

A38 Derby Junctions

TR010022

Volume 6

**6.3 Environmental Statement
Appendices**

**Appendix 8.6a: Great Crested Newt
Surveys in 2017**

Regulation 5(2)(a)

Planning Act 2008

Infrastructure Planning (Applications: Prescribed
Forms and Procedure) Regulations 2009

April 2019

Infrastructure Planning

Planning Act 2008

**The Infrastructure Planning
(Applications: Prescribed Forms
and Procedure) Regulations 2009**

A38 Derby Junctions
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6.3 Environmental Statement Appendices
Appendix 8.6a: Great Crested Newt Surveys in 2017

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A38 Derby Junctions

Great Crested Newt Survey Report

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Contents

1	Introduction	1
1.1	Background and Scope	1
1.2	Study Site	1
1.3	Relevant Legislation	2
2	Methodology.....	4
2.1	Desk-based Study	4
2.2	Great Crested Newt Survey.....	7
2.3	Limitations.....	11
3	Results.....	12
3.1	Desk-based Study	12
3.2	Pond Descriptions and HSI Results	12
3.3	Survey Results.....	17
4	Summary	19
5	References	20

List of Tables

Table 1: Desk Study and Site Observations to Determine Necessary GCN Survey.....	5
Table 2: Habitat Suitability Indices.....	7
Table 3: GCN Survey Conditions.....	9
Table 4: Waterbody/ Pond Descriptions and HSI Values	12
Table 5: Results Summary – GCN Presence/ Likely Absence Surveys and Other Amphibians.....	18

Appendices

- Appendix A Figures**
- Appendix B GCN Habitat Suitability Index**
- Appendix C eDNA Test Results from SureScreen**
- Appendix D Full Survey Results**

1 INTRODUCTION

1.1 Background and Scope

- 1.1.1 AECOM Infrastructure & Environment UK Limited (AECOM) has been commissioned by Highways England to provide design services with regards to the A38 Derby Junctions scheme (referred to as the proposed scheme herein).
- 1.1.2 The proposed scheme concerns the grade separation of three junctions on the A38 in Derby, namely:
- A38/ A61 Little Eaton junction;
 - A38/ A52 Markeaton junction; and
 - A38/ A5111 Kingsway junction.
- 1.1.3 These three junctions are located along an approximate 5.5km length of the A38 national trunk road, to the west and north of Derby.
- 1.1.4 In order to assist with the assessment of the proposed scheme's potential environmental effects, a range of environmental surveys has been undertaken to define prevailing baseline conditions.
- 1.1.5 The Phase 1 Habitat survey conducted by AECOM in 2015 across the proposed scheme highlighted the potential for Great Crested Newts (GCN) *Triturus cristatus* and the requirement for further surveys (AECOM(a), 47071319-URS-05-RP-EN-009, 2016). Habitat Suitability Index (HSI) assessments and presence/ likely absence surveys were conducted on 26 ponds within 500m of the 2015 proposed scheme boundary. No GCN were recorded during those surveys. A notable population of common toads *Bufo bufo* were, however, found in ponds located within 50m of Markeaton junction. Refer to the A38 Derby Junctions GCN Report for further details (AECOM(b), 47071319-URS-05-RP-EN-003, 2016).
- 1.1.6 The proposed scheme boundary was revised in 2017 to include additional areas proposed for potential flood storage, construction compounds and ecological compensation. A desk study review was carried out in 2017 to identify any new ponds within 500m of the updated proposed scheme boundary. Any new ponds identified were subjected to HSI and presence/ likely absence surveys in 2017 (where applicable). Additionally, the ponds previously surveyed in 2015 were subject to an updated HSI assessment and presence/ likely absence surveys (where applicable) to ensure that up to date baseline survey information was available for GCN to support the environmental impact assessment (EIA), and potential licence applications, where applicable, in 2018.
- 1.1.7 Results of the 2017 GCN survey are documented herein, together with the updated desktop data.

1.2 Study Site

- 1.2.1 The proposed scheme under appraisal encompasses land between Kingsway and Markeaton junctions, west of the City of Derby (SK 32801 36103) and Little Eaton junction north of Derby (SK 36402 39990). A plan showing the proposed scheme boundary is presented in Figure 1 (Appendix A). The ecological study area as referred to herein extends up to 500m beyond the proposed scheme boundary.

- 1.2.2 The A38 is an existing and busy arterial 'A' road carrying traffic around the west and north of the City of Derby. South of Kingsway junction, the road enters a cutting and is bordered by semi-improved grassland and scrub covered verges. The central reservation south of Kingsway junction and the junction island in this location support a mosaic of habitat types, including semi-improved neutral grassland and native broadleaved woodland. Bramble Brook flows from the west of the proposed scheme in this location through culverts located under the north-bound carriageway and the central reservation before connecting with further culverts located between the junction islands. North of Kingsway junction there is an area of mixed plantation represented by semi-mature trees on embankment.
- 1.2.3 Markeaton junction is bordered to the east by residential properties and to the west by parkland with veteran trees. The outfall from Markeaton Lake and Markeaton Brook flows through culverts beneath the existing A38 at the northern extent of proposed scheme at Markeaton junction.
- 1.2.4 The western boundary of the proposed scheme at Little Eaton junction borders the road bridge over the River Derwent. The existing A38 is on embankment in this location, with the embankments themselves represented by areas of scrub and immature broadleaved plantation habitats. A variety of grassland habitats exist at the base of the embankments in this location.

1.3 Relevant Legislation

- 1.3.1 The GCN is listed under Schedule 5 of the Wildlife and Countryside Act 1981 (as amended) and Schedule 2 of the Conservation of Habitats and Species Regulations 2010 (as amended). This legislation, when taken together, results in a level of protection that prohibits the intentional, deliberate or reckless:
- Killing, injuring, taking or disturbance of GCN;
 - Damaging, destroying or obstructing any place used by GCN for the purposes of breeding or sheltering/ protection; and
 - Selling and/ or advertising for sale a GCN or any part thereof.
- 1.3.2 The common toad, along with common frog *Rana temporaria*, smooth newt *Lissotriton vulgaris* and palmate newt *Lissotriton helveticus* are listed under Section 9(5) of the Wildlife and Countryside Act (as amended), which protects them against sale only.
- 1.3.3 GCN is listed under Section 41 of the Natural Environment and Rural Communities (NERC) Act 2006 as Species of Principal Importance for Conservation in England. Section 40 of the same Act requires that local and regional authorities have regard to the conservation of biodiversity in England, when carrying out their normal functions. Common toads are also listed under Section 41 of the NERC Act (2006), but do not receive the same level of legal protection as the aforementioned species. The common toad is also a priority species in England under Biodiversity 2020: A strategy for England's wildlife and ecosystem services (DEFRA, 2011).
- 1.3.4 GCN and common toads are also identified in the Local Biodiversity Action Plan (LBAP) as priority species (Lowland Derbyshire Biodiversity Partnership, 2011).
- 1.3.5 Highways England, through the national Road Investment Strategy (RIS), has set an aspiration that the operation, maintenance, and enhancement of the Strategic Road Network (SRN) should move to a position that delivers no net loss of biodiversity; and, in the long term, Highways England should deliver a net gain in biodiversity across its

broader range of works. Highways England published a Biodiversity Plan in 2015 (Highways England, 2015) to show how it will work with service providers to halt overall biodiversity loss, and maintain and enhance habitats and ecological networks. The Government requires Highways England to demonstrate progress against the 2015 Biodiversity Plan, to secure an ongoing annual reduction in the loss of net biodiversity due to its activities. The 2015 Biodiversity Plan provides a general plan to protect and increase biodiversity. The 2015 Biodiversity Plan supersedes the preceding 2002 Highways Agency (now Highways England) Biodiversity Action Plan (BAP), which still however carries some relevance as it lists specific species of conservation concern.

- 1.3.6 GCN are listed in the 2002 Highways BAP as priority species. The objectives of this species action plan for GCN is to maintain and enhance GCN populations that occur within the soft estate, through protection and appropriate management of suitable habitat, and to prevent or adequately mitigate any adverse impacts of new road schemes on the species and its habitat.

2 METHODOLOGY

2.1 Desk-based Study

Data Search

- 2.1.1 A desk-based study was undertaken to identify internationally, nationally and locally designated statutory sites, local designated non-statutory sites and records of protected and/ or notable amphibians within 1km of the proposed scheme boundary. Online data resources were reviewed e.g. the Multi-Agency Geographic Information Centre (MAGIC). A data search to identify any further notable or protected amphibian records and ponds within 1km of the proposed scheme boundary was also requested from the Derbyshire Wildlife Trust (DWT). Furthermore, OS maps, online aerial photographs, data from local record centres and the 2016 AECOM GCN report (AECOM(a), 2016) were used to identify waterbodies.
- 2.1.2 Highways England's own record database, Environmental Information System (EnvIS), was also searched for any amphibian records.

Determination of Ponds to be Surveyed

- 2.1.3 The desk study identified the presence of 40 waterbodies within 500m of the proposed scheme boundary. Twenty six of these ponds had already been scoped into the survey during 2015, whilst 14 were new ponds. Twenty four of these 40 waterbodies were recorded as having been destroyed, fish present, pond dry, flowing water or not found. The other 16 waterbodies, however, were identified as being potentially suitable to support GCN.
- 2.1.4 Three ponds to the west of the proposed scheme (>900 m away) were reported to have recent GCN records by the DWT. Although these ponds were located more than 500m from the proposed scheme boundary, they were considered to potentially be ecologically linked to the proposed scheme by optimum GCN terrestrial habitats. As such, it was considered that any GCN discovered in ponds within 500m of the proposed scheme at Kingsway junction may be part of a wider population. It was therefore considered necessary to gather data on these wider ponds to ensure sufficient information was available to support a Natural England European Protected Species Mitigation Licence (EPSML) application (if required). These ponds, as well as 6 more in the vicinity, were also included in the survey area as a precaution and are referred to as the 'Investigation Area' in this report.
- 2.1.5 The Investigation Area comprised 9 ponds; 5 of which had their access not granted or had been destroyed, dried or not found; whilst 4 were identified as being potentially suitable to support GCN.
- 2.1.6 Ponds within 500m of Markeaton Park (a potential area for ecological compensation) were scoped out of GCN surveys. It was considered that any potential enhancement measures/ ecological works within the park would be low impact and non-licensable.
- 2.1.7 Table 1 summarises the desk study process undertaken and subsequent in-situ ground-truthing to determine the ponds to be scoped in/ out of survey for GCN presence/ likely absence in 2017.

