 Date of issue: 21/06/2024

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[†] Recorded as the number of positive replicate reactions at expected C_t value. If the expected C_t value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.

[§] No degradation is expected within time frame of kit preparation, sample collection and analysis.

[#] Additional positive controls (10⁻¹, 10⁻², 10⁻³ ng/μL) are also routinely run, results not shown here.

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Sample ID: ADAS-5685 Condition on Receipt: Low Sediment Volume: Passed
Client Identifier: P11 Hallgates Description: pond water samples in preservative
Date of Receipt: 14/06/2024 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	19/06/2024
Degradation Control [§]	Within Limits	Real Time PCR	19/06/2024
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	19/06/2024
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by: Dr Helen Rees Report Issued by: Dr Ben Maddison

Signed:  Signed: 

Position: Director: Biotechnology Position: MD: Biotechnology

Date of preparation: 21/06/2024 Date of issue: 21/06/2024

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[§] No degradation is expected within time frame of kit preparation, sample collection and analysis.

[#] Additional positive controls (10⁻¹, 10⁻², 10⁻³ ng/μL) are also routinely run, results not shown here.

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Sample ID: ADAS-5686 Condition on Receipt: Good Volume: Passed
Client Identifier: P34 Hallgates Description: pond water samples in preservative
Date of Receipt: 14/06/2024 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	19/06/2024
Degradation Control [§]	Within Limits	Real Time PCR	19/06/2024
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	19/06/2024
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by: Dr Helen Rees Report Issued by: Dr Ben Maddison

Signed:

Signed:

Position: Director: Biotechnology Position: MD: Biotechnology

Date of preparation: 21/06/2024 Date of issue: 21/06/2024

eDNA analysis was carried out in accordance with the stipulated methodology found in the Technical Advice Note (WC1067 Appendix 5 Technical Advice Note) published by DEFRA and adopted by Natural England.

** If all PCR controls and extraction blanks give the expected results a sample is considered: negative for great crested newt if all of the replicates are negative; positive for great crested newt if one or more of the replicates are positive.*

[†] Recorded as the number of positive replicate reactions at expected C_t value. If the expected C_t value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.

[§] No degradation is expected within time frame of kit preparation, sample collection and analysis.

[#] Additional positive controls (10⁻¹, 10⁻², 10⁻³ ng/μL) are also routinely run, results not shown here.

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Sample ID: ADAS-5687 Condition on Receipt: Medium Sediment Volume: Passed
Client Identifier: P33 Hallgates Description: pond water samples in preservative
Date of Receipt: 14/06/2024 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	19/06/2024
Degradation Control [§]	Within Limits	Real Time PCR	19/06/2024
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	19/06/2024
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by: Dr Helen Rees Report Issued by: Dr Ben Maddison

Signed:

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Position: Director: Biotechnology Position: MD: Biotechnology

Date of preparation: 21/06/2024 Date of issue: 21/06/2024

eDNA analysis was carried out in accordance with the stipulated methodology found in the Technical Advice Note (WC1067 Appendix 5 Technical Advice Note) published by DEFRA and adopted by Natural England.

** If all PCR controls and extraction blanks give the expected results a sample is considered: negative for great crested newt if all of the replicates are negative; positive for great crested newt if one or more of the replicates are positive.*

[†] Recorded as the number of positive replicate reactions at expected C_t value. If the expected C_t value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.

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[#] Additional positive controls (10⁻¹, 10⁻², 10⁻³ ng/μL) are also routinely run, results not shown here.

Appendix 1: Interpretation of results

Sample Condition

Upon sample receipt we score your samples according to quality: good, low sediment, medium sediment, high sediment, white precipitate, and presence of algae.

There are three reasons as to why sediment should be avoided:

1. It is possible for DNA to persist within the sediment for longer than it would if it was floating in the water which could lead to a false positive result i.e. in this case GCN not recently present but present a long time ago
2. In some cases sediment can cause inhibition of the PCR analysis used to detect GCN eDNA within samples which could lead to an indeterminate result.
3. In some cases sediment can interfere with the DNA extraction procedure resulting in poor recovery of the eDNA which in turn can lead to an indeterminate result.

Algae can make the DNA extraction more difficult to perform so if it can be avoided then this is helpful.

Sometimes samples contain a white precipitate which we have found makes the recovery of eDNA very difficult. This precipitate can be present in such high amounts that it interferes with the eDNA extraction process meaning that we cannot recover the degradation control (nor most likely the eDNA itself) at sufficient levels for the control to be within the acceptable limits for the assay, therefore we have to classify these type of samples as indeterminate.

What do my results mean?

A positive result means that great crested newts are present in the water or have been present in the water in the recent past (eDNA degrades over around 7-21 days).

A negative result means that DNA from the great crested newt has not been detected in your sample.

On occasion an inconclusive result will be issued. This occurs where the DNA from the great crested newt has not been detected but the controls have indicated that either: the sample has been degraded and/or the eDNA was not fully extracted (poor recovery); or the PCR inhibited in some way. This may be due to the water chemistry or may be due to the presence of high levels of sediment in samples which can interfere with the DNA extraction process. A re-test could be performed but a fresh sample would need to be obtained. We have successfully performed re-tests on samples which have had high sediment content on the first collection and low sediment content (through improved sample collection) on the re-test. If water chemistry was the cause of the indeterminate then a re-test would most likely also return an inconclusive result.

The results will be recorded as indeterminate if the GCN result is negative and the degradation result is recorded as:

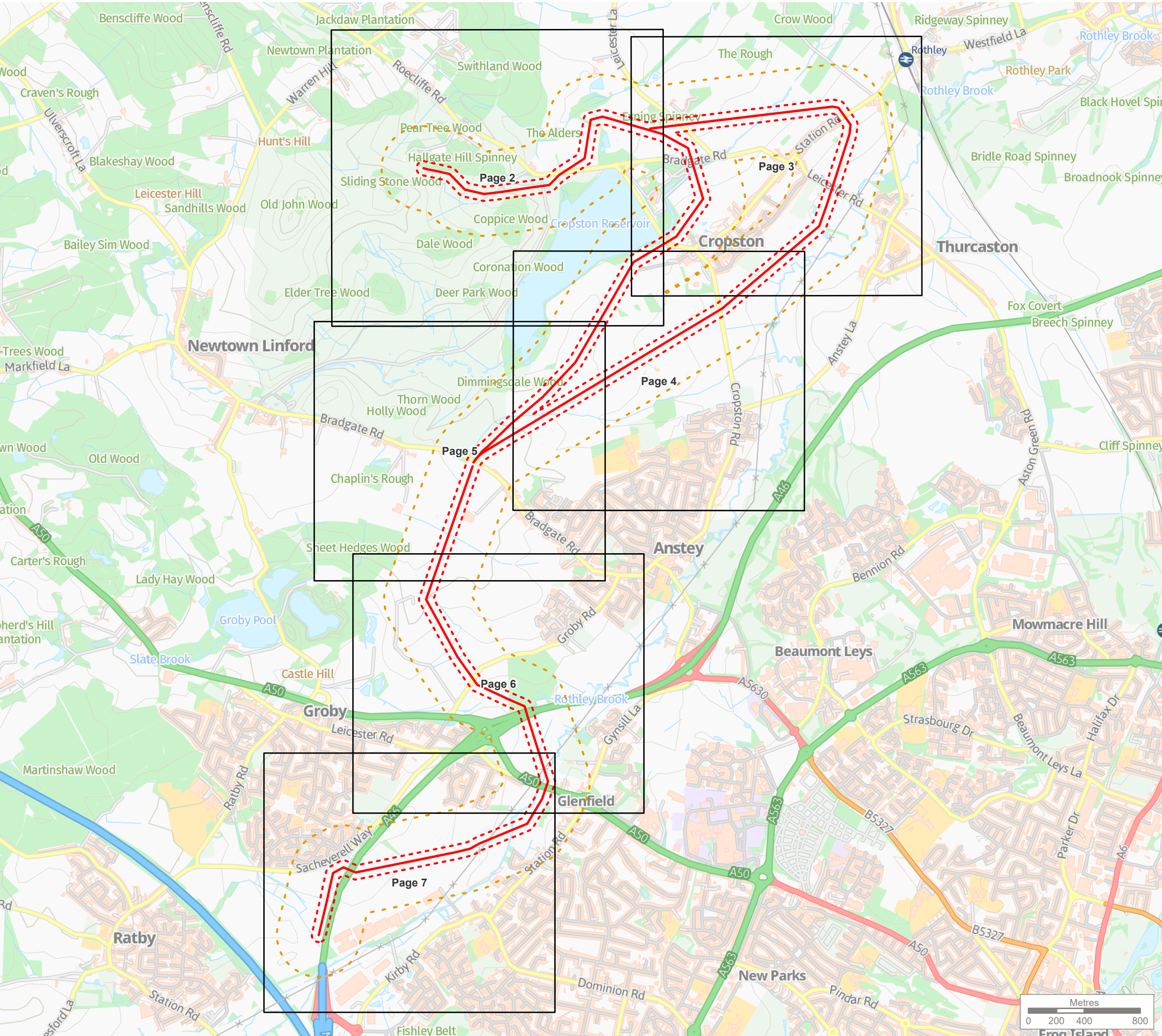
1. evidence of decay - meaning that the degradation control was outside of accepted limits
2. evidence of degradation or residual inhibition - meaning that the degradation control was outside of accepted limits but that this could have been due to inhibitors not being removed sufficiently by the dilution of inhibited samples (according to the technical advice note)

Drawings

Drawing 1: Pond Location Plan (G10480.001)

Drawing 2: Great Crested Newt Habitat Suitability Index Assessment Results
(G10480.012)

Drawing 3: Great Crested Newt eDNA Survey Results (G10480.014)



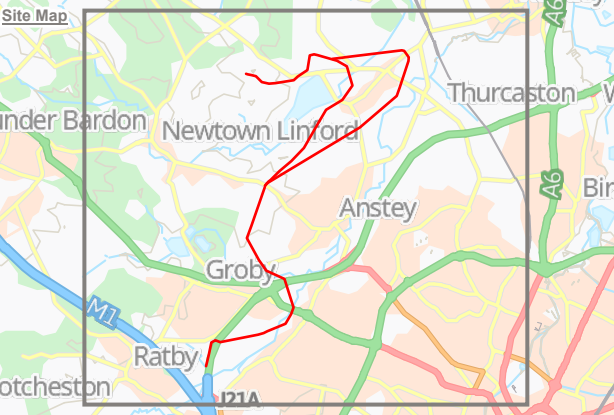
KEY

- Page and page number
- Pipeline Route
- Pipeline Route - 50 m buffer (the site)
- Pipeline Route - 300 m buffer (within 250 m of the site)

Note:
The locations of habitats and habitat features are indicative.

Ponds between 250 m and 500 m of the site were not subject to survey and are displayed with transparency

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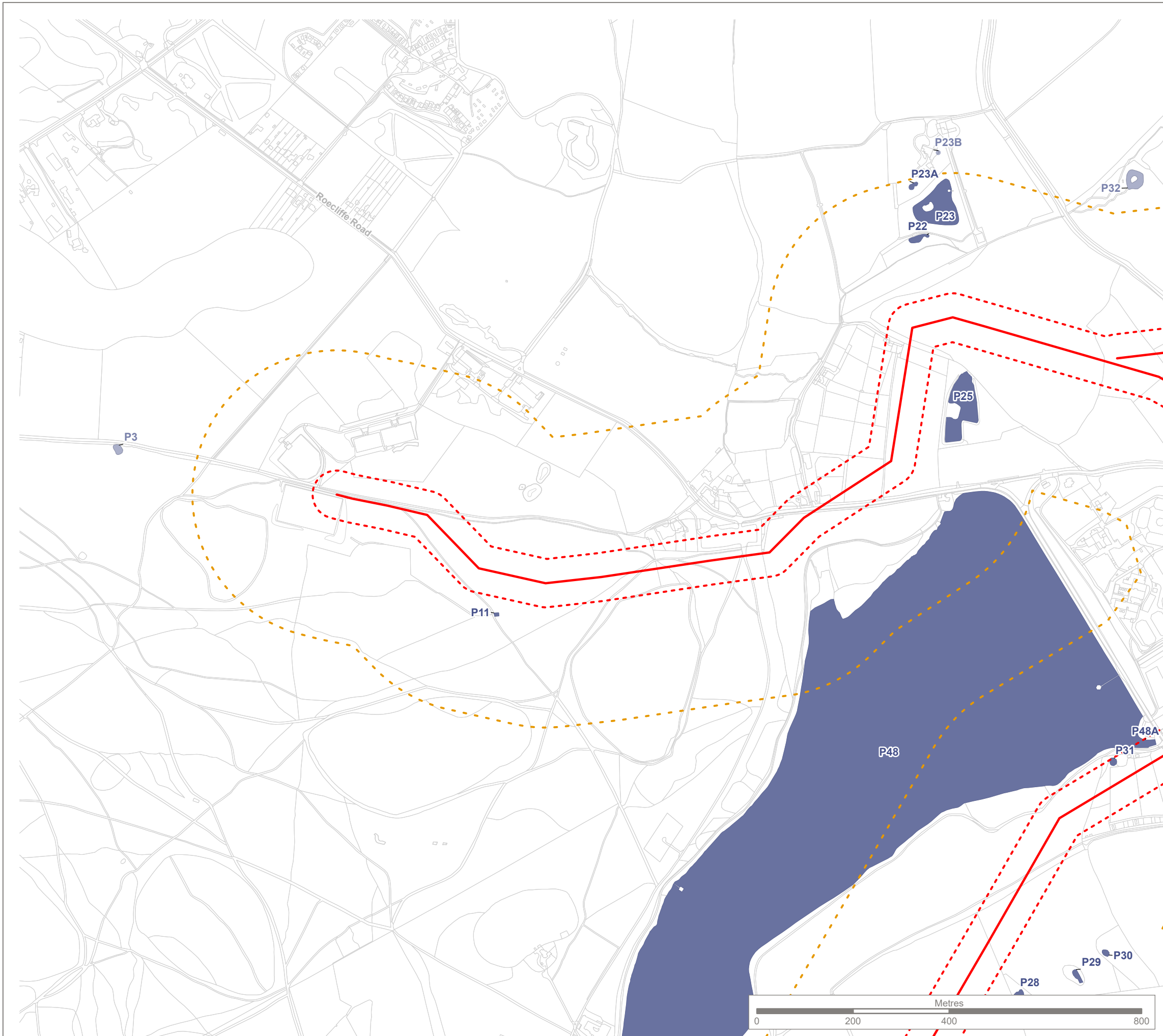
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Project
Hallgates to Elms Farm Pipeline, Leicestershire

