# Southampton to London Pipeline Project

# Volume 6

Environmental Statement (Volume D)

Appendix 7.10: Great Crested Newt Factual Report

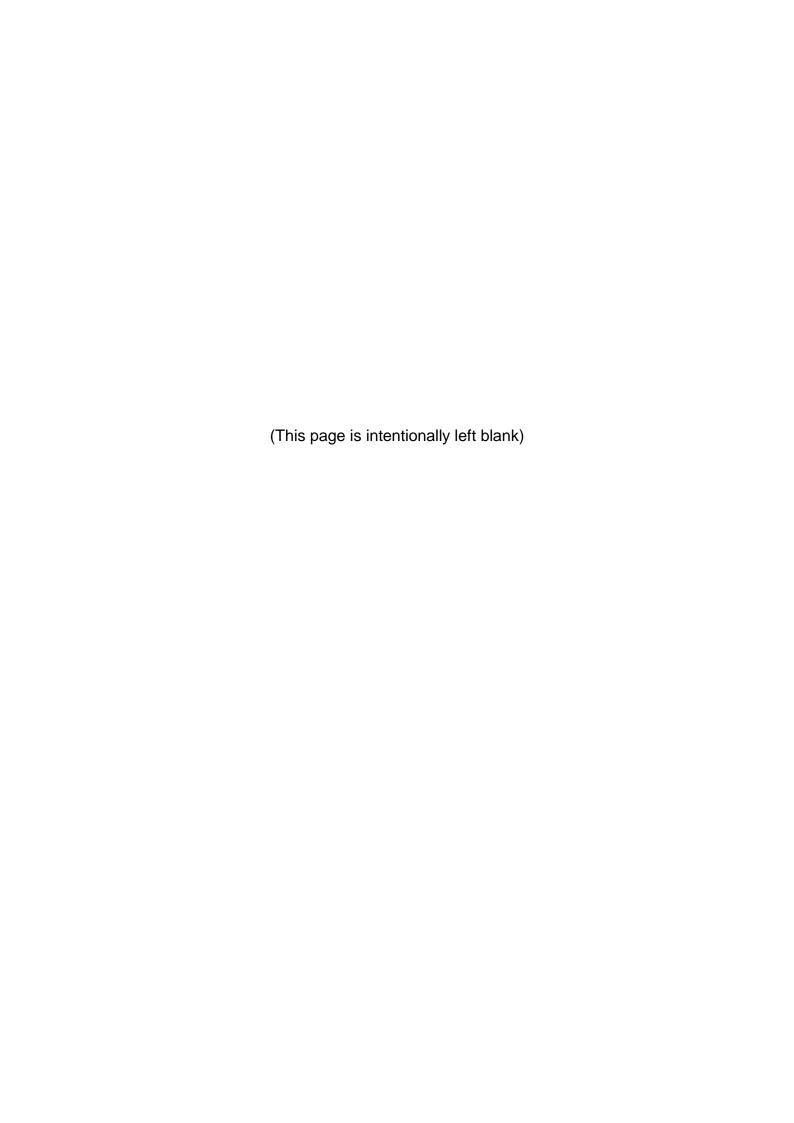
Application Document: 6.4

Planning Inspectorate Reference Number: EN070005

APFP Regulation No. 5(2)(a)

Revision No. 1.0

May 2019





# Southampton to London Pipeline Project

Esso Petroleum Company, Limited

Appendix 7.10: Great Crested Newt Factual Report

B2325300-JAC-000-ENV-APP-000067

May 2019



#### **Southampton to London Pipeline Project**

Project No: B2325300

Document Title: Appendix 7.10: Great Crested Newt Factual Report

Document No.: B2325300-JAC-000-ENV-APP-000067

Revision: R1.0 Date: May 2019

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### 1 Introduction

#### 1.1 Overview

- 1.1.1 Esso Petroleum Company, Limited (Esso) is making an application for development consent to replace 90km (56 miles) of its existing aviation fuel pipeline that runs from the Fawley Refinery near Southampton, to the West London Terminal storage facility in Hounslow.
- 1.1.2 This Great Crested Newt (GCN) Factual Report has been produced to support the application for development consent under the Planning Act 2008, and the accompanying Environmental Statement (ES).

### 1.2 Scheme Description

- 1.2.1 Esso Petroleum Company, Limited (Esso) intends to replace 90km (56 miles) of its 105km (65 miles) long aviation fuel pipeline that runs from its Fawley Refinery near Southampton to its West London Terminal storage facility in Hounslow. The replacement pipeline is 97km (60 miles) long, and is referred to as 'the project'.
- 1.2.2 The replacement pipeline starts near Boorley Green at the end point of the previously replaced pipeline. The route runs generally in a northeast direction via the Pumping Station in Alton. It terminates at the Esso West London Terminal storage facility. A description of the project is provided in Chapter 3 Project Description.

### 1.3 Legal Context

- 1.3.1 GCN are fully protected under European and UK wildlife legislation, including:
  - Conservation of Habitats and Species Regulations 2017; and
  - Wildlife and Countryside Act 1981 (as amended).
- Further to this, GCN are listed as a species of principal importance under Section 41 of the National Environment and Rural Communities (NERC) Act 2006.

# 2 Methodology

#### 2.1 Introduction

2.1.1 The survey methodology described below is based on the methodology detailed in the project's Scoping Report (Esso, 2018). The methodology has also been informed by consultation and engagement with consultees (e.g. Natural England), the results of desk studies, and professional judgement.

### 2.2 Desk Study

2.2.1 Historic records of GCN were requested from Greenspace Information for Greater London (GiGL), Hampshire Biodiversity Information Centre (HBIC), Surrey Biodiversity Information Centre (SBIC) and Surrey Amphibian and Reptile Group



(SARG). Data requests were made to GiGL, HBIC and SBIC in January 2018, requests were made to SARG in August 2018. Results were received from GiGL in January 2018, results from HBIC were received in February 2018 and results from SARG were received in August 2018. At the time of writing this report results have not been received from SBIC.

- 2.2.2 The study area for the desk study is as set out in Chapter 7 Biodiversity.
- 2.2.3 The Multi-Agency Geographic Information for the Countryside (MAGIC) website was reviewed to identify the locations of any European Protected Species (EPS) licences, with respect to GCN within the desk study area.
- 2.2.4 A desk study identified all water bodies within 250m of the Order Limits. Current guidance advises that suitable habitats within 250m of a breeding pond are likely to be used most frequently by GCN if there is an absence of barriers to movement. Small-scale losses of terrestrial habitat, especially over 250m from the breeding pond, are considered unlikely to have effects on GCN (English Nature, 2001). The 250m study area is considered proportionate due to the localised, temporary and reversible nature of pipelaying. The use of a 250m wide buffer is considered standard practice for pipeline projects of this nature. In the context of this project, the buffer extends 250m from the outer edge of the Order Limits in all directions.
- 2.2.5 Ordnance Survey (OS) maps, Geographical Information Systems (GIS) OS MasterMap layers and aerial photography were examined to identify all water bodies within the 250m study area. Ponds located on the limit of the 250m buffer, or just beyond it, were also included. Every pond identified was given a unique reference number.
- 2.2.6 Where necessary, consideration was given to assessing ponds beyond the 250m buffer. For example, where terrestrial habitat likely to be affected by installation may be of importance to a GCN metapopulation located beyond the 250m buffer. A metapopulation is defined as a series of sub-populations that are linked