Table 1: Desk Study and Site Observations to Determine Necessary GCN Survey

Waterbody	Desk Study				2017 Survey		
	Recorded on OS map/ aerial photography	Location recorded by DWT	Pond recorded in-situ in 2015	Notes from 2015 Survey	Pond observed in-situ in 2017	Reasons for removal from 2017 GCN survey scope	Ponds scoped in for GCN surveys in 2017
Waterbodies within 500m of proposed scheme							
Pa1	✓		✓		✓		✓
Pa2	✓	✓	✓		✓		✓
Pa3	✓		✓		✓		✓
Pa4	✓		✓		✓		✓
Pa5	✓	✓	✓		✓		✓
Pa6	✓	✓	✓	Fish present	✓	Assumed fish presence from 2015 data	
Pa7	✓	✓	✓	Fish present	✓	Assumed fish presence from 2015 data	
Pa8	✓	✓	✓	Fish present	✓	Assumed fish presence from 2015 data	
Pa9		✓	✓		✓		✓
Pa10	✓		✓			Pond destroyed	
Pa11	✓		✓			Pond destroyed	
Pa12	✓		✓		✓		✓
Pa13	✓		✓			Pond destroyed	
Pa14	✓		✓	Pond dry			
Pa15	✓		✓			Pond destroyed	
Pb1	✓		✓		✓		✓
Pb2a	✓		✓	Fish present	✓	Assumed fish presence from 2015 data	
Pb2b	✓		✓	Fish present	✓	Assumed fish presence from 2015 data	
Pb3a	✓		✓	Fish present	✓	Assumed fish presence from 2015 data	
Pb3b	✓		✓	Fish present	✓	Assumed fish presence from 2015 data	
Pb4	✓		✓		✓		✓
Pb5	✓	✓	✓		✓		✓

Waterbody	Desk Study				2017 Survey		
	Recorded on OS map/ aerial photography	Location recorded by DWT	Pond recorded in-situ in 2015	Notes from 2015 Survey	Pond observed in-situ in 2017	Reasons for removal from 2017 GCN survey scope	Ponds scoped in for GCN surveys in 2017
Pb6	✓		✓		✓		✓
Pb7	✓		✓		✓	Pond dry	
Pb8	✓		✓		✓		✓
Pb9	✓		✓		✓		✓
Pc9	✓				✓	Flowing water	
Pc10	✓				✓	Pond isolated	
Pc27	✓	✓			✓	Fish present	
Pc28	✓	✓			✓	Fish present	
Pc29	✓	✓			✓	Fish present	
Pc54	✓	✓					
Pc55		✓			✓	Pond isolated	
Pc58	✓				✓	Pond dry	
Pc59	✓				✓		✓
Pc60	✓				✓	Fish present	
PC61	✓				✓		✓
Pc62	✓				✓	Fish present	
Pc65		✓				Flowing water	
Pc66	✓				✓		✓
Investigation Area							
Pc11	✓	✓			✓	Access not granted	
Pc12	✓	✓			✓		✓
Pc13	✓	✓			✓	Flowing water	
Pc14	✓	✓			✓		✓
Pc16	✓	✓ GCN recorded			✓		✓
Pc17	✓	✓ GCN recorded			✓	Access not granted	
Pc18	✓	✓ GCN recorded			✓	Access not granted	
Pc63	✓				✓		✓
Pc64		✓				Pond dry	

2.2 Great Crested Newt Survey

Habitat Suitability Index (HSI)

2.2.1 All 16 waterbodies within 500m of the proposed scheme and the 4 accessible waterbodies within the Investigation Area identified as being potentially suitable to support GCN were described and assessed for their suitability for supporting GCN using the standardised Habitat Suitability Index (HSI) (*Oldham et al.*, 2000). The HSI is a mathematical model that incorporates ten suitability indices, all of which are thought to influence the likelihood of the presence of GCN in a waterbody. The output of an HSI assessment is a score between 0 (unsuitable) and 1 (optimal). The HSI is a tool for assessing the suitability of waterbodies for GCN; however, it is not a substitute for surveys. It does provide useful baseline data for the creation of new ponds, or the restoration of existing ponds. The HSI is calculated using the following formula (*Oldham et al.*, 2000):

$HSI = (SI_1 \times SI_2 \times SI_3 \times SI_4 \times SI_5 \times SI_6 \times SI_7 \times SI_8 \times SI_9 \times SI_{10})^{1/10}$, where SI represent Suitability Indices, as detailed in Table 2.

Table 2: Habitat Suitability Indices

Suitability Index	
SI ₁	Location
SI ₂	Pond Area
SI ₃	Pond Drying
SI ₄	Water Quality
SI ₅	Shade
SI ₆	Fowl
SI ₇	Fish
SI ₈	Ponds Within 1km
SI ₉	Terrestrial Habitat
SI ₁₀	Macrophyte Cover

2.2.2 Based on the categorisation of HSI scores, a pond's suitability for GCN is rated as follows:

- <0.5 = poor;
- 0.5 – 0.59 = below average;
- 0.6 – 0.69 = average;
- 0.7 – 0.79 = good; or
- >0.8 = excellent.

Field Techniques

eDNA Sampling

2.2.3 Environmental DNA (eDNA) analysis is a new method for species monitoring in waterbodies approved by Natural England. eDNA analysis gives a quick GCN presence/ absence result from a water sample which is collected following a specific protocol.

- 2.2.4 The eDNA sampling technique was used on 14 of the 16 waterbodies scoped in for surveys within 500m of the proposed scheme (Pa1, Pa2, Pa3, Pa4, Pa5, Pa9, Pa12, Pa14, Pb1, Pb4, Pb6, Pb8, Pb9, Pc61; see Figures 3 and 4 in Appendix A). The remaining 2 ponds (Pb5 and Pc59; see Figures 3 and 4 in Appendix A) were unsuitable for eDNA sampling: Pond Pb5 was too turbid with floating sediments and Pond Pc59 had high volumes of debris.
- 2.2.5 The eDNA sampling technique was also used on one of the four ponds scoped in for surveys within the Investigation Area (Pc14) (see Figure 3 in Appendix A). The remaining three ponds (Pc12, Pc16 and Pc63; see Figure 3 in Appendix A) were not sampled using the eDNA sampling technique as Pc12 was found to be dry at the time of the survey; and GCN presence in Pc16 and Pc63 had already been confirmed using conventional survey techniques.
- 2.2.6 Sampling was carried out according to the protocol described in the Technical advice note for field and laboratory sampling of GCN environmental DNA (Biggs *et al*, 2014), using the equipment provided by SureScreen, the laboratory undertaking the analysis. Water sampling was undertaken by two AECOM ecologists, with at least one Natural England GCN survey licence holder. The dates, weather conditions and temperatures at the time of pond sampling are shown in Table 3.
- 2.2.7 Separate sampling kits were provided by SureScreen for each waterbody. The kits comprised: a sampling ladle, a sealable plastic bag, a pipette, two pairs of latex gloves and six sample bottles each containing absolute ethanol as a fixing agent, sodium acetate and other markers.
- 2.2.8 The protocol for sampling was as follows:
- Put on first pair of gloves;
 - Take sampling ladle and plastic bag;
 - Using the ladle, take around 20 full (30ml) ladle samples of water from around the pond edge, first gently agitating the water to stir up the water column, but not too much to avoid disturbing or collecting sediment in the sample;
 - Seal the plastic bag and shake to mix the sample;
 - Put on new gloves and, taking pipette, put 15ml into each of the six replicated sample bottles;
 - Screw the lid tight and then shake to mix the sample with the ethanol; and
 - Once samples have been taken, keep in cool boxes until delivered to the laboratory within two days of sampling.

Conventional Survey Methodology

- 2.2.9 As the eDNA test can only be used to determine presence/ likely absence and not to estimate population size, at least 2 conventional GCN surveys were also undertaken alongside the eDNA sampling to ensure that if eDNA sampling results were returned as positive, a population size estimate survey could still be undertaken within the survey window in spring 2017 (representing 6 visits, including 3 within the peak period mid-April to mid-May).

2.2.10 Where feasible, two survey visits were undertaken for the 16 waterbodies scoped in for surveys within 500m of the proposed scheme and the 4 ponds within the Investigation Area. The dates, weather conditions and temperatures at the time of each survey are shown in Table 3. All surveys were undertaken by Natural England licensed ecologists from AECOM.

2.2.11 Where possible, and in accordance with published guidance (*English Nature*, 2001; *Gent & Gibson*, 1998), each survey visit utilised a minimum of 3 approved survey techniques: namely bottle trapping, egg searching, torching and/ or netting also used where appropriate. A brief description of each technique is described below:

- Bottle trapping involved the placement of traps (constructed from 2l plastic bottles) around the pond margin at 2m intervals, where access permitted;
- Vegetation and other submerged materials were inspected for the presence of GCN eggs for a minimum of 15 minutes per pond;
- Surveyors walked the periphery of the pond (where possible) using powerful handheld torches (Clulite – 1M candlelight) to search the water for adult newts and their larvae;
- Refuge searching involved looking underneath rocks, logs, moss, and discarded debris, especially in the vicinity of ponds; and
- Netting involved using a sturdy dip-net with a 2 - 4mm mesh, which can be a useful survey technique, although in general it is not as likely to reveal the presence of newts as are egg searching, torching or bottle trapping.