Title
Pond Location Plan
OS

Drawing Number G10480.001.1	Page 1 of 7
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Drawn	Checked	Approved	Scale	Date
OH	SP	AP	1:28,000 @ A3	15/08/2024



KEY

- Pipeline Route
- Pipeline Route - 50 m buffer (the site)
- Pipeline Route - 300 m buffer (within 250 m of the site)
- Ponds
- Ponds outside of 300 m buffer

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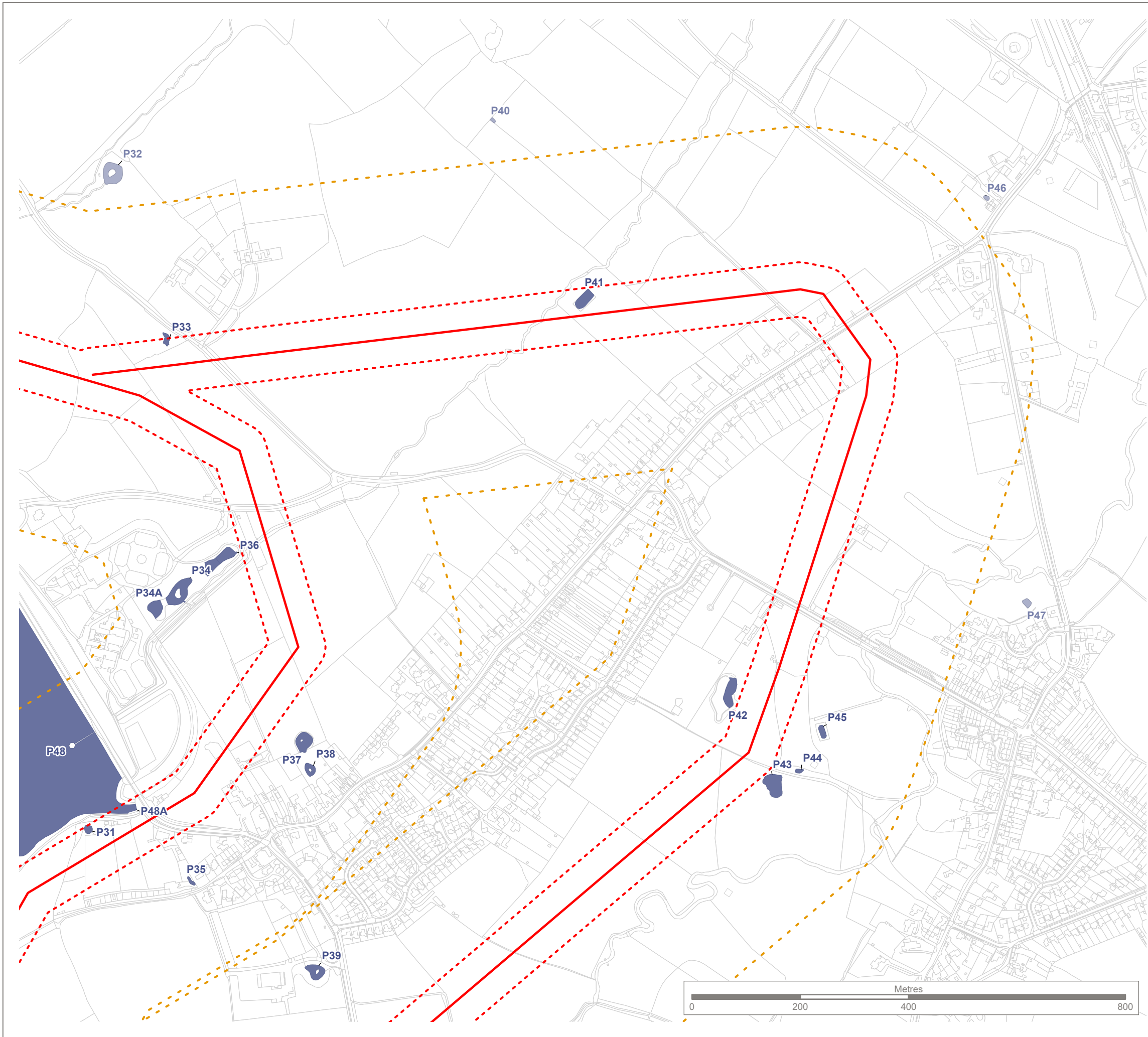
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Project
Hallgates to Elms Farm Pipeline, Leicestershire

Title
Pond Location Plan
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Drawing Number G10480.001.2	Page 2 of 7
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KEY

- Pipeline Route
- Pipeline Route - 50 m buffer (the site)
- Pipeline Route - 300 m buffer (within 250 m of the site)
- Ponds
- Ponds outside of 300 m buffer

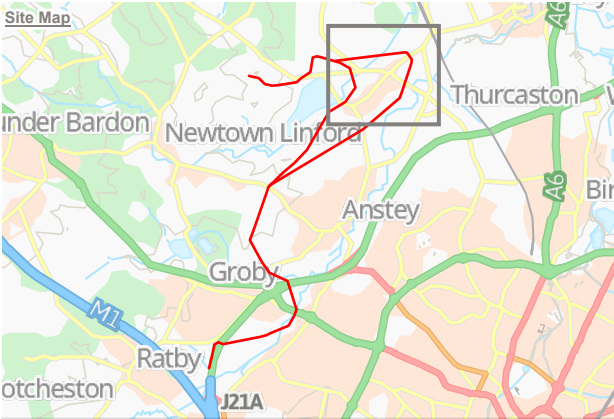
Note:

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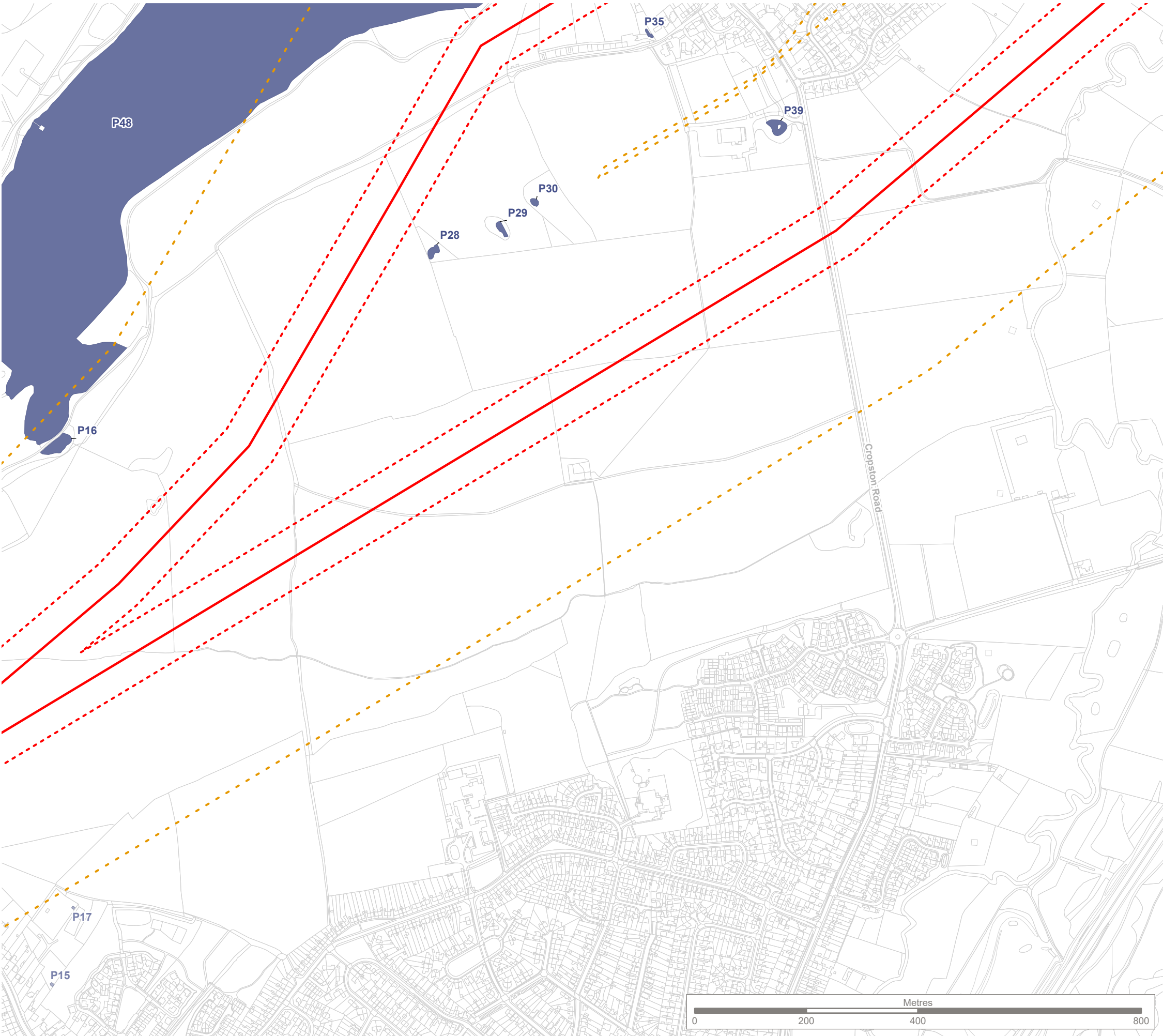
Project
Hallgates to Elms Farm Pipeline, Leicestershire

Title
Pond Location Plan
OS

Drawing Number
G10480.001.3

Page 3 of 7

Drawn	Checked	Approved	Scale	Date
OH	SP	AP	1:7,000 @ A3	15/08/2024



KEY

- Pipeline Route
- Pipeline Route - 50 m buffer (the site)
- Pipeline Route - 300 m buffer (within 250 m of the site)
- Ponds
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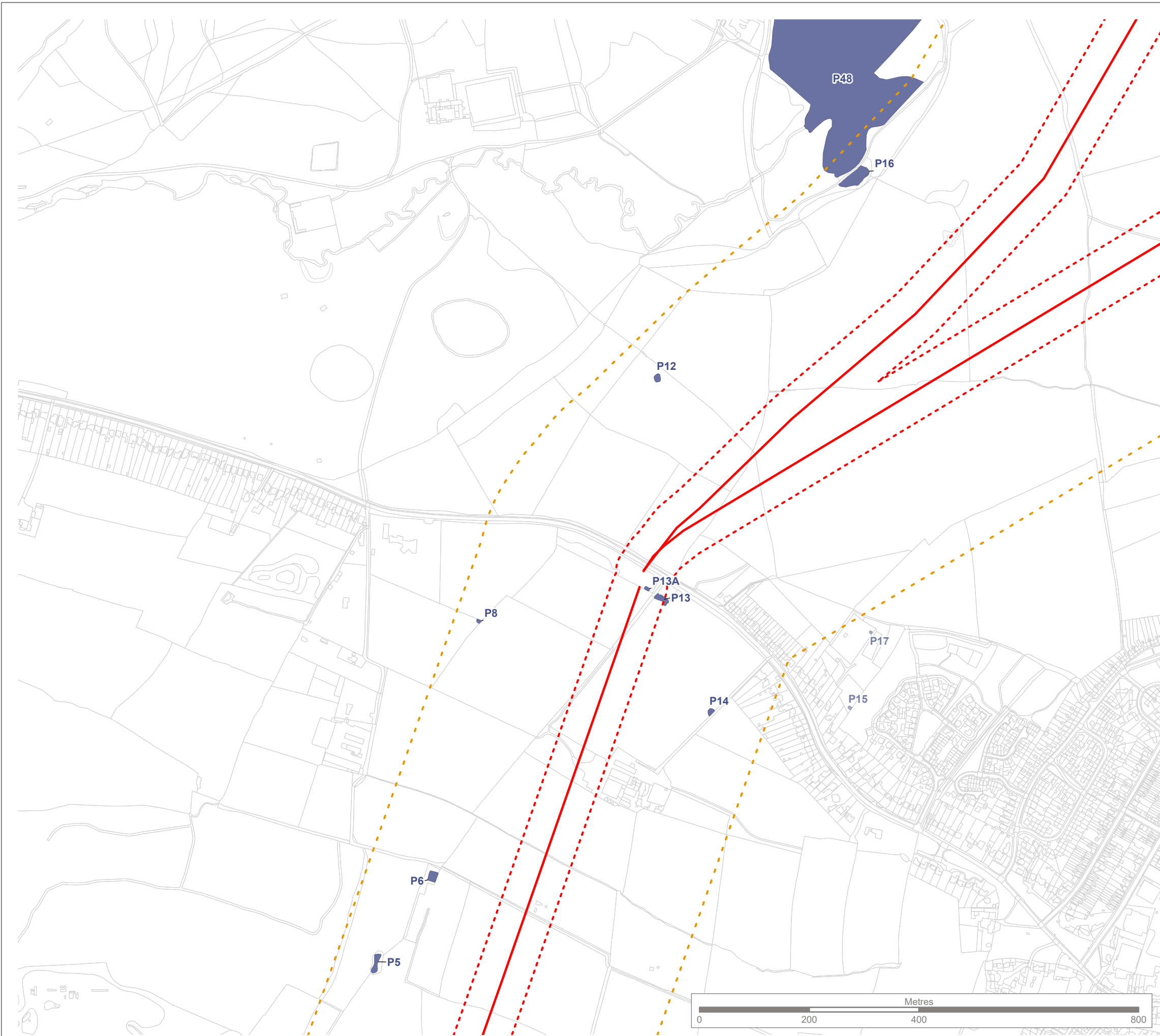
Project
Hallgates to Elms Farm Pipeline, Leicestershire

Title
Pond Location Plan
OS

Drawing Number
G10480.001.4

Page 4 of 7

Drawn OH	Checked SP	Approved AP	Scale 1:7,000 @ A3	Date 15/08/2024
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KEY

- Pipeline Route
- Pipeline Route - 50 m buffer (the site)
- Pipeline Route - 300 m buffer (within 250 m of the site)
- Ponds
- Ponds outside of 300 m buffer

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Site Map



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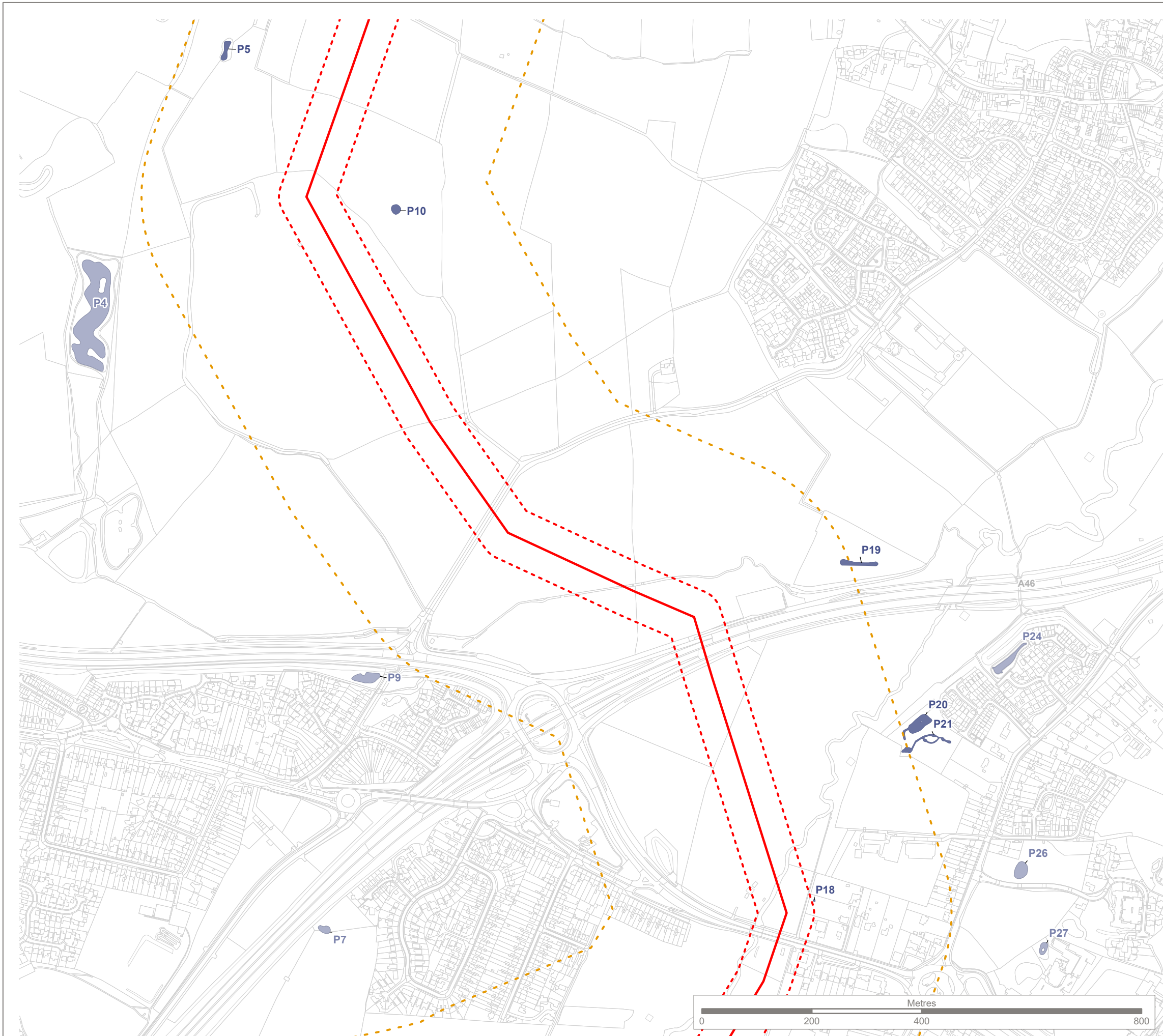
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Project
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Title
Pond Location Plan

OS

Drawing Number G10480.001.5		Page 5 of 7	
Drawn OH	Checked SP	Approved AP	Date 15/08/2024



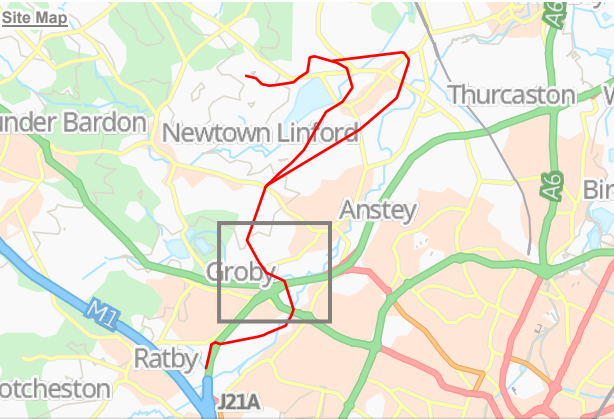
KEY

- Pipeline Route
- Pipeline Route - 50 m buffer (the site)
- Pipeline Route - 300 m buffer (within 250 m of the site)
- Ponds
- Ponds outside of 300 m buffer

Note:
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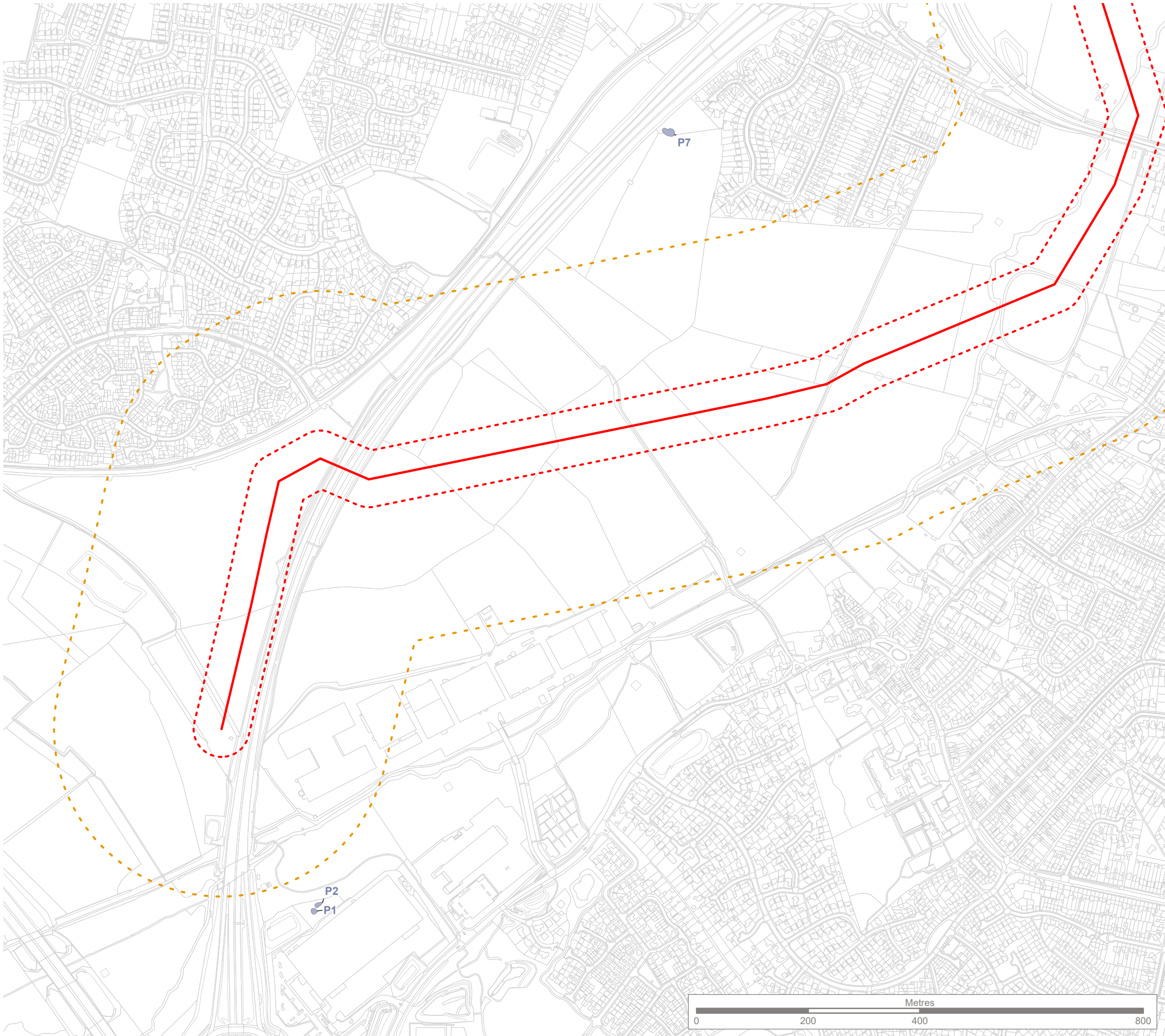
Project
Hallgates to Elms Farm Pipeline, Leicestershire

Title
Pond Location Plan
OS

Drawing Number
G10480.001.6

Page 6 of 7

Drawn	Checked	Approved	Scale	Date
OH	SP	AP	1:7,000 @ A3	15/08/2024



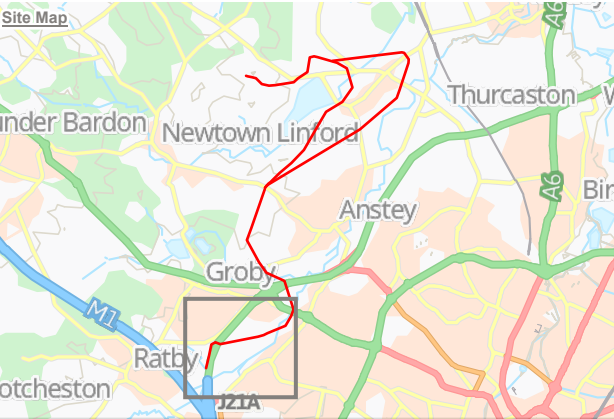
KEY

- Pipeline Route
- Pipeline Route - 50 m buffer (the site)
- Pipeline Route - 300 m buffer (within 250 m of the site)
- Ponds
- Ponds outside of 300 m buffer