Table 3: GCN Survey Conditions

Waterbody	Conventional Survey												eDNA sampling date
	Visit 1						Visit 2						
	Date	Torching Temperature (°C) (Overnight minimum Temperature (°C))	Technique used				Date	Torching T° (Overnight min. T°)	Technique used				
			Bottle trapping	Egg searching	Torching	Netting/hand search			Bottle trapping	Egg searching	Torching	Netting/hand search	
Waterbodies within 500m of proposed scheme													
Pa1	03/04/2017	11 (5)	✓	✓	✓		18/04/2017	7 (3)	✓	✓	✓		18/04/2017
Pa2	04/04/2017	6 (4)	✓	✓	✓		19/04/2017	10 (7)	✓	✓	✓	✓	18/04/2017
Pa3	06/04/2017	7 (4)	✓	✓		✓	19/04/2017	10 (7)	✓	✓	✓	✓	20/04/2017
Pa4	04/04/2017	8 (4)	✓	✓		✓	No evening access						19/04/2017
Pa5	04/04/2017	8 (4)	✓	✓	✓		19/04/2017	10 (7)	✓	✓	✓	✓	19/04/2017
Pa9	03/04/2017	11 (5)	✓	✓		✓	18/04/2017	7 (3)	✓	✓	✓	✓	18/04/2017
Pa12	04/04/2017	8 (4)	✓	✓	✓		19/04/2017	10 (7)	✓	✓	✓	✓	20/04/2017
Pb1	05/04/2017	7 (4)	✓	✓	✓		20/04/2017	12 (8)	✓	✓	✓		20/04/2017

Waterbody	Conventional Survey												eDNA sampling date	
	Visit 1						Visit 2							
	Date	Torching Temperature (°C) (Overnight minimum Temperature (°C))	Technique used				Date	Torching T° (Overnight min. T°)	Technique used					
Bottle trapping			Egg searching	Torching	Netting/hand search	Bottle trapping			Egg searching	Torching	Netting/hand search			
Pb4	05/04/2017	7 (4)		✓	✓	✓	20/04/2017	12 (8)		✓	✓	✓	20/04/2017	
Pb5	05/04/2017	7 (4)		✓	✓	✓	20/04/2017	12 (8)		✓	✓	✓	Pond nearly dry – eDNA test not undertaken	
Pb6	05/04/2017	7 (4)	✓	✓	✓	✓	20/04/2017	13 (8)	✓	✓	✓	✓	20/04/2017	
Pb8	05/04/2017	7 (4)	✓	✓	✓		20/04/2017	12 (8)	✓	✓	✓		20/04/2017	
Pb9	05/04/2017	7 (4)		✓	✓	✓	20/04/2017	12 (8)		✓	✓	✓	20/04/2017	
Pc59	04/04/2017	8 (4)	✓	✓	✓	✓	19/04/2017	10 (7)	✓	✓	✓	✓	Unsuitable for eDNA sampling - too much debris. Visit 3 undertaken on the 16/05/2017 and Visit 4 on the 19/06/2017 (bottle trapping, egg search and Torching used on both additional visits).	
Pc61	04/04/2017	8 (4)	✓	✓		✓	19/04/2017	10 (7)	✓	✓		✓	19/04/2017	
Pc66	05/04/2017	7 (4)	✓	✓	✓	✓	High numbers of sticklebacks present – survey aborted						eDNA test not undertaken	
Investigation Area														
Pc12	03/04/2017	9 (5)	✓	✓		✓	19/07/2017	Pond dry						Pond dry
Pc14	03/04/2017	9 (5)	✓	✓	✓		18/04/2017	7 (3)	✓	✓	✓	✓	19/04/2017	
Pc16	03/04/2017	9 (5)	✓	✓	✓		18/04/2017	7 (3)	✓	✓	✓		No eDNA test required as GCN presence	

Waterbody	Conventional Survey											eDNA sampling date	
	Visit 1						Visit 2						
	Date	Torching Temperature (°C) (Overnight minimum Temperature (°C))	Technique used				Date	Torching T° (Overnight min. T°)	Technique used				
Bottle trapping			Egg searching	Torching	Netting/hand search	Bottle trapping			Egg searching	Torching	Netting/hand search		
												confirmed	
Pc63	03/04/2017	11 (5)	✓	✓		✓	18/04/2017	7 (3)	✓	✓	✓	✓	No eDNA test required as GCN presence confirmed

2.3 Limitations

- 2.3.1 No significant constraints to the validity of the surveys were identified.
- 2.3.2 On nights when the waterbodies were turbid, torchlight surveys could only be undertaken in the margins. On these occasions, other survey techniques such as refuge search or netting were also used to complement the survey.
- 2.3.3 On 18 April 2017, the overnight minimum temperature was 3°C, below the 5°C recommended by Natural England for survey. However, whilst this is a limitation for conventional survey methods, the principal survey method was eDNA analysis of water samples and so is not considered to be a significant constraint to the results.
- 2.3.4 The waterbodies were identified from OS maps, online aerial photographs, pond records from DWT, information from locals, and the results of the AECOM Phase 1 Habitat survey (dating from 2015, 2016 and 2017). Considering the urban landscape of the proposed scheme, it is possible that there are small ponds located within private gardens, but these could not be confirmed. However, it is not considered that this represents a significant constraint with regard to the validity of the surveys as reported herein, given the ponds that could be surveyed in the wider area.
- 2.3.5 Access was not granted to 3 out of the 9 ponds within the Investigation Area (located >900m from the proposed scheme at Kingsway junction). However, this is not considered to be a constraint as data gathered from the other 5 ponds was sufficient to confirm presence of GCN in the Investigation Area.
- 2.3.6 The survey constraints relative to each waterbody on different visits, and how these were considered/ addressed, are highlighted in the full survey results in Appendix D

3 RESULTS



3.1 Desk-based Study





- 3.1.1 Biological records were provided by DWT as part of the updated desk based investigations in 2016 (see Figure 2 in Appendix A).
- 3.1.2 The nearest record of GCN to the proposed scheme from within the last ten years is located at approximately 900m from the proposed scheme boundary in Mickelover Meadows, to the west of Kingsway junction.
- 3.1.3 No designated site noted for GCN is located within 2km of the proposed scheme boundary.
- 3.1.4 No amphibian records were provided from EnvIS.

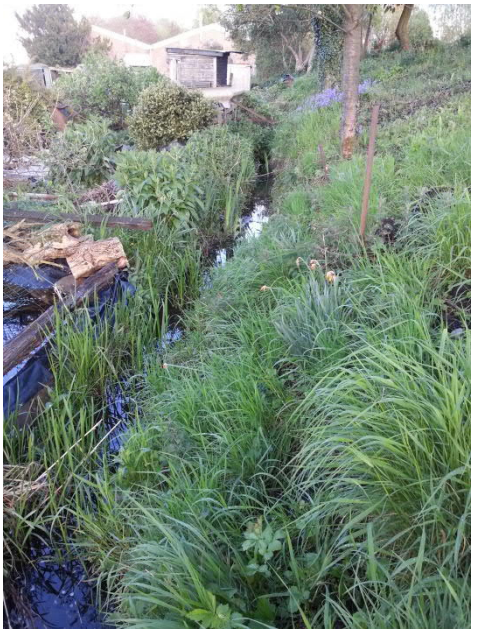



3.2 Pond Descriptions and HSI Results





- 3.2.1 The habitat descriptions, HSI results and photographs of the 16 waterbodies within 500m of the proposed scheme, and the 4 accessible waterbodies within the Investigation Area identified as being potentially suitable to support GCN, are shown in Table 4. The full details of the HSI calculations are presented in Appendix B.





Table 4: Waterbody/ Pond Descriptions and HSI Values


Waterbody	Habitat Description	HSI Value	Photograph
Waterbodies within 500m of proposed scheme			
Pa1	Pa1 was a very large waterbody with an area of approximately 2,000m ² . Bulrush <i>Typha latifolia</i> was present all around the margins. It was located to the west of the carriageway at SK 32110 35459. It had been installed to accept run-off from the Girton Way residential development. This pond was located amongst semi-improved grassland with scrub and plantation woodland.	0.89 Excellent	
Pa2	Pa2 was a very large (approximately 1,500m ²) balancing pond south of Kingsway junction, and to the east of the carriageway. It is understood that this pond was installed to accept run-off from the Derby Royal Hospital overflow car park. The pond was cut in two by a large strip of common reed <i>Phragmites australis</i> . The western bank of the pond was bare and sloped gently towards the water. The eastern side was within scrub and immature trees. The pond was surrounded by semi-improved grassland.	0.86 Excellent	

Waterbody	Habitat Description	HSI Value	Photograph
Pa3	Pa3 was a shallow medium sized pond created in 2013 to receive water from drainage channels dug across the Mackworth allotments. This pond was located within disturbed ground with minimal marginal vegetation. Submerged macrophytes include <i>Elodea canadensis</i> and the surface was dominated by duckweed <i>Lemna spp.</i> The surrounding habitats consisted of scrub and semi-improved grassland.	0.73 Good	
Pa4	Pa4 was a small, recently dug, wildlife pond within the grounds of Brackensdale Junior School at SK 32870 36498 near Kingsway junction. This pond was located within amenity grassland and was considered to have limited connectivity with surrounding habitats.	0.64 Average	
Pa5	Pa5 was a balancing pond for the University of Derby Arts building. The northern bank was steep and maintained with gabions. The southern side slopes gently within amenity grassland towards the water. Two large stands of common reed were located within the water.	0.86 Excellent	
Pa9	Pa9 was a new pond located within Bramble Brook and Margins Local Wildlife Site, south of the Kingsway junction, and east of the carriageway. The pond covered an area of approximately 200m ² . It was located within dense woodland and was entirely shaded. No marginal or aquatic vegetation were recorded. It was set within terrestrial habitat with potential to support GCN.	0.64 Average	

Waterbody	Habitat Description	HSI Value	Photograph
Pa12	Pa12 was located within Mill Dam allotment. It was a narrow and shallow ditch running east-west and then north-south. Dense aquatic or marginal vegetation was recorded.	0.88 Excellent	
Pb1	Pb1 was a ditch surrounding the eastern and southern edges of the Severn Trent Water ponds (Pb2, Pb3). This waterbody was surrounded by good terrestrial habitat.	0.78 Good	
Pb4	Pb4 was a spring-fed duck pond with a septic tank overflow also entering the pond at SK 36780 40247. This waterbody was surrounded by good terrestrial habitat.	0.48 Poor	
Pb5	Pb5 was a small shallow pond receiving run-off from road gullies on Little Eaton junction. The pond was heavily silted with dense reed sweetgrass <i>Glyceria maxima</i> and duckweed present. The surrounding habitat offered with limited habitat connectivity.	0.56 Below average	

Waterbody	Habitat Description	HSI Value	Photograph
Pb6	Pb6 was a concrete lined overflow tank to the east of Little Eaton junction taking water from the enclosed potable water reservoir. This pond was located in a small area of coniferous woodland.	0.71 Good	
Pb8	Pb8 was a long ditch with slow flowing water flows from Little Eaton alongside the Alfreton Road to Little Eaton junction at SK 36422 40173. It was surrounded by good terrestrial habitat.	0.61 Average	
Pb9	Pb9 was a temporary inundation area. No aquatic vegetation was recorded.	0.38 Poor	
Pc59	Pc59 was a very small garden pond, lined and protected with a metal mesh. No aquatic vegetation was recorded.	0.36 Poor	

Waterbody	Habitat Description	HSI Value	Photograph
Pc61	Pc61 was a very small garden pond, lined and elevated with dense Canadian pondweed <i>Elodea sp.</i> and leaf litter.	0.40 Poor	
Pc66	Recently dug shallow pond within turf farm. No shoreline shade. Only a few reeds in the north east corner. Dense water star-wort <i>Callitriche sp.</i> and blanket weed in places.	0.46 Poor	
Investigation Area			
Pc12	Pc12 was a pond of approximately 55m ² in size surrounded by scrub habitat. Aquatic vegetation including floating sweet-grass <i>Glyceria fluitans</i> occupies approximately 15% of the pond.	0.77 Good	
Pc14	Pc14 was a pond of approximately 85m ² and of medium depth. It was surrounded by moderate terrestrial habitat consisting of oak <i>Quercus robur</i> , hawthorn <i>Crataegus monogyna</i> and willow <i>Salix L.</i> Litter pollution was recorded in the pond. Reed mace <i>Typha latifolia</i> , marsh marigold <i>Caltha palustris</i> , fools watercress <i>Apium nodiflorum</i> and floating sweet-grass were recorded, occupying 10% of the pond surface area.	0.67 Average	

Waterbody	Habitat Description	HSI Value	Photograph
Pc16	Pc16 was a small pond within a residential garden. No pollution, invasive species, fowl or fish were recorded. No shoreline shade was present with 10% of the pond area occupied by macrophyte cover. Spike rush <i>Eleocharis palustris</i> was found around the perimeter of the pond.	0.56 Below Average	No photograph
Pc63	Pc63 was built by DWT in 2009 and fenced off from the public in 2011. No signs of pollution were recorded. The pond has no shoreline shade with minor water fowl impact, covering an area of approximately 130m ² . Vegetation present included soft rush <i>Juncus effusus</i> and reed mace.	0.73 Good	

3.3 Survey Results

Waterbodies within 500m of Proposed Scheme

- 3.3.1 No GCN were recorded during the course of the surveys (both eDNA and conventional survey methods). All the eDNA tests came back negative. The laboratory results for the eDNA tests are available in Appendix C.
- 3.3.2 Smooth newts were found in Ponds Pa1, Pa2, Pa3, Pa4, Pa5, Pa9, Pa12, Pb1, Pc59 and Pc61. Smooth newts were also recorded during the 2015 GCN surveys in ponds Pa6 and Pa7. These ponds were scoped out for the 2017 GCN surveys due to the presence of fish.
- 3.3.3 Common frogs (either adults, eggs or tadpoles) were found in Ponds Pa3, Pa4, Pa9, Pa12, Pb9, Pc59 and Pc61. Common frogs were also recorded during the 2015 GCN surveys for Ponds Pa1, Pa2, Pa5, Pa6, Pa7, Pa8, Pb4, Pb6 and Pb8. Pa6, Pa7 and Pa8 were scoped out for the 2017 GCN surveys due to the presence of fish.
- 3.3.4 No adult common toads were found in any of the ponds surveyed in 2017. However, a population of toads was recorded in Ponds Pa6, Pa7 and Pa8 in 2015. These ponds were scoped out for the 2017 GCN surveys due to the presence of fish.
- 3.3.5 Table 5 summarises the findings of the presence/ likely absence survey. Full details of the survey results are provided in Appendix E.

Investigation Area

- 3.3.6 GCN were recorded in Ponds Pc16 and Pc63 using conventional surveys and in Pc14 from the eDNA test. The laboratory result for the eDNA test is available in Appendix D.
- 3.3.7 Smooth newts were found in Ponds Pc14, Pc16 and Pc63.
- 3.3.8 Common frogs were found in Pond Pc63.
- 3.3.9 No adult common toads were found in any of the ponds surveyed.

3.3.10 Table 5 summarises the findings of the survey. Full details of the survey results are provided in Appendix E.

Table 5: Results Summary – GCN Presence/ Likely Absence Surveys and Other Amphibians

Waterbody	GCN	Smooth newt	Frog	Toads
Waterbodies within 500m of proposed scheme				
Pa1		✓	✓*	
Pa2		✓	✓*	
Pa3		✓	✓	
Pa4		✓	✓	
Pa5		✓	✓*	
Pa6	Pond not surveyed for GCN in 2017 due to fish presence	✓*	✓*	✓*
Pa7	Pond not surveyed for GCN in 2017 due to fish presence	✓*	✓*	✓*
Pa8	Pond not surveyed for GCN in 2017 due to fish presence		✓*	✓*
Pa9		✓	✓	
Pa12		✓	✓	
Pb1		✓		
Pb4			✓*	
Pb5				
Pb6			✓*	
Pb8			✓*	
Pb9			✓	
Pc59		✓	✓	
Pc61		✓	✓	
Pc66				
Investigation Area				
Pc12				
Pc14	✓	✓		
Pc16	✓	✓		
Pc63	✓	✓	✓	

* Species not recorded during 2017 surveys, but recorded during 2015 surveys

4 SUMMARY

- 4.1.1 GCN surveys were undertaken between April and June 2017 using HSI, eDNA sampling techniques and conventional survey methods.
- 4.1.2 The surveys identified that GCN were likely to be absent from those habitats on and within 500m of the proposed scheme. Several ponds outside the standard 500m survey buffer were also investigated in Mickleover Meadows where records of GCN were reported by DWT. GCN presence was confirmed with proof that the GCN territory had expanded slightly to the east, however, these would not be impacted by the proposed scheme. Other amphibians found within 500m of the proposed scheme were smooth newts, toads and frogs.
- 4.1.3 A population of toads was found at Markeaton in 2015 on ponds Pa6, Pa7 and Pa8. These three ponds were scoped out for the 2017 GCN surveys due to the presence of fish. However, it is considered that this population of toads is still present in this area and will be considered within the next stage of assessment.

5 REFERENCES

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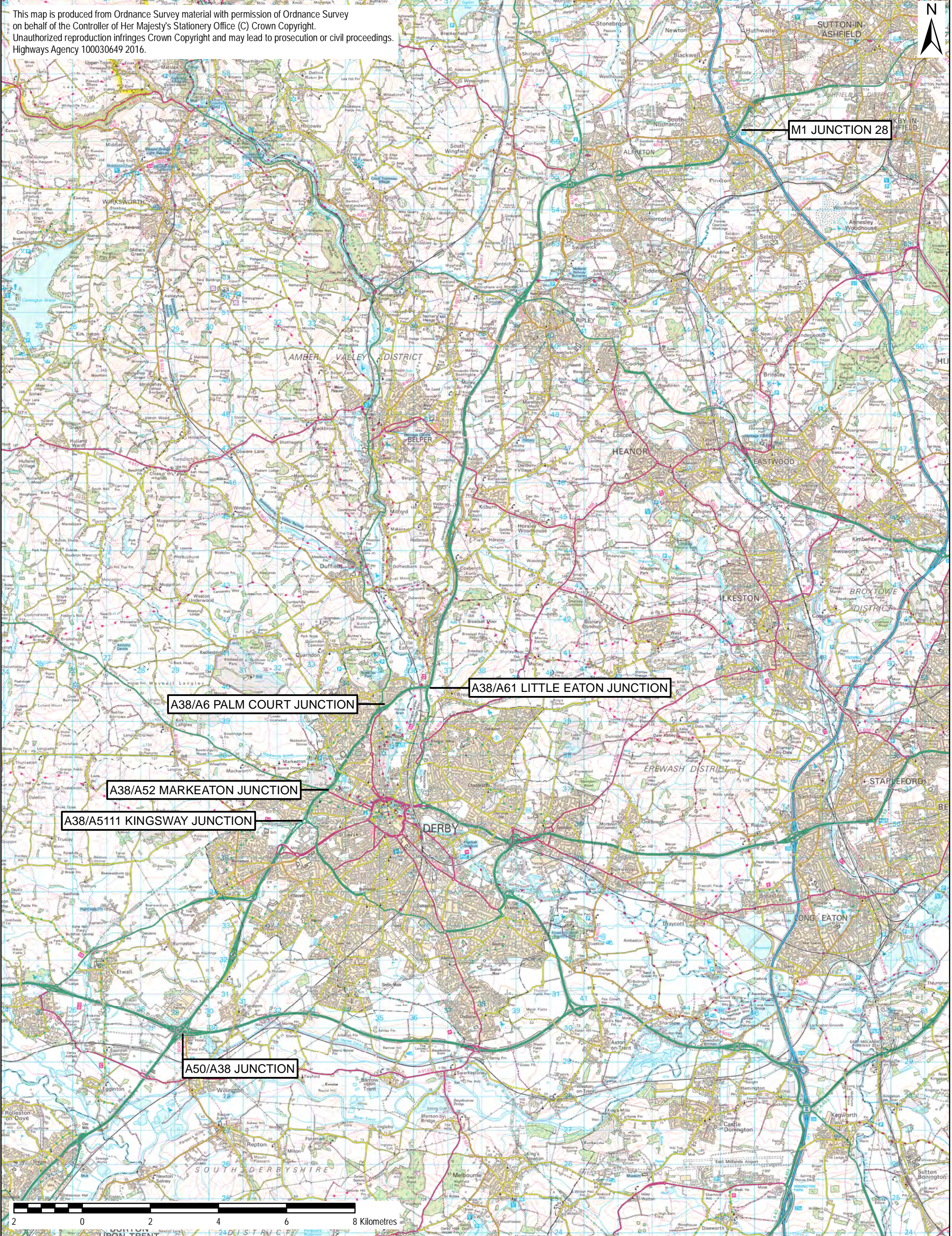
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Appendix A Figures