Note:
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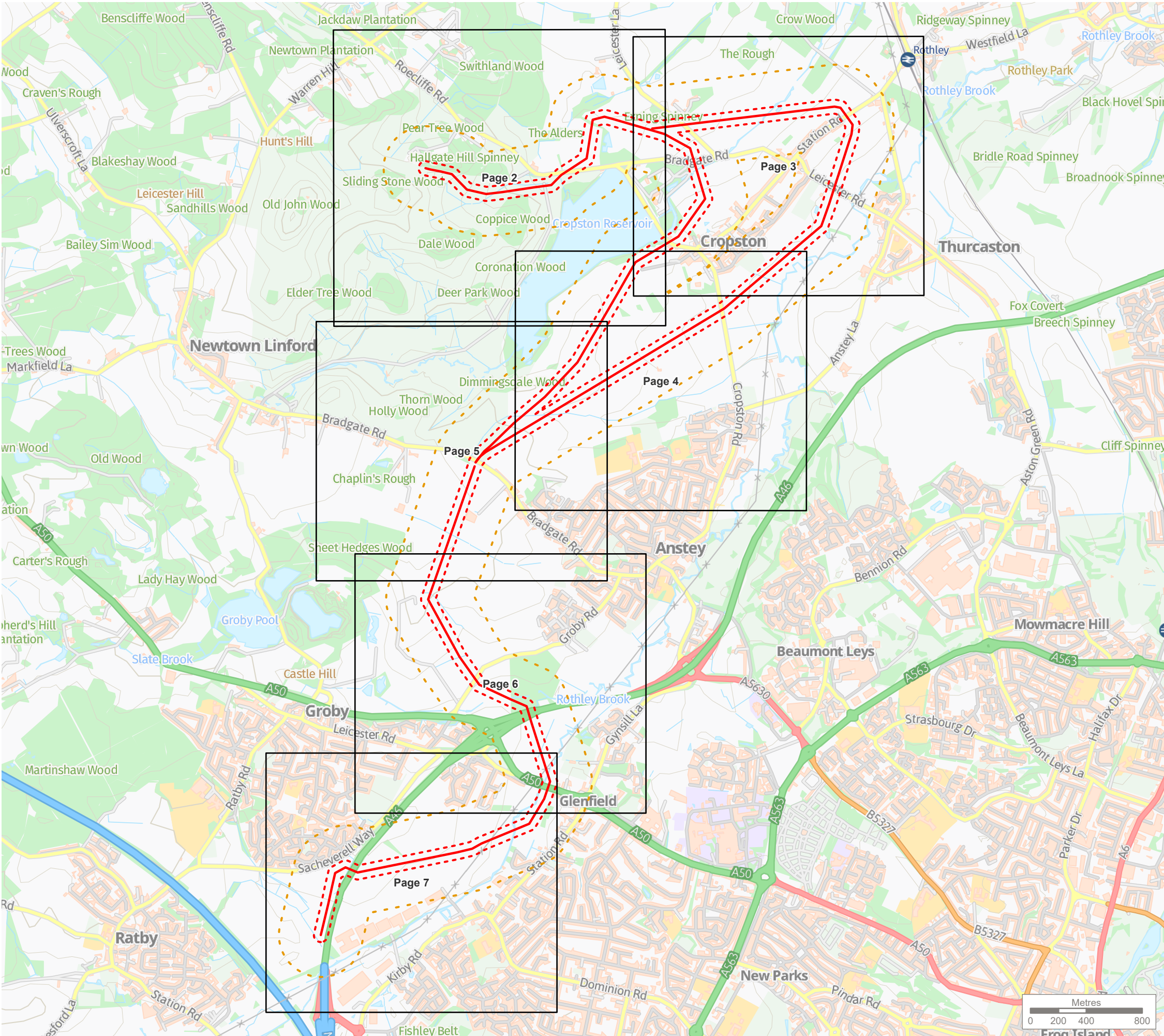
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Project
Hallgates to Elms Farm Pipeline, Leicestershire

Title
Pond Location Plan
OS

Drawing Number G10480.001.7	Page 7 of 7
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Drawn OH	Checked SP	Approved AP	Scale 1:7,000 @ A3	Date 15/08/2024
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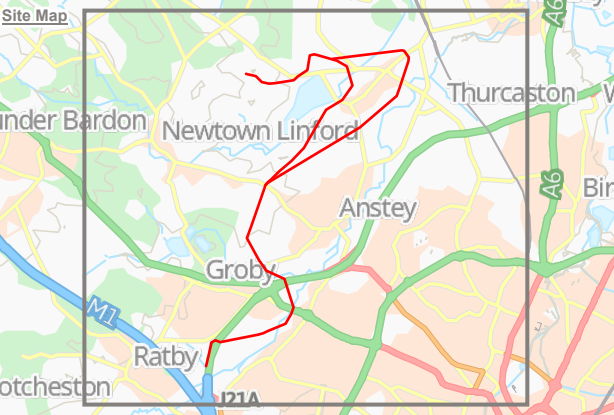
KEY

- Page and page number
- Pipeline Route
- Pipeline Route - 50 m buffer (the site)
- Pipeline Route - 300 m buffer (within 250 m of the site)

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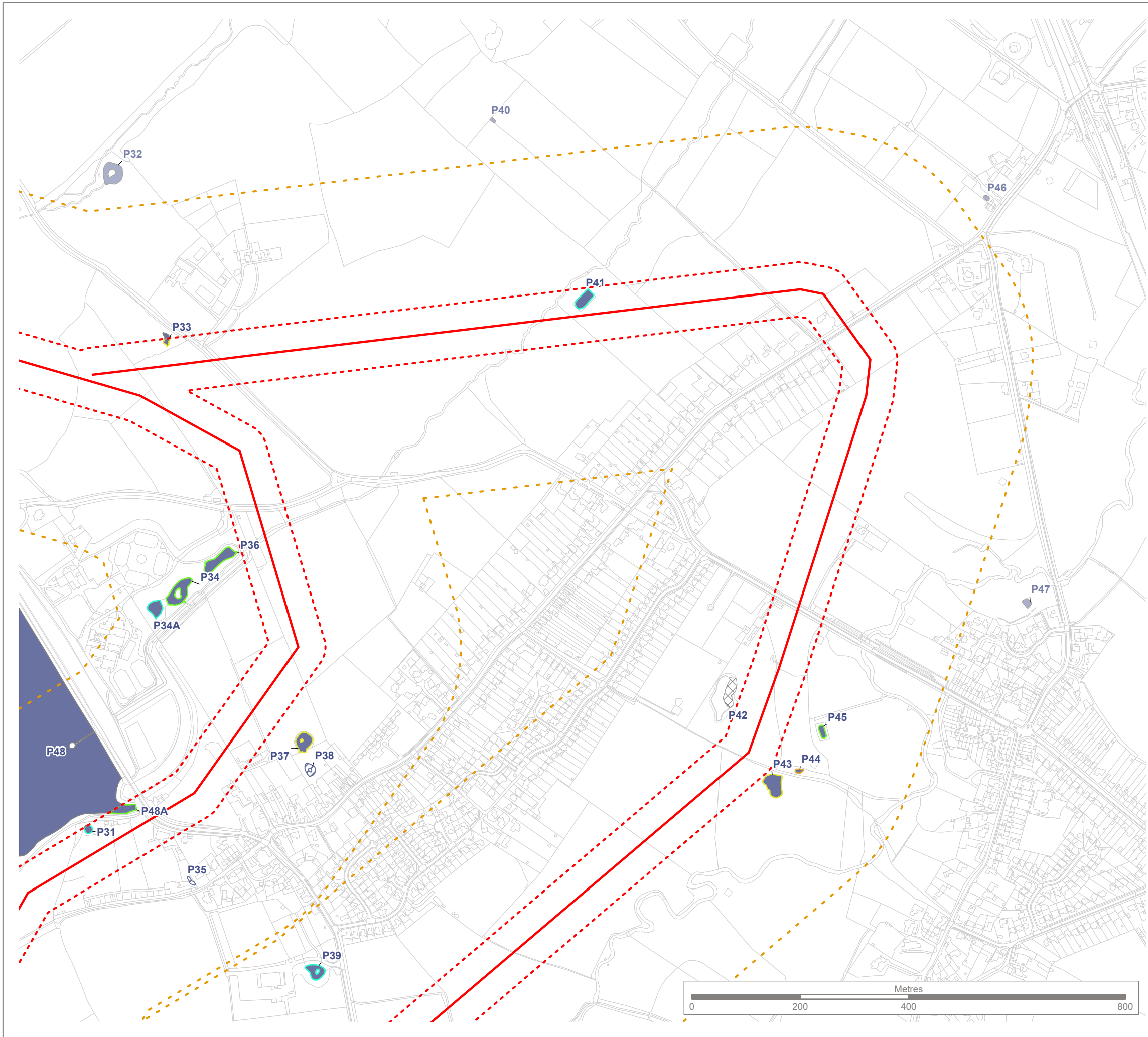
Project
Hallgates to Elms Farm Pipeline, Leicestershire

Title
GCN HSI Assessment Results 2024
OS

Drawing Number
G10480.012.1

Page 1 of 7

Drawn	Checked	Approved	Scale	Date
OH	SP	AP	1:28,000 @ A3	15/08/2024



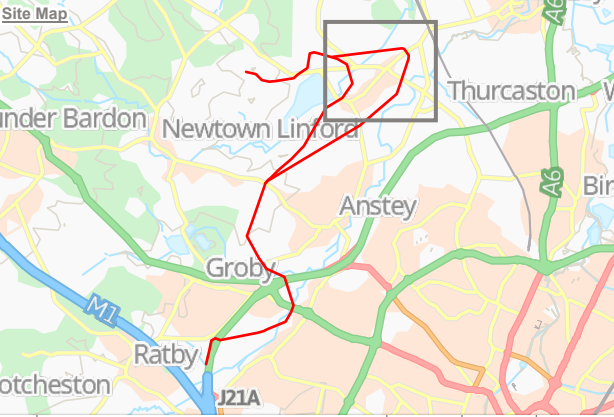
KEY

- Pipeline Route
- Pipeline Route - 50 m buffer (the site)
- Pipeline Route - 300 m buffer (within 250 m of the site)
- Habitat Suitability Index (HSI) Results**
 - Excellent
 - Good
 - Average
 - Below Average
- Pond outside of 300 m buffer
- Pond no longer exists
- No access

Note:
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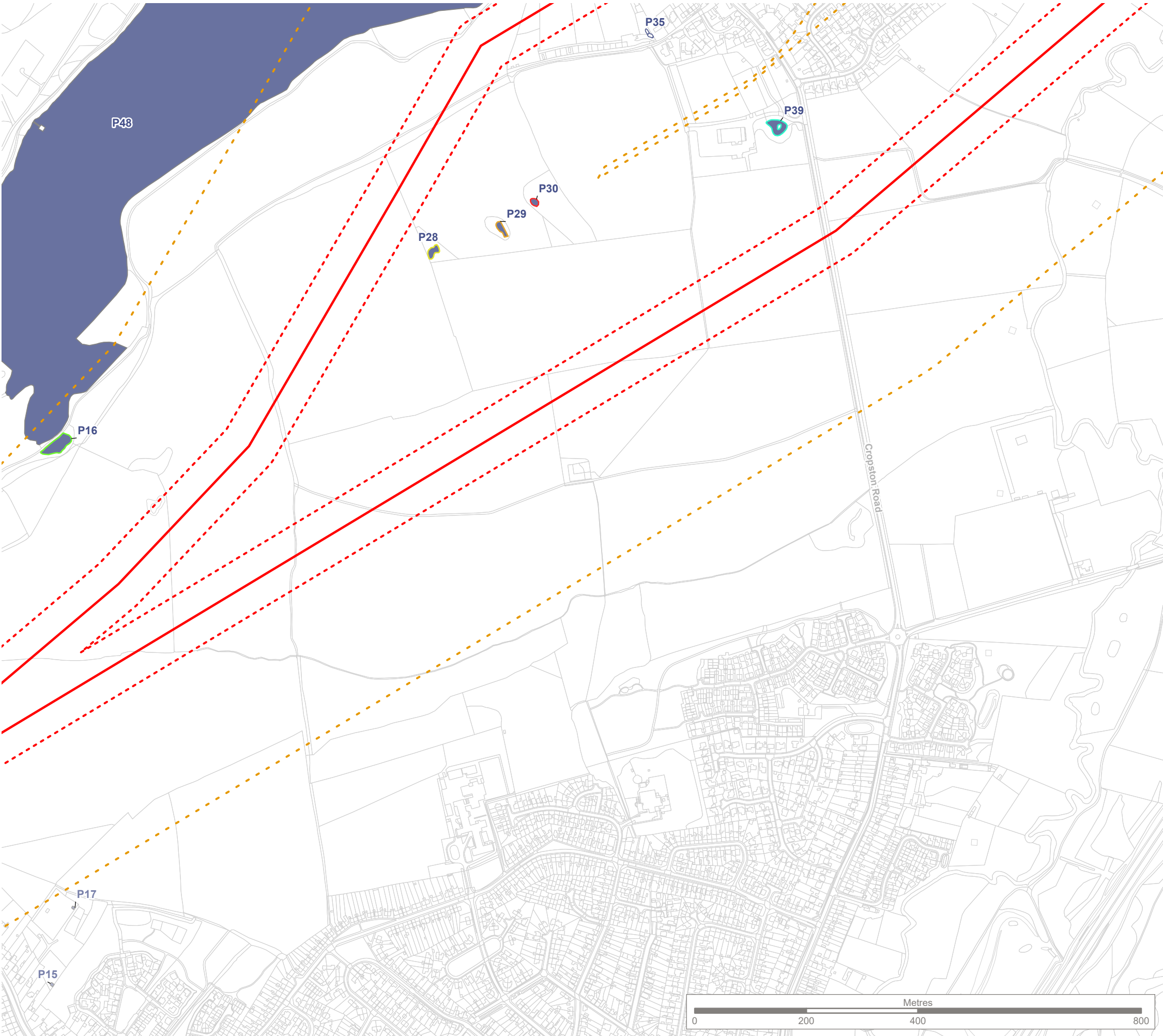
Project
Hallgates to Elms Farm Pipeline, Leicestershire

Title
GCN HSI Assessment Results 2024
OS

Drawing Number
G10480.012.3

Page 3 of 7

Drawn	Checked	Approved	Scale	Date
OH	SP	AP	1:7,000 @ A3	15/08/2024



KEY

- Pipeline Route
- Pipeline Route - 50 m buffer (the site)
- Pipeline Route - 300 m buffer (within 250 m of the site)

Habitat Suitability Index (HSI) Results

- Excellent
- Good
- Average
- Below Average
- Poor
- Pond outside of 300 m buffer
- No access

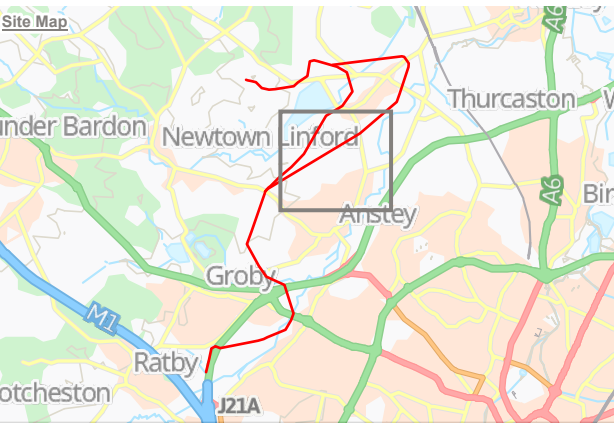
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Site Map



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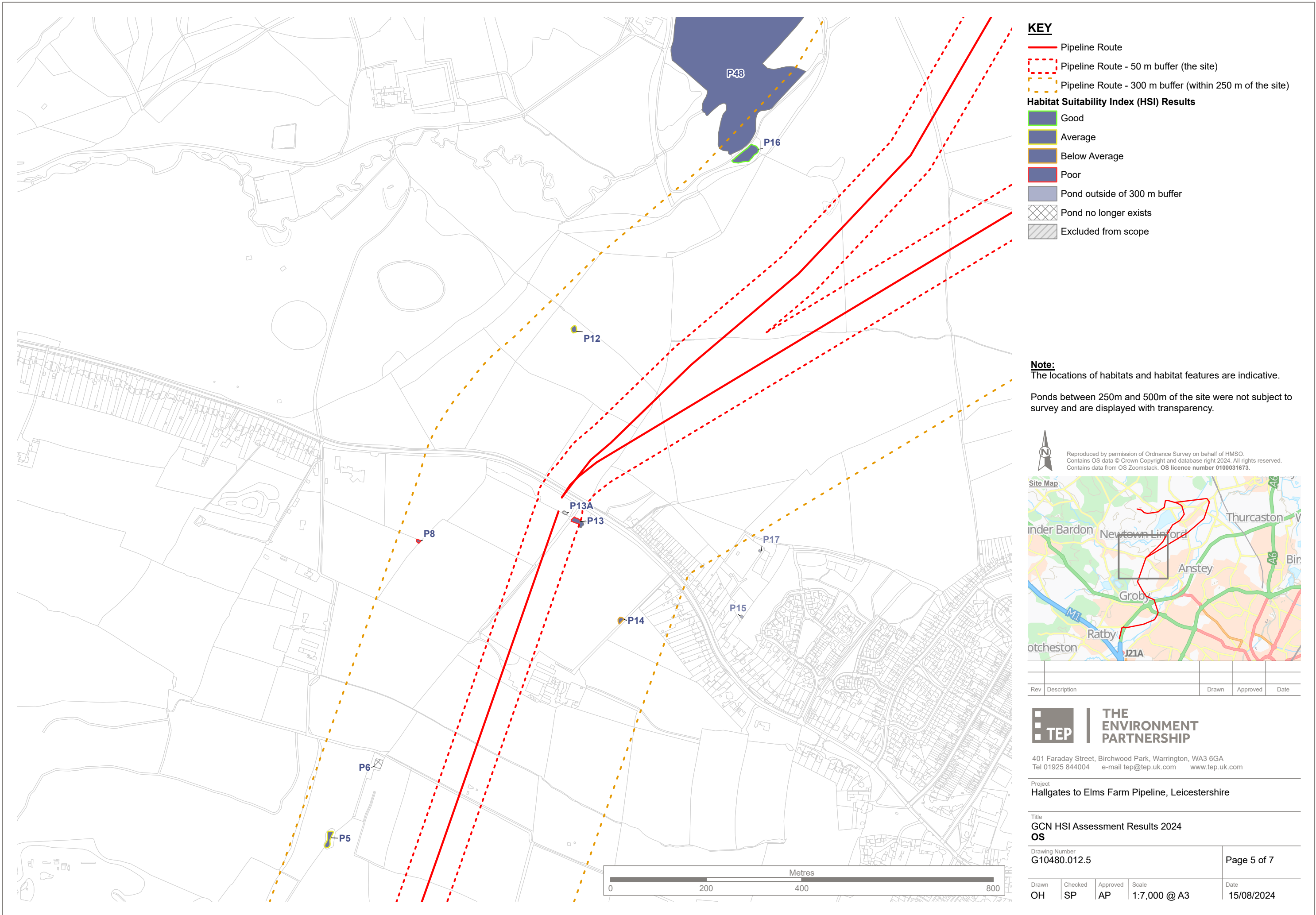
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Tel 01925 844004 e-mail tep@tep.uk.com www.[tep.uk.com](http://www.tep.uk.com)

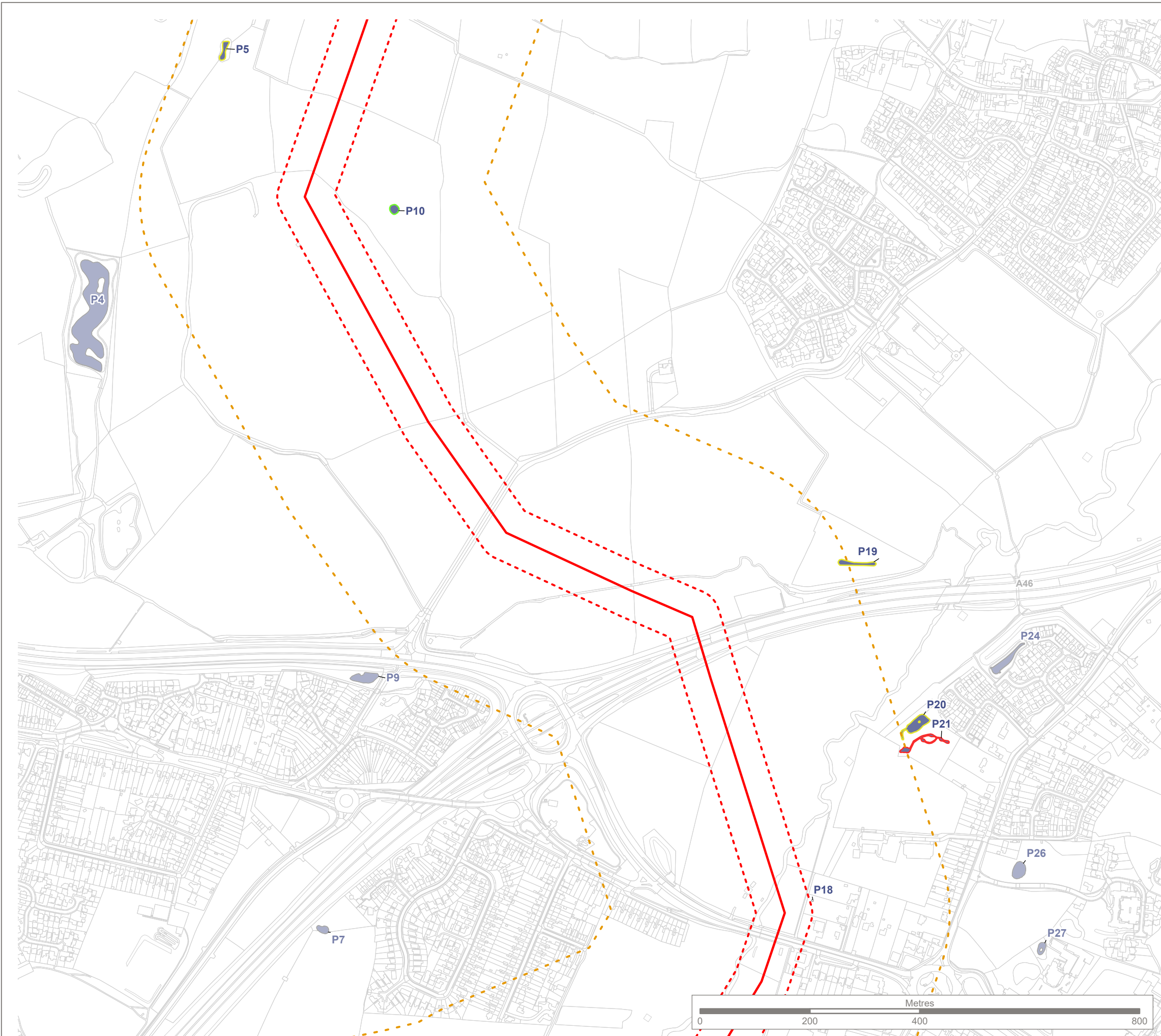
Project
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Title
GCN HSI Assessment Results 2024
OS

Drawing Number G10480.012.4	Page 4 of 7
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Drawn OH	Checked SP	Approved AP	Scale 1:7,000 @ A3	Date 15/08/2024
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KEY

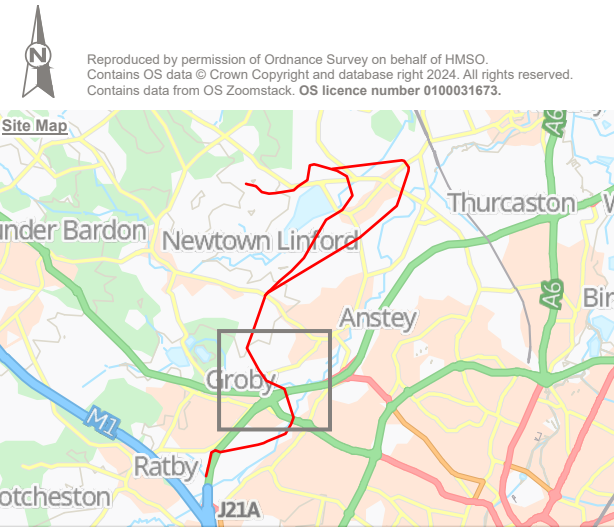
- Pipeline Route
- Pipeline Route - 50 m buffer (the site)
- Pipeline Route - 300 m buffer (within 250 m of the site)

Habitat Suitability Index (HSI) Results

- Good
- Average
- Poor
- Pond outside of 300 m buffer
- Pond no longer exists

Note:
The locations of habitats and habitat features are indicative.

Ponds between 250m and 500m of the site were not subject to survey and are displayed with transparency.