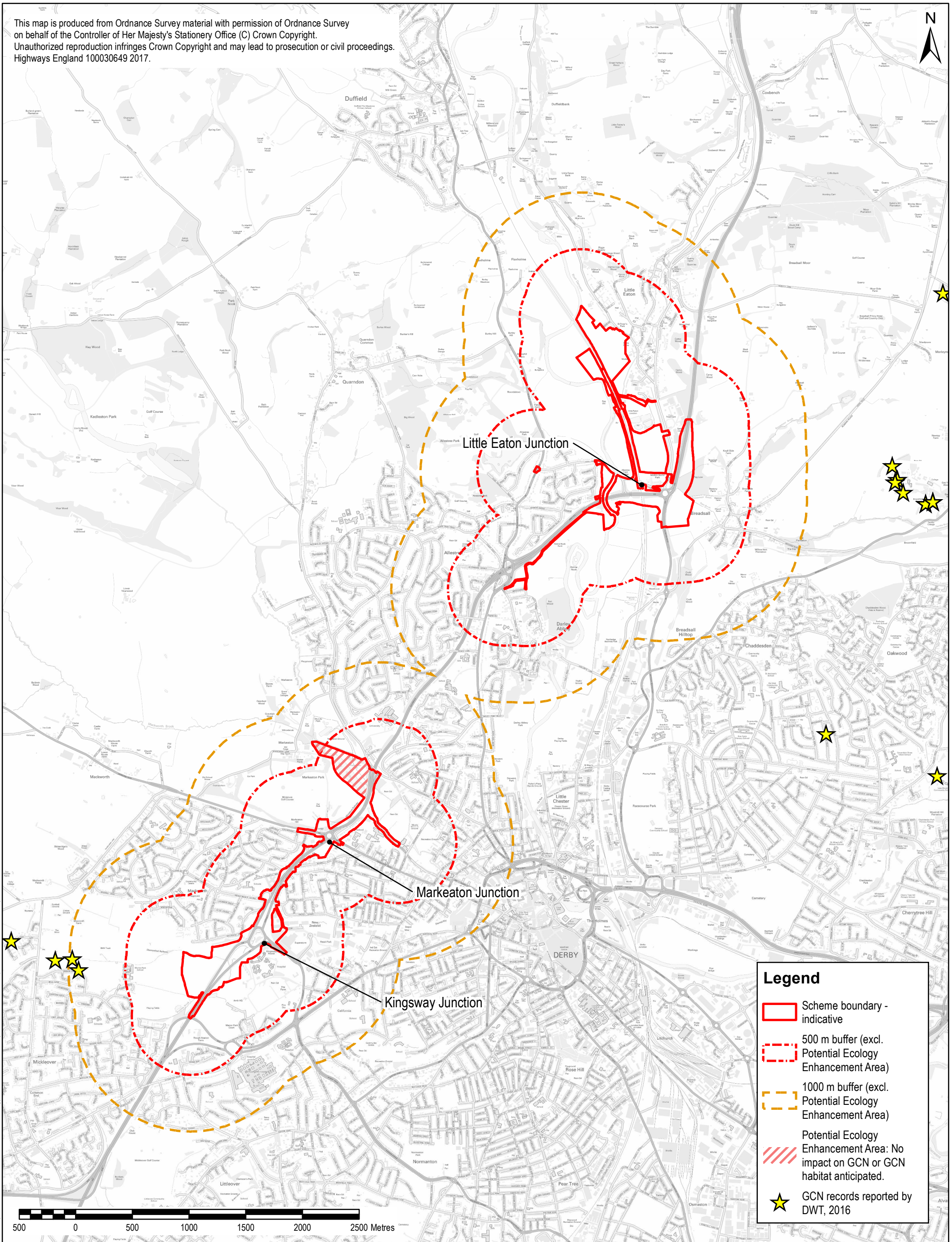
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	Drawn GB	Checked SW	Approved SW		
	Date 04/02/2016	Scale @ A3 1:100,000	Purpose of issue FINAL	AECOM Royal Court Basil Close, Chesterfield Derbyshire, S41 7SL +44 (0) 1246 209221 +44 (0) 1246 209229 www.aecom.com	
Drawing Number Figure 1			Rev 1F		

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File Name: \\ch-wip-001\CH_Roads\A38 Derby Jns - POT3912 CAD\12.1 WIP\FIGURE 1.1 - LOCATION PLAN F1.mxd

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Project Title/Drawing Title			AECOM Internal Project Number			A38 Derby Junctions Project	
A38 DERBY JUNCTIONS DESK STUDY GREAT CRESTED NEWT RECORDS (2016 DATA REQUEST)			60533462			Highways England, Floor 5	
			Drawn	Checked	Approved	2 Colmore Square	
			GSB	HP	PB	38 Colmore Circus	
Date			Scale @ A3			AECOM	
18/10/2017			1:30,000			Royal Court	
Drawing Number			Purpose of issue			Basil Close, Chesterfield	
Figure 2			FINAL			Derbyshire, S41 7SL	
			Rev			+44 (0) 1246 209221	
						+44 (0) 1246 209229	
						www.aecom.com	

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Legend

Scheme boundary - indicative

500 m buffer (excl. Potential Ecology Enhancement Area)

Potential Ecology Enhancement Area: No impact on GCN or GCN habitat anticipated.

Investigation Area: Mickleover Meadows

Desk study:

Pond recorded on OS map, aerial, DWT record or survey observation

Pond surveyed in 2015 (AECOM)

Nearest GCN records reported by DWT, 2016

Ponds scoped in for 2017 GCN surveys:

Pond surveyed for GCN

No access

GCN presence confirmed > 500 m from scheme boundary

No GCN recorded within 500 m of the scheme boundary

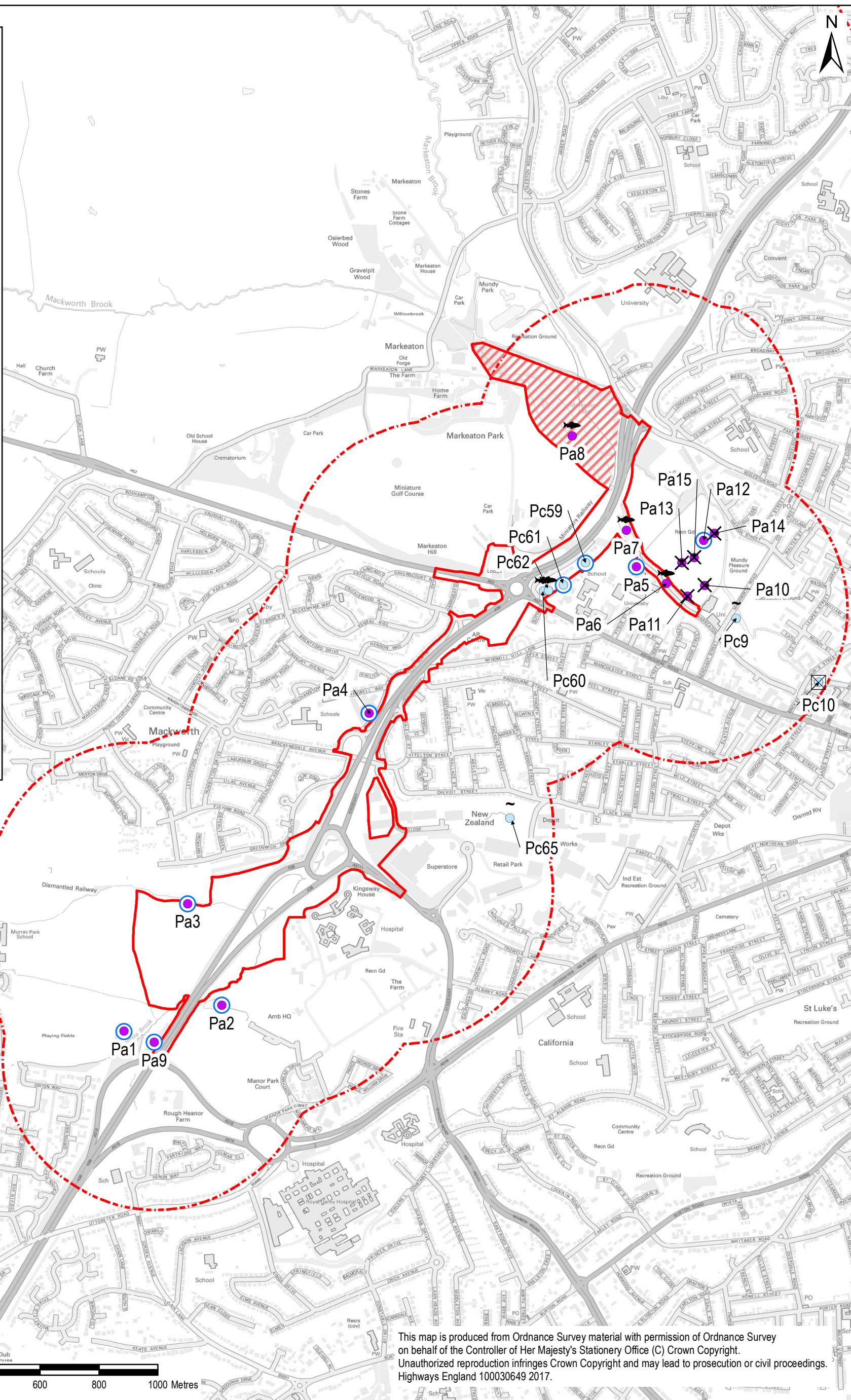
Ponds scoped out from 2017 GCN surveys:

Fish present

Isolated/no habitat connection

Flowing water

Pond destroyed, dry or not found



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Project Title/Drawing Title	
A38 DERBY JUNCTIONS KINGSWAY MARKEATON GREAT CRESTED NEWT SURVEY 2017	
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AECOM Internal Project Number 60533462		
Drawn GSB	Checked HP	Approved PB
Date 18/10/2017	Scale @ A3 1:13,000	Purpose of issue FINAL
Drawing Number Figure 3		Rev .

A38 Derby Junctions Project Highways England, Floor 5 2 Colmore Square 38 Colmore Circus Birmingham B4 6BN	
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Legend

Scheme boundary - Indicative

GCN survey area - 500 m buffer

Running water

Desk study:

Pond recorded on OS map, aerial, DWT record or survey observation

Pond surveyed in 2015 (AECOM) - No GCN recorded

No GCN records reported by DWT for within the GCN survey area in 2016

Ponds scoped in for 2017 GCN surveys:

Pond surveyed for GCN

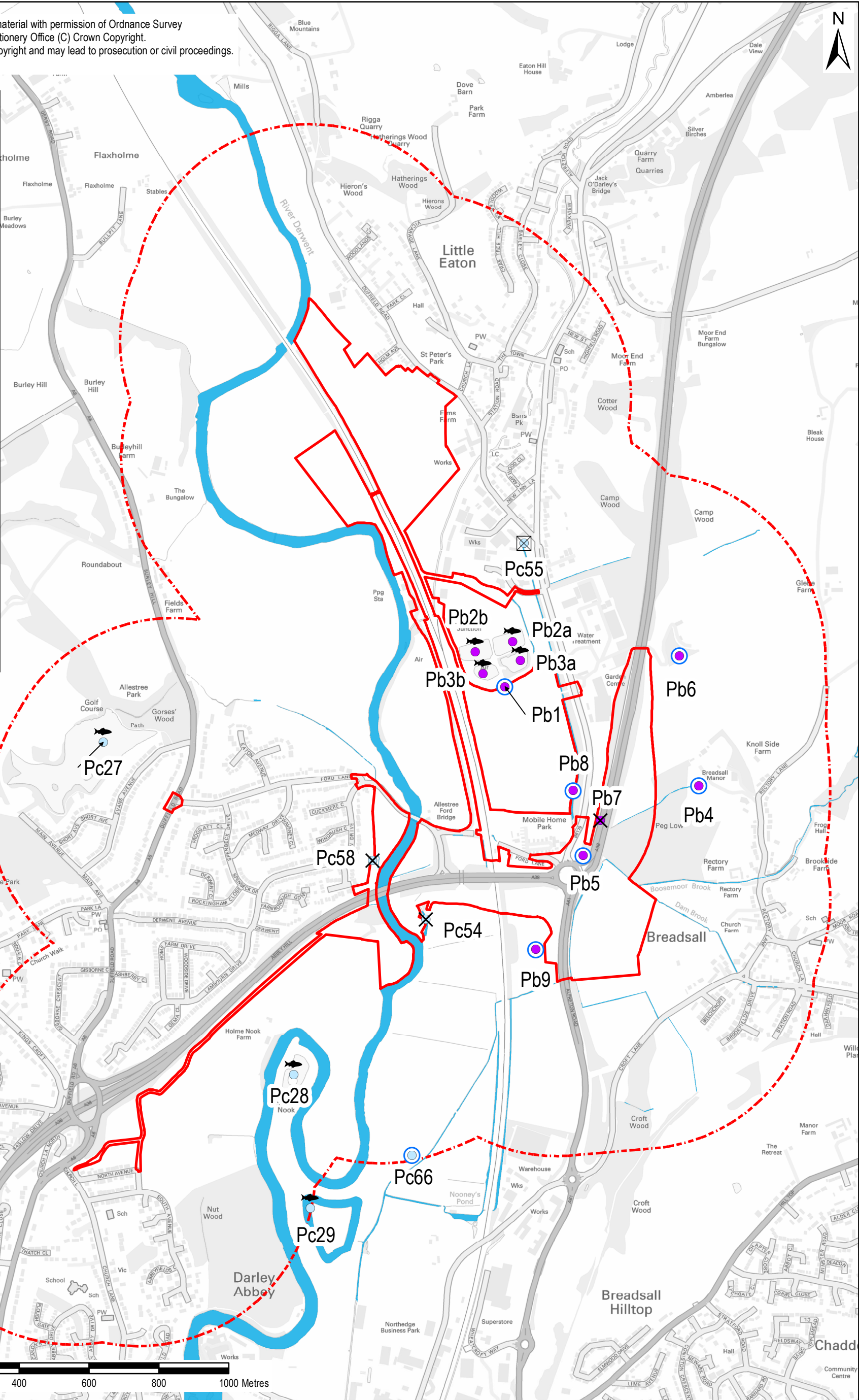
GCN absence confirmed within the GCN survey area


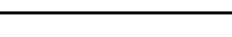
Ponds scoped out from 2017 GCN surveys:

Fish present

Isolated/no habitat connection

Pond destroyed, dry or not found



Project Title/Drawing Title		AECOM Internal Project Number 60533462			A38 Derby Junctions Project Highways England, Floor 5 2 Colmore Square 38 Colmore Circus Birmingham B4 6BN			
A38 DERBY JUNCTIONS LITTLE EATON GREAT CRESTED NEWT SURVEY 2017		Drawn GSB	Checked HP	Approved PB				
		Date 18/10/2017	Scale @ A3 1:11,000	Purpose of issue FINAL				
		Drawing Number Figure 4						
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Appendix B GCN Habitat Suitability Index

		Pa1		Pa2		Pa3	
		Results	Scores	Results	Scores	Results	Scores
SI ₁	Location	A	1.00	A	1.00	A	1.00
SI ₂	Pond area	2,000m ²	0.80	1,500m ²	0.88	150m ²	0.30
SI ₃	Pond drying	Never	0.90	Never	0.90	Never	0.90
SI ₄	Water quality	Good	1.00	Good	1.00	Moderate	0.67
SI ₅	Shoreline shade	10%	1.00	20%	1.00	10%	1.00
SI ₆	Fowl	Minor	0.67	Minor	0.67	Minor	0.67
SI ₇	Fish	Absent	1.00	Absent	1.00	Absent	1.00
SI ₈	Pound count	4	0.72	4	0.72	4	0.72
SI ₉	Terrestrial habitat	Good	1.00	Good	1.00	Good	1.00
SI ₁₀	Macrophytes	60%	0.90	30%	0.60	20%	0.50
HSI			0.89		0.86		0.73
Pond suitability			Excellent		Excellent		Good

		Pa4		Pa5		Pa9	
		Results	Scores	Results	Scores	Results	Scores
SI ₁	Location	A	1.00	A	1.00	A	1.00
SI ₂	Pond area	20m ²	0.04	450m ²	0.90	200m ²	0.40
SI ₃	Pond drying	Never	0.90	Never	0.90	Never	0.90
SI ₄	Water quality	Good	1.00	Moderate	0.67	Moderate	0.67
SI ₅	Shoreline shade	0%	1.00	10%	1.00	100%	0.20
SI ₆	Fowl	Minor	0.67	Minor	0.67	Minor	0.67
SI ₇	Fish	Absent	1.00	Absent	1.00	Absent	1.00
SI ₈	Pound count	5	0.78	8	0.88	1	0.38
SI ₉	Terrestrial habitat	Good	1.00	Good	1.00	Good	1.00
SI ₁₀	Macrophytes	30%	0.60	40%	0.70	60%	0.90
HSI			0.64		0.86		0.64
Pond suitability			Average		Excellent		Average

		Pa12		Pb1		Pb4	
		Results	Scores	Results	Scores	Results	Scores
SI ₁	Location	A	1.00	A	1.00	A	1.00
SI ₂	Pond area	1,000m ²	0.95	1,000m ²	0.95	300m ²	0.60
SI ₃	Pond drying	Rarely	1.00	Never	0.90	Never	0.90
SI ₄	Water quality	Moderate	0.67	Moderate	0.67	Good	1.00
SI ₅	Shoreline shade	5%	1.00	90%	0.40	70%	0.80
SI ₆	Fowl	Absent	1.00	Minor	0.67	Major	0.01
SI ₇	Fish	Absent	1.00	Absent	1.00	Absent	1.00
SI ₈	Pound count	8	0.88	3	0.65	4	0.72
SI ₉	Terrestrial habitat	Good	1.00	Good	1.00	Moderate	0.67
SI ₁₀	Macrophytes	20%	0.50	50%	0.80	0%	0.30
HSI			0.88		0.78		0.48
Pond suitability			Excellent		Good		Poor

		Pb5		Pb6		Pb8	
		Results	Scores	Results	Scores	Results	Scores
SI ₁	Location	A	1.00	A	1.00	A	1.00
SI ₂	Pond area	100m ²	0.20	300m ²	0.60	200m ²	0.40
SI ₃	Pond drying	Annually	0.10	Rarely	1.00	Sometimes	0.50
SI ₄	Water quality	Poor	0.33	Moderate	0.67	Poor	0.33
SI ₅	Shoreline shade	50%	1.00	90%	0.40	95%	0.30
SI ₆	Fowl	Absent	1.00	Absent	1.00	Absent	1.00
SI ₇	Fish	Absent	1.00	Absent	1.00	Absent	1.00
SI ₈	Pound count	4	0.72	4	0.72	3	0.65
SI ₉	Terrestrial habitat	Good	1.00	Good	1.00	Good	1.00
SI ₁₀	Macrophytes	30%	0.60	0%	0.30	30%	0.60
HSI			0.56		0.71		0.61
Pond suitability			Below average		Good		Average