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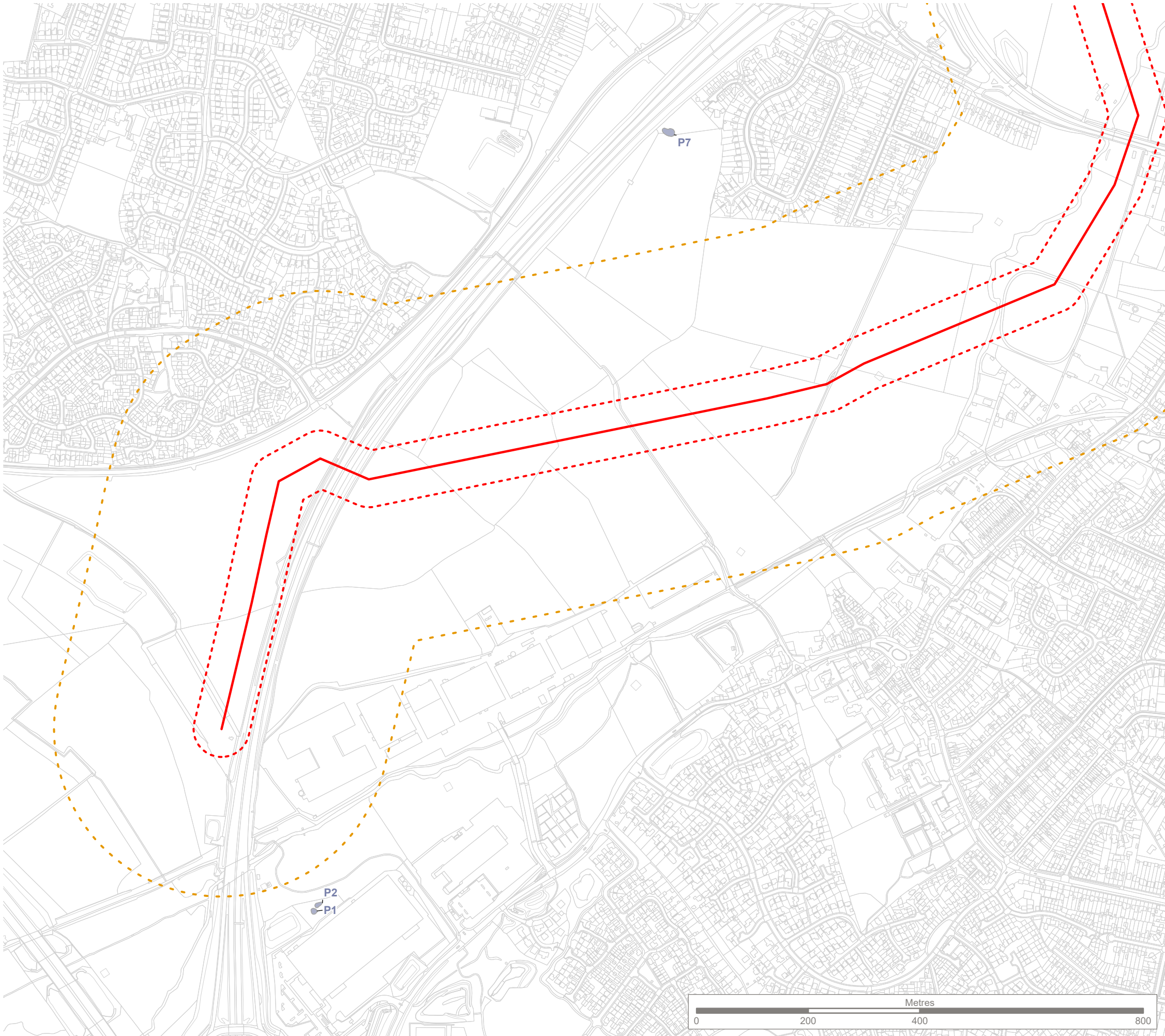
Project
Hallgates to Elms Farm Pipeline, Leicestershire

Title
GCN HSI Assessment Results 2024
OS

Drawing Number
G10480.012.6

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KEY

Pipeline Route

Pipeline Route - 50 m buffer (the site)

Pipeline Route - 300 m buffer (within 250 m of the site)

Habitat Suitability Index (HSI) Results

Pond outside of 300 m buffer

Pond no longer exists

Note:
The locations of habitats and habitat features are indicative.

Ponds between 250m and 500m of the site were not subject to survey and are displayed with transparency.

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Site Map

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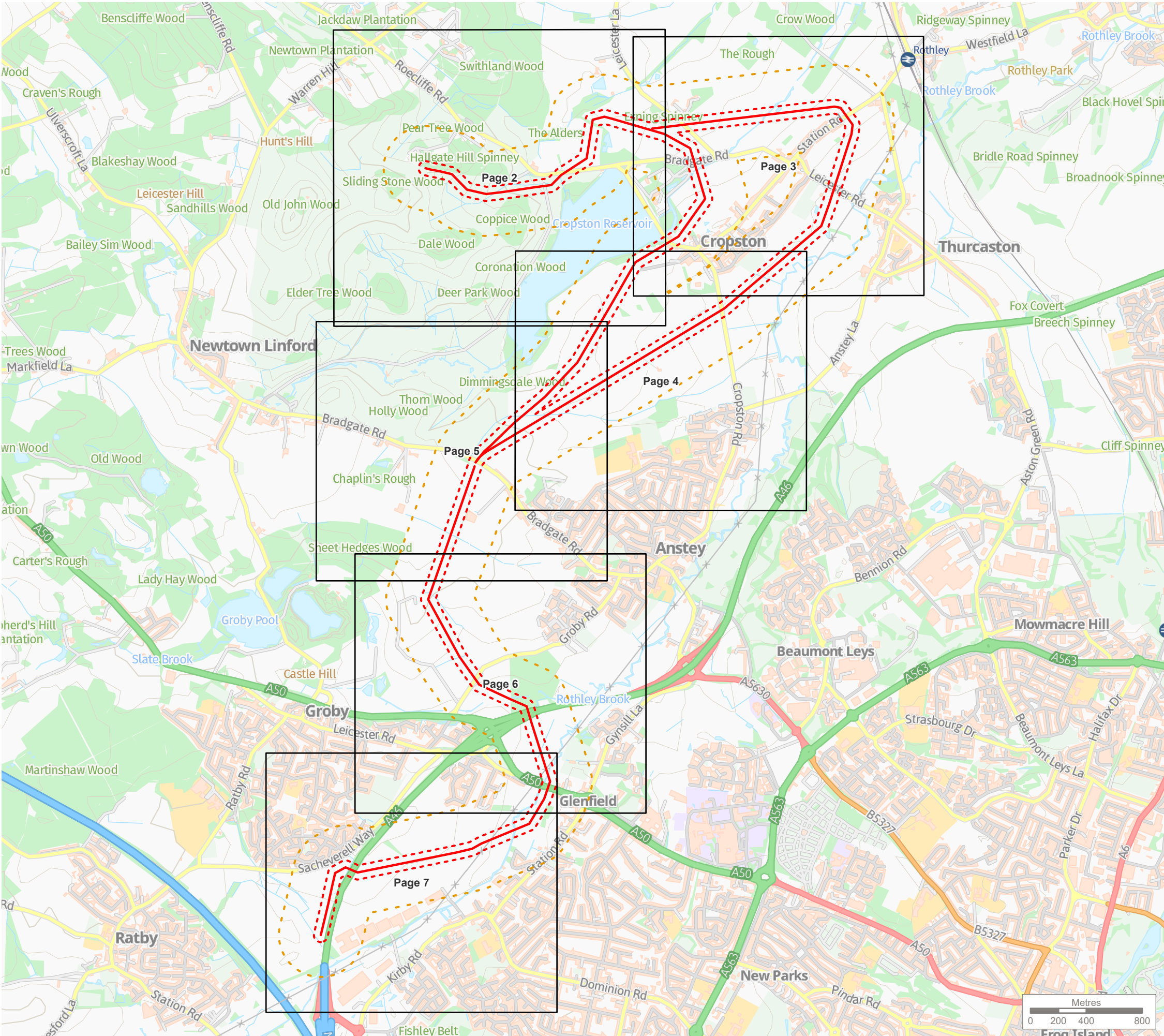
Project
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Title
GCN HSI Assessment Results 2024
OS

Drawing Number
G10480.012.7

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Drawn	Checked	Approved	Scale	Date
OH	SP	AP	1:7,000 @ A3	15/08/2024



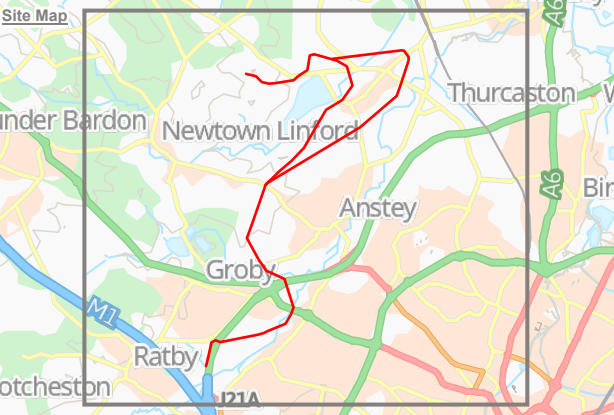
KEY

- Page and page number
- Pipeline Route
- Pipeline Route - 50 m buffer (the site)
- Pipeline Route - 300 m buffer (within 250 m of the site)

Note:
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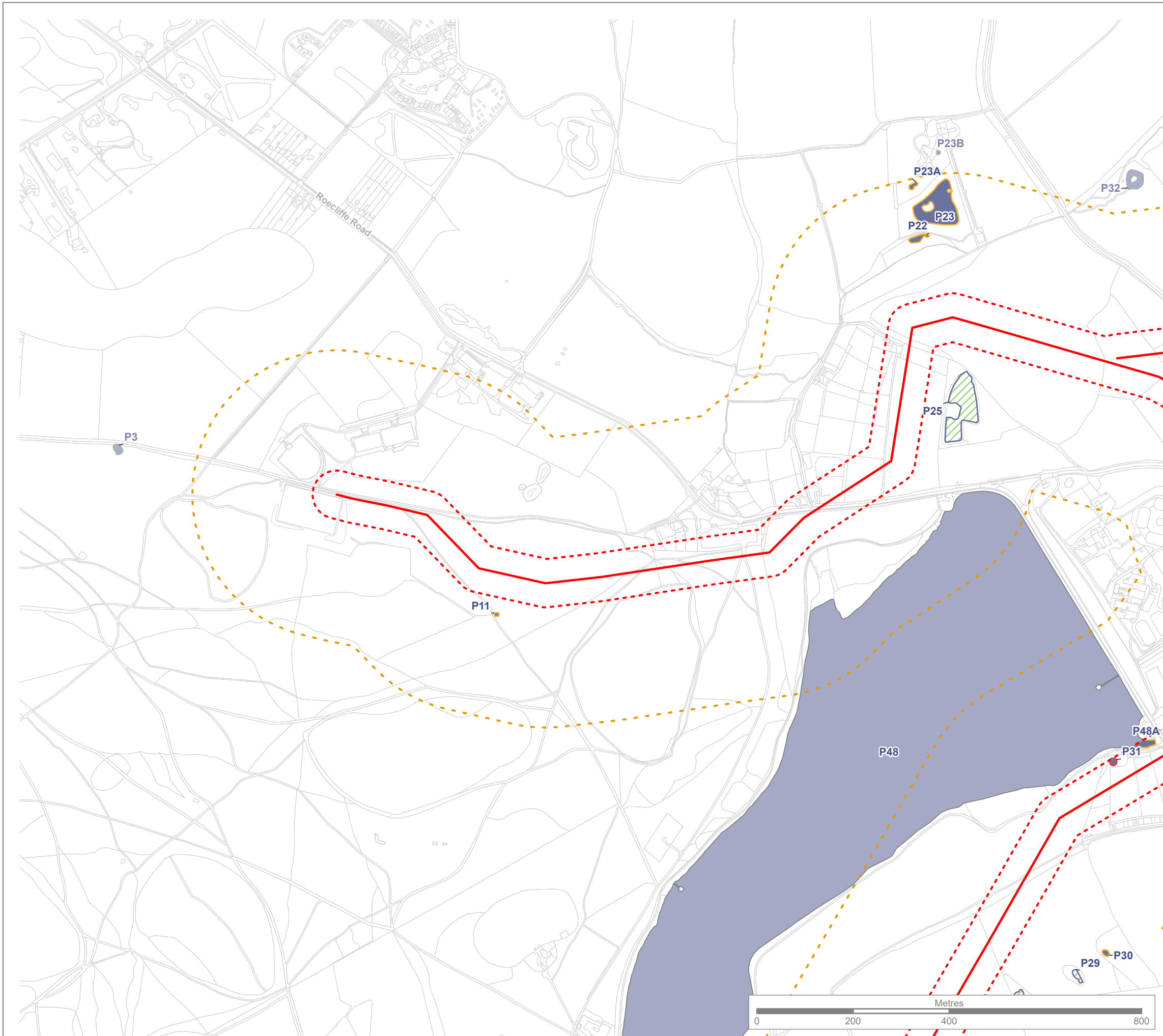
Project
Hallgates to Elms Farm Pipeline, Leicestershire

Title
GCN eDNA Survey Results 2024
OS

Drawing Number
G10480.014.1

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Drawn	Checked	Approved	Scale	Date
OH	SP	AP	1:28,000 @ A3	15/08/2024



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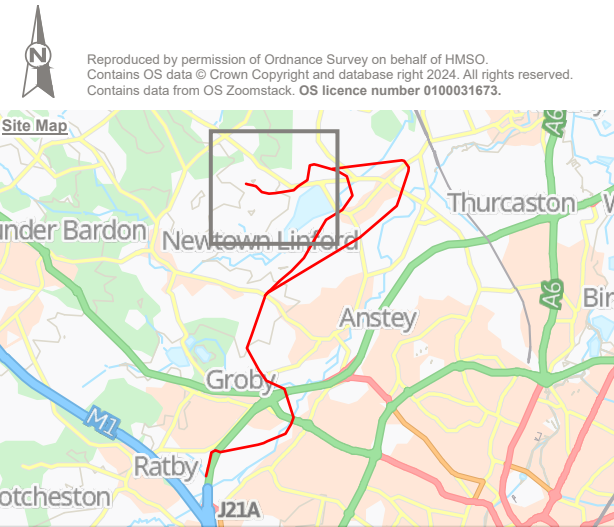
- Pipeline Route
- Pipeline Route - 50 m buffer (the site)
- Pipeline Route - 300 m buffer (within 250 m of the site)

eDNA Results

- Pond with great crested newt presence
- Pond with great crested newt absence
- Pond outside of 300 m buffer
- Vegetation restricted access - could not eDNA

Note:
The locations of habitats and habitat features are indicative.

Ponds between 250m and 500m of the site were not subject to survey and are displayed with transparency.



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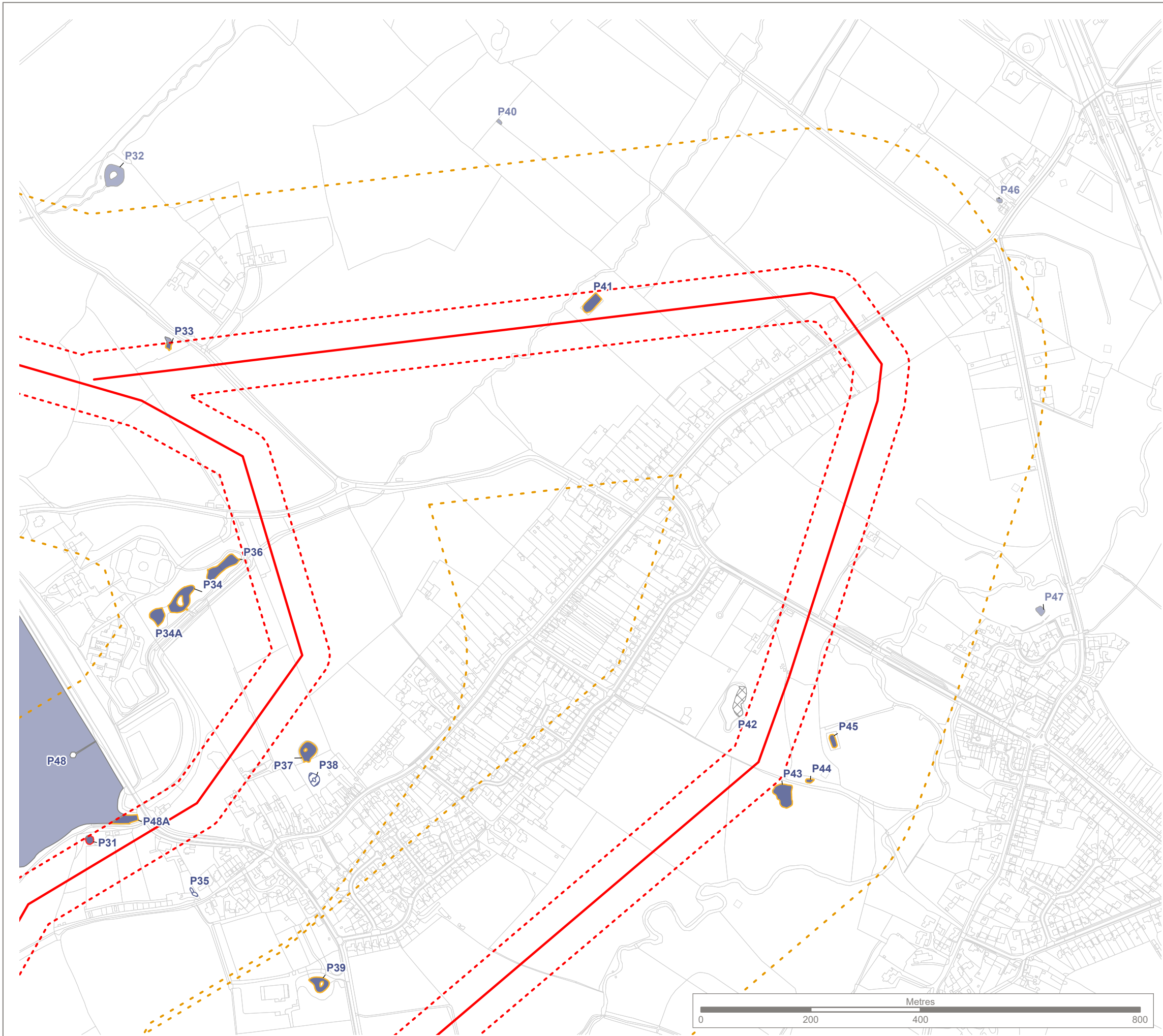
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Title
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Drawn OH	Checked SP	Approved AP	Scale 1:8,000 @ A3	Date 15/08/2024
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KEY

- Pipeline Route
- Pipeline Route - 50 m buffer (the site)
- Pipeline Route - 300 m buffer (within 250 m of the site)

eDNA Results

- Pond with great crested newt presence
- Pond with great crested newt absence
- Pond outside of 300 m buffer
- No access to pond
- Pond no longer exists

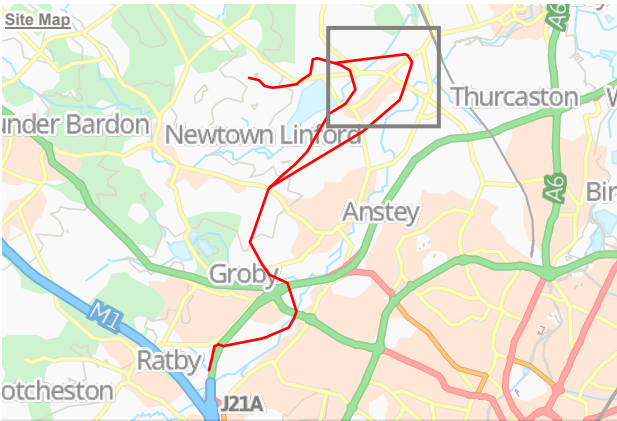
Note:

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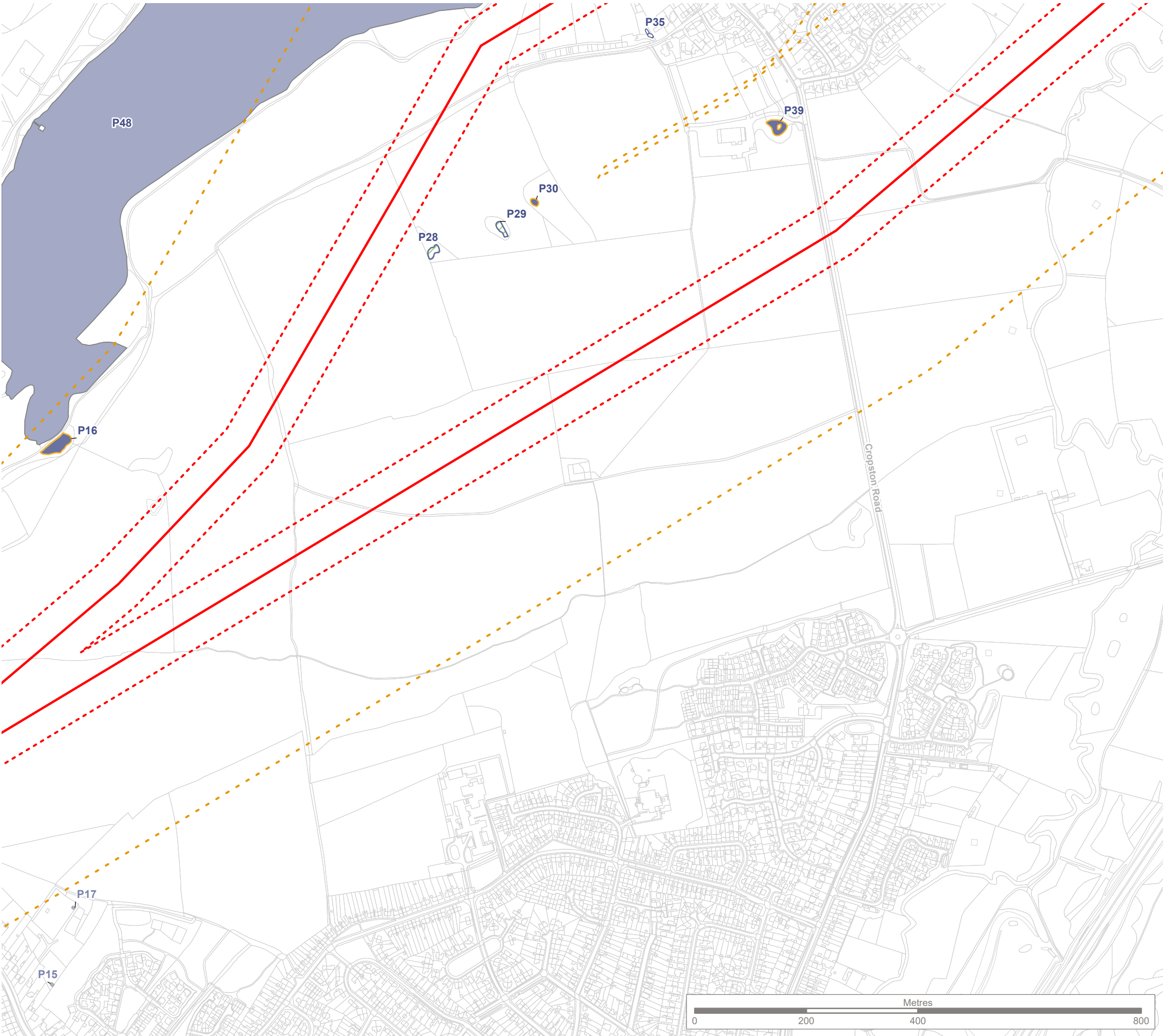
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Title
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Drawn OH	Checked SP	Approved AP	Scale 1:7,000 @ A3	Date 15/08/2024
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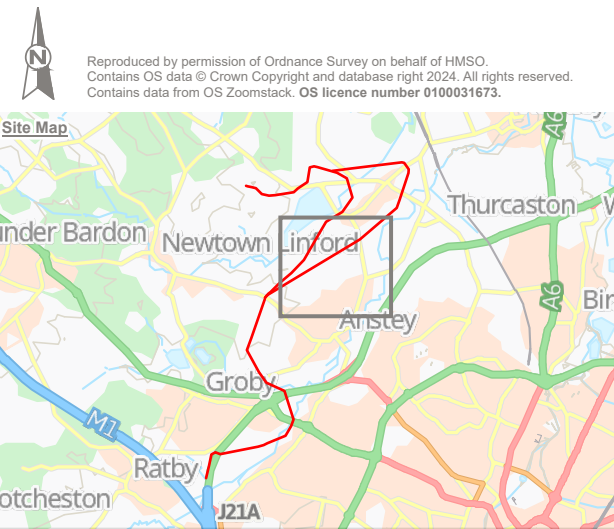
- Pipeline Route
- Pipeline Route - 50 m buffer (the site)
- Pipeline Route - 300 m buffer (within 250 m of the site)

eDNA Results

- Pond with great crested newt absence
- Pond outside of 300 m buffer
- Vegetation restricted access - could not eDNA
- No access to pond

Note:
The locations of habitats and habitat features are indicative.

Ponds between 250m and 500m of the site were not subject to survey and are displayed with transparency.



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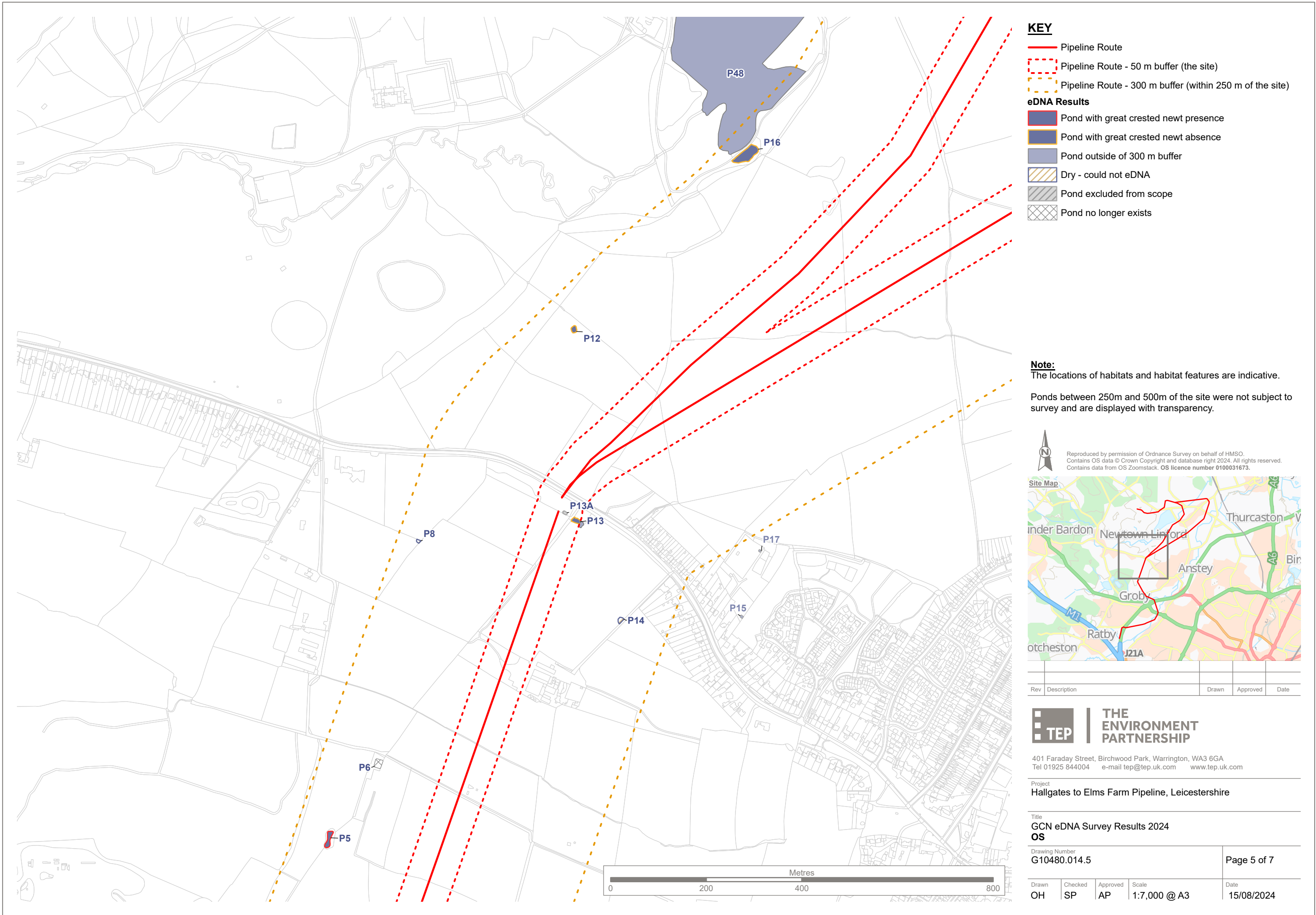
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Drawn OH	Checked SP	Approved AP	Scale 1:7,000 @ A3	Date 15/08/2024
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KEY