		Pb9		Pc59		Pc61	
		Results	Scores	Results	Scores	Results	Scores
SI ₁	Location	A	1.00	A	1.00	A	1.00
SI ₂	Pond area	4,000m ²	0.49	2m ²	0.00	3m ²	0.01
SI ₃	Pond drying	Annually	0.10	Never	0.90	Never	0.90
SI ₄	Water quality	Moderate	0.67	Poor	0.33	Poor	0.33
SI ₅	Shoreline shade	10%	1.00	100%	0.20	100%	0.20
SI ₆	Fowl	Major	0.01	Absent	1.00	Absent	1.00
SI ₇	Fish	Absent	1.00	Absent	1.00	Absent	1.00
SI ₈	Pound count	1	0.38	5	0.78	5	0.78
SI ₉	Terrestrial habitat	Good	1.00	Moderate	0.67	Moderate	0.67
SI ₁₀	Macrophytes	20%	0.50	0%	0.30	30%	0.60
HSI			0.38		0.36		0.40
Pond suitability			Poor		Poor		Poor

		Pc66		Pc12		Pc14	
		Results	Scores	Results	Scores	Results	Scores
SI ₁	Location	A	1.00	A	1.00	A	1.00
SI ₂	Pond area	1,000m ²	0.95	120m ²	0.24	85m ²	0.17
SI ₃	Pond drying	Never	0.90	Rarely	1.00	Never	0.90
SI ₄	Water quality	Moderate	0.67	Moderate	0.67	Moderate	0.67
SI ₅	Shoreline shade	0%	1.00	70%	0.80	60%	1.00
SI ₆	Fowl	Minor	0.67	Absent	1.00	Minor	0.67
SI ₇	Fish	Major	0.01	Absent	1.00	Absent	1.00
SI ₈	Pound count	4	0.72	7	0.85	12	0.98
SI ₉	Terrestrial habitat	Poor	0.33	Good	1.00	Moderate	0.67
SI ₁₀	Macrophytes	20%	0.50	40%	0.70	10%	0.40
HSI			0.46		0.77		0.67
Pond suitability			Poor		Good		Average

		Pc16		Pc63	
		Results	Scores	Results	Scores
SI ₁	Location	A	1.00	A	1.00
SI ₂	Pond area	6m ²	0.01	130m ²	0.26
SI ₃	Pond drying	Never	0.90	Never	0.90
SI ₄	Water quality	Good	1.00	Moderate	0.67
SI ₅	Shoreline shade	0%	1.00	0%	1.00
SI ₆	Fowl	Absent	1.00	Minor	0.67
SI ₇	Fish	Absent	1.00	Absent	1.00
SI ₈	Pound count	12	0.98	12	0.98
SI ₉	Terrestrial habitat	Moderate	0.67	Moderate	0.67
SI ₁₀	Macrophytes	10%	0.40	30%	0.60
HSI			0.56		0.73
Pond suitability			Below Average		Good

Appendix C eDNA Test Results from SureScreen

Folio No: E0235
Report No: 1
Order No: 60533462
Client: AECOM INFRASTRUCTURE &
ENVIRONMENT
Contact: Gaelle Bardsley
Contact Details: gaelle.bardsley@aecon.com
Date: 04/05/2017

TECHNICAL REPORT

ANALYSIS OF ENVIRONMENTAL DNA IN POND WATER FOR THE DETECTION OF GREAT CRESTED NEWTS

Date sample received at Laboratory: 20/04/2017
Date Reported: 04/05/2017
Matters Affecting Results: None

RESULTS

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
31198	Pa 5, Derby University	N/A	Pass	Pass	Pass	Negative	0
31199	Pa 4, Bracken Dale junior School, Derby	N/A	Pass	Pass	Pass	Negative	0
31201	Pa 12, allotments, Muchworth Road, Derby	N/A	Pass	Pass	Pass	Negative	0
31202	Pc 14, Murray Road, Community Park	N/A	Pass	Pass	Pass	Positive	8
31205	Pa 3, Greenwich Drive South, Allotment, A38, Derby	N/A	Pass	Fail	Pass	Negative	0

31207	Pc 61, 6 Queensway, Derby	N/A	Pass	Pass	Pass	Negative	0
31211	Pa 2, A38 Kingsway Hospital, Derbyshire	N/A	Pass	Pass	Pass	Negative	0
31213	Pa 9, A38, Girton Way, Playing Field	N/A	Pass	Pass	Pass	Negative	0
31217	Pa 1, Aiton Way Playing fields, Derby	N/A	Pass	Pass	Pass	Negative	0

SUMMARY

When Great Crested Newts (GCN); *Triturus cristatus* inhabit a pond, they deposit traces of their DNA in the water as evidence of their presence. By sampling the water, we can analyse these small environmental DNA (eDNA) traces to confirm GCN habitation, or establish GCN absence.

The water samples detailed below were submitted for eDNA analysis to the protocol stated in DEFRA WC1067 (Latest Amendments). Details on the sample submission form were used as the unique sample identity.

RESULTS INTERPRETATION

Lab Sample No.- When a kit is made it is given a unique sample number. When the pond samples have been taken and the kit has been received back in to the laboratory, this sample number is tracked throughout the laboratory.

Site Name- Information on the pond.

O/S Reference - Location/co-ordinates of pond.

SIC- Sample Integrity Check. Refers to quality of packaging, absence of tube leakage, suitability of sample (not too much mud or weed etc.) and absence of any factors that could potentially lead to results errors. Inspection upon receipt of sample at the laboratory. To check if the Sample is of adequate integrity when received. Pass or Fail.

DC- Degradation Check. Analysis of the spiked DNA marker to see if there has been degradation of the kit since made in the laboratory to sampling to analysis. Pass or Fail.

IC- Inhibition Check- PCR inhibitors can cause false results. Inhibitors are analysed to check the quality of the result. Every effort is made to clean the sample pre-analysis however some inhibitors cannot be extracted. An unacceptable inhibition check will cause an indeterminate sample and must be sampled again.

Result- NEGATIVE means that GCN eDNA was not detected or is below the threshold detection level and the test result should be considered as no evidence of GCN presence. POSITIVE means that GCN eDNA was found at or above the threshold level and the presence of GCN at this location at the time of sampling or in the recent past is confirmed. Positive or Negative.

Positive Replicates- To generate the results all of the tubes from each pond are combined to produce one eDNA extract. Then twelve separate analyses are undertaken. If one or more of these analyses are positive the pond is declared positive for the presence of GCN. It may be assumed that small fractions of positive analyses suggest low level presence but this cannot currently be used for population studies. In accordance with Natural England protocol, even a score of 1/12 is declared positive.

METHODOLOGY

The laboratory testing adheres to strict guidelines laid down in WC1067 Analytical and Methodological Development for Improved Surveillance of The Great Crested Newt, Version 1.1

The analysis is conducted in two phases. The sample first goes through an extraction process where all six tubes are pooled together to acquire as much eDNA as possible. The pooled sample is then tested via real time PCR (also called q-PCR). This process amplifies select part of DNA allowing it to be detected and measured in 'real time' as the analytical process develops. qPCR combines PCR amplification and detection into a single step. This eliminates the need to detect products using gel electrophoresis. With qPCR, fluorescent dyes specific to the target sequence are used to label PCR products during thermal cycling. The accumulation of fluorescent signals during the exponential phase of the reaction is measured for fast and objective data analysis. The point at which amplification begins (the Ct value) is an indicator of the quality of the sample. True positive controls, negatives and blanks as well as spiked synthetic DNA are included in every analysis and these have to be correct before any result is declared so they act as additional quality control measures.

The primers used in this process are specific to a part of mitochondrial DNA only found in GCN ensuring no DNA from other species present in the water is amplified. The unique sequence appropriate for GCN analysis is quoted in DEFRA WC 1067 and means there should be no detection of closely related species. We have tested our system exhaustively to ensure this is the case in our laboratory. We can offer eDNA analysis for most other species including other newts.

Analysis of eDNA requires scrupulous attention to detail to prevent risk of contamination. Kits are manufactured by SureScreen Scientifics to strict quality procedures in a separate building and with separate staff, adopting best practice from WC1067 and WC1067 Appendix 5. Kits contain a 'spiked' DNA marker used as a quality control tracer (SureScreen patent pending) to ensure any DNA contained in the sampled water has not deteriorated in transit. Stages of the DNA analysis are also conducted in different buildings at our premises for added

SureScreen Scientifics Ltd also participate in Natural England's proficiency testing scheme and we also carry out inter-laboratory checks on accuracy of results as part of our quality procedures.

Reported by: Sam Humphrey

Approved by: Harry Neal

End Of Report

Folio No: E0262
Report No: 1
Order No: 60533462
Client: AECOM INFRASTRUCTURE &
ENVIRONMENT
Contact: Gaelle Bardsley
Contact Details: gaelle.bardsley@aecom.com
Date: 28/04/2017

TECHNICAL REPORT

ANALYSIS OF ENVIRONMENTAL DNA IN POND WATER FOR THE DETECTION OF GREAT CRESTED NEWTS

Date sample received at Laboratory: 21/04/2017
Date Reported: 28/04/2017
Matters Affecting Results: None

RESULTS

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
31204	PB6 - A38	N/A	Pass	Pass	Pass	Negative	0
31208	PB1 - A38	N/A	Pass	Pass	Pass	Negative	0
31212	PB9 - A38	N/A	Pass	Pass	Pass	Negative	0
31214	PB8 - A38	N/A	Pass	Pass	Pass	Negative	0
31216	PB4 - A38	N/A	Pass	Pass	Pass	Negative	0

SUMMARY

When Great Crested Newts (GCN); *Triturus cristatus* inhabit a pond, they deposit traces of their DNA in the water as evidence of their presence. By sampling the water, we can analyse these small environmental DNA (eDNA) traces to confirm GCN habitation, or establish GCN absence.

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The analysis is conducted in two phases. The sample first goes through an extraction process where all six tubes are pooled together to acquire as much eDNA as possible. The pooled sample is then tested via real time PCR (also called q-PCR). This process amplifies select part of DNA allowing it to be detected and measured in 'real time' as the analytical process develops. qPCR combines PCR amplification and detection into a single step. This eliminates the need to detect products using gel electrophoresis. With qPCR, fluorescent dyes specific to the target sequence are used to label PCR products during thermal cycling. The accumulation of fluorescent signals during the exponential phase of the reaction is measured for fast and objective data analysis. The point at which amplification begins (the Ct value) is an indicator of the quality of the sample. True positive controls, negatives and blanks as well as spiked synthetic DNA are included in every analysis and these have to be correct before any result is declared so they act as additional quality control measures.

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Analysis of eDNA requires scrupulous attention to detail to prevent risk of contamination. Kits are manufactured by SureScreen Scientifics to strict quality procedures in a separate building and with separate staff, adopting best practice from WC1067 and WC1067 Appendix 5. Kits contain a 'spiked' DNA marker used as a quality control tracer (SureScreen patent pending) to ensure any DNA contained in the sampled water has not deteriorated in transit. Stages of the DNA analysis are also conducted in different buildings at our premises for added

SureScreen Scientifics Ltd also participate in Natural England's proficiency testing scheme and we also carry out inter-laboratory checks on accuracy of results as part of our quality procedures.

Reported by: Sam Humphrey

Approved by: Derry Hickman

End Of Report

Appendix D Full Survey Results

Survey Results - Ponds Located within 500m of Proposed Scheme

Waterbody	Visit occasion	Date	Conventional Survey Methods						eDNA Sampling	
			Water turbidity	GCN	Other amphibians Smooth newt (SN), frog and toad	Fish	Methods used	Constraints / Notes	eDNA kit number	eDNA test result
Pa1	Visit 1	03/04/2017	1	0	Bottles: 2 male SN, 1 female SN	No	Bottle trap Egg search Torch	Very difficult to bottle due to <i>Typha</i>		
	Visit 2	18/04/2017	1	0	Bottle: 2 male SN	No	Bottle trap Egg search Torch	Limited access to water due to reeds	-	
	eDNA sampling	18/04/2017	-	-	-	-	-	-	Kit number 31217	Negative
Pa2	Visit 1	04/04/2017	1	0	Bottle: 5 male SN, 3 female SN. Torching: 1 male SN, 1 female SN	No	Bottle trap Egg search Torch	-		
	Visit 2	19/04/2017	1	0	Bottle: 1 female SN Torch: 7 male SN, 6 female SN	No	Bottle trap Egg search Net Torch	-		
	eDNA sampling	18/04/2017	-	-	-	-	-	-	Kit number 31211	Negative
Pa3	Visit 1	06/04/2017	2	0	Bottle: 1 male SN, 3 female SN	No	Bottle trap Egg search Hand Search/ Net	Unable to torch as pond obscured by vegetation	-	
	Visit 2	19/04/2017	2	0	Torch: 4 male SN, 5 female SN, 1 Frog Bottle: 2 male SN, 5 female SN Lots of frog spawn and 5 SN eggs found	No	Bottle trap Egg search Hand Search/ Net Torch	-		
	eDNA sampling	20/04/2017	-	-	-	-	-	-	Kit number 31205	Negative

Waterbody	Visit occasion	Date	Conventional Survey Methods						eDNA Sampling	
			Water turbidity	GCN	Other amphibians Smooth newt (SN), frog and toad	Fish	Methods used	Constraints / Notes	eDNA kit number	eDNA test result
Pa4	Visit 1	04/04/2017	-	0	Bottle: 3 male SN, Lots of Frog spawn	No	Bottle trap Egg search Hand Search/ Net	Unable to torch due to no access after dark	-	
	Visit 2	19/04/2017						No evening access		
	eDNA sampling	19/04/2017	-	-	-	-	-	-	Kit number 31199	Negative
Pa5	Visit 1	04/04/2017	2	0	Bottle: 15 male SN, 1 female SN Torch: 1 male SN	No	Bottle trap Egg search Torch	-		
	Visit 2	19/04/2017	3	0	Torch: 10 male SN, 5 female SN Bottle: 3 male SN, 11 female SN	No	Bottle trap Egg search Hand Search/ Net Torch	-		
	eDNA sampling	19/04/2017	-	-	-	-	-	-	Kit number 31198	Negative
Pa9	Visit 1	03/04/2017	1	0	Bottles: 6 male SN, 6 female SN	No	Bottle trap Egg search Hand Search/ Net	Very high macrophyte cover, no torching possible. X2 Mallard present		
	Visit 2	18/04/2017	1	0	Bottle: 1 frog, 2 larvae	No	Bottle trap Egg search Hand Search/ Net Torch	High surface macrophyte cover	-	
	eDNA sampling	18/04/2017	-		-	-	-	-	Kit number 31213	Negative

Waterbody	Visit occasion	Date	Conventional Survey Methods						eDNA Sampling	
			Water turbidity	GCN	Other amphibians Smooth newt (SN), frog and toad	Fish	Methods used	Constraints / Notes	eDNA kit number	eDNA test result
Pa12	Visit 1	04/04/2017	2	0	Bottle: 13 male SN, 3 female SN Torch: 8 male SN, 7 female SN	No	Bottle trap Egg search Torch	Only ditch along Mill Dam deep enough for bottles		
	Visit 2	19/04/2017	4	0	Torch: 1 female SN, 1 Frog Bottle: 18 male SN, 1 female SN	No	Bottle trap Egg search Hand Search/ Net Torch	Heavy cover of surface weed	-	
	eDNA sampling	20/04/2017	-		-	-	-	-	Kit number 31201	Negative
Pb1	Visit 1	05/04/2017	2	0	Bottle: 4 male SN	No	Torch Bottle Trap Egg search	Water Quality poor- evidence of leakage entering		
	Visit 2	20/04/2017	2	0	Torch: 2 male SN, 1 female SN Bottle: female SN	No	Torch Bottle Trap Egg search	-	-	
	eDNA sampling	20/04/2017	-	-	-	-	-	-	Kit number 31208	Negative
Pb4	Visit 1	05/04/2017	2	0	None	No	Torch Egg search Hand Search/ Net	Very heavy silt, very little water - Unable to bottle trap		
	Visit 2	20/04/2017	2	0	None	No	Torch Egg search Hand Search/ Net	Very heavy silt, very little water - Unable to bottle trap	-	
	eDNA sampling	20/04/2017	-	-	-	-	-	-	Kit number 31216	Negative
Pb5	Visit 1	05/04/2017	2	0	None	No	Torch Egg search	Heavy silting - No bottle		

Waterbody	Visit occasion	Date	Conventional Survey Methods						eDNA Sampling	
			Water turbidity	GCN	Other amphibians Smooth newt (SN), frog and toad	Fish	Methods used	Constraints / Notes	eDNA kit number	eDNA test result
							Hand Search/ Net	trapping possible		
	Visit 2	20/04/2017	2	0	None	No	Torch Egg search Hand Search/ Net	Nearly dry		
	eDNA sampling	N/A	-	-	-	-	-	eDNA not undertaken too murky	-	-
Pb6	Visit 1	05/04/2017	0	0	None	No	Torch Bottle Trap Egg search Hand Search/ Net	Poor quality water and poor access for safety		
	Visit 2	20/04/2017	0	0	None	No	Torch Bottle Trap Egg search Hand Search/ Net			
	eDNA sampling	20/04/2017	-	-	-	-	-	-	Kit number 31204	Negative
Pb8	Visit 1	05/04/2017	2	0	None	No	Torch Bottle Trap Egg search	Water quality poor		
	Visit 2	20/04/2017	2	0	None	No	Torch Bottle Trap Egg search	Water quality poor		
	eDNA sampling	20/04/2017	-	-	-	-	-	-	Kit number 31214	Negative
Pb9	Visit 1	05/04/2017	1	0	None	No	Torch Egg search Hand Search/	Very shallow and silt in margins, not safe to		

Waterbody	Visit occasion	Date	Conventional Survey Methods						eDNA Sampling	
			Water turbidity	GCN	Other amphibians Smooth newt (SN), frog and toad	Fish	Methods used	Constraints / Notes	eDNA kit number	eDNA test result
							Net	access. Livestock present could interfere with traps. Bottle traps not used. New Zealand pygmyweed <i>Crassula helmsii</i> present		
	Visit 2	20/04/2017	1	0	Torch: 1 frog	No	Torch Egg search Hand Search/ Net	Very shallow and silt in margins, not safe to access. Livestock present could interfere with traps. Bottle traps not used. New Zealand pygmyweed present		
	eDNA sampling	20/04/2017	-	-	-	-	-	-	Kit number 31212	Negative
Pc59	Visit 1	04/04/2017	3	0	Torch: 1 male SN, 1 Frog	No	Torch Bottle Trap Egg search Hand Search/ Net			
	Visit 2	19/04/2017	3	0	None	No	Torch Bottle Trap Egg search Hand Search/ Net	-		

Waterbody	Visit occasion	Date	Conventional Survey Methods						eDNA Sampling	
			Water turbidity	GCN	Other amphibians Smooth newt (SN), frog and toad	Fish	Methods used	Constraints / Notes	eDNA kit number	eDNA test result
	eDNA sampling	N/A	-	-	-	-	-	Unsuitable for eDNA –Too much debris	N/A	-
	Visit 3	16/05/2017	4	0	Torch: 1 juv. frog	No	Torch Bottle Trap Egg search			
	Visit 4	13/06/2017	2	0	None	No	Torch Bottle Trap Egg search			
Pc61	Visit 1	04/04/2017	3	0	Bottle: 4 male SN, 2 female SN Frog spawn and tadpoles	No	Bottle Trap Egg search Hand Search/ Net	No after dark access		
	Visit 2	19/04/2017	3	0	Bottle: 8 male SN, several frog larvae	No	Bottle Trap Egg search Hand Search/ Net	No after dark access		
	eDNA sampling	19/04/2017	-	-	-	-	-	-	Kit number 31207	Negative
Pc66	Visit 1	05/04/2017	0	0	None	Yes	Torch Bottle Trap Egg search Hand Search/ Net	Sticklebacks in 35 of 50 traps Survey aborted due to high density of fish		
	eDNA sampling	-	-	-	-	-	-	-	N/A	-

* The water turbidity was assessed on a scale for 0 to 5, where 0 is very clear and 5 is very turbid.