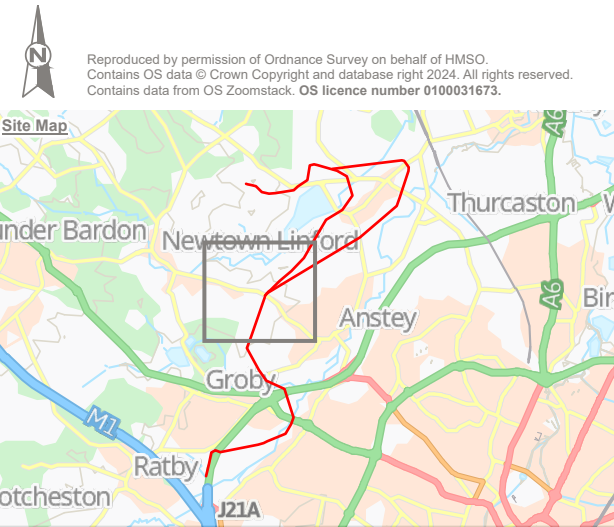
- Pipeline Route
- Pipeline Route - 50 m buffer (the site)
- Pipeline Route - 300 m buffer (within 250 m of the site)

eDNA Results

- Pond with great crested newt presence
- Pond with great crested newt absence
- Pond outside of 300 m buffer
- Dry - could not eDNA
- Pond excluded from scope
- Pond no longer exists

Note:
The locations of habitats and habitat features are indicative.

Ponds between 250m and 500m of the site were not subject to survey and are displayed with transparency.



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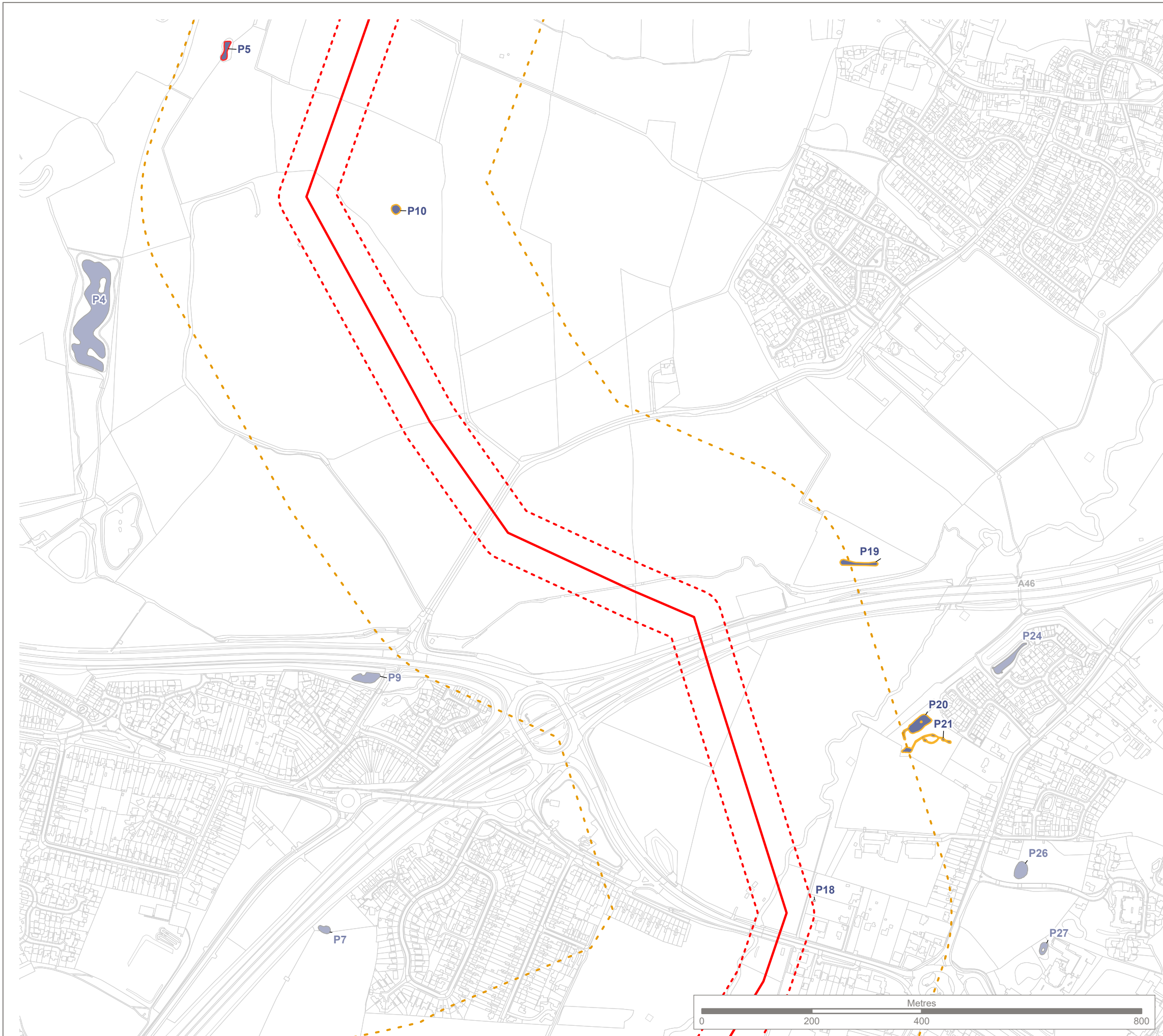
Project
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Title
GCN eDNA Survey Results 2024
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G10480.014.5

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Drawn	Checked	Approved	Scale	Date
OH	SP	AP	1:7,000 @ A3	15/08/2024



KEY

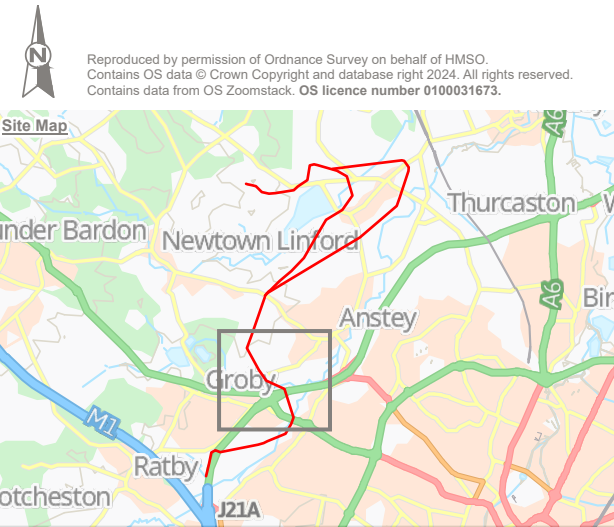
- Pipeline Route
- Pipeline Route - 50 m buffer (the site)
- Pipeline Route - 300 m buffer (within 250 m of the site)

eDNA Results

- Pond with great crested newt presence
- Pond with great crested newt absence
- Pond outside of 300 m buffer
- Pond no longer exists

Note:
The locations of habitats and habitat features are indicative.

Ponds between 250m and 500m of the site were not subject to survey and are displayed with transparency.



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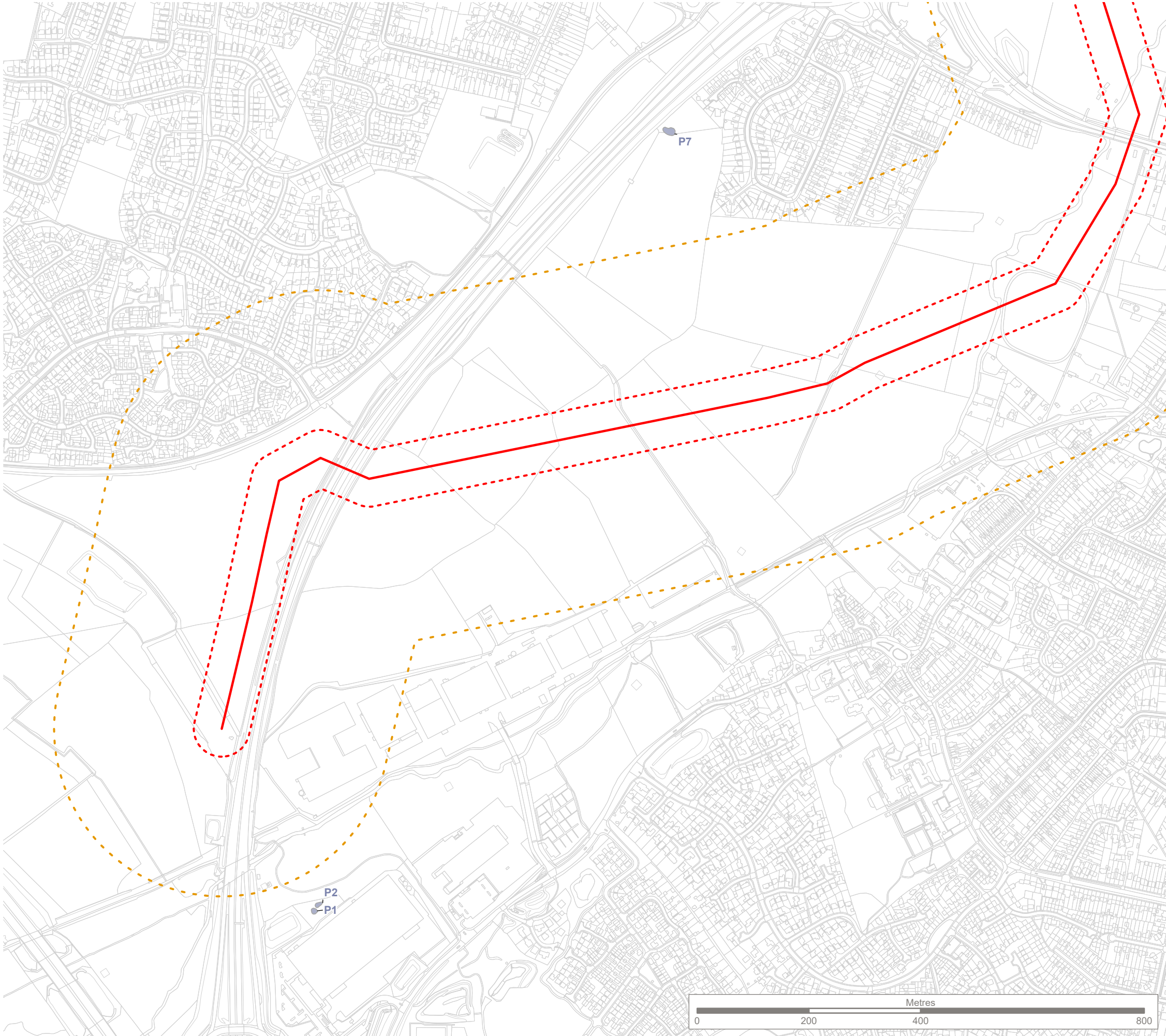
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KEY

- Pipeline Route
- Pipeline Route - 50 m buffer (the site)
- Pipeline Route - 300 m buffer (within 250 m of the site)

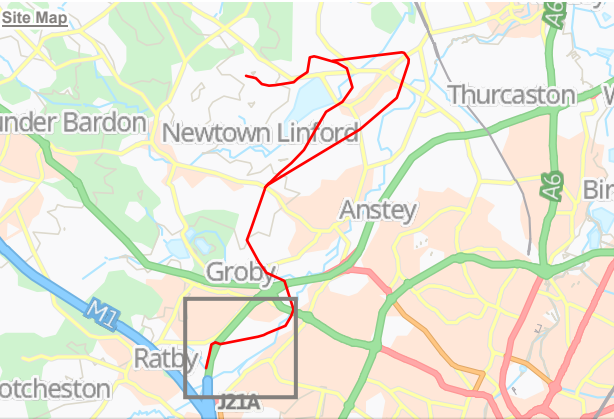
eDNA Results

- Pond outside of 300 m buffer
- Pond no longer exists

Note:
The locations of habitats and habitat features are indicative.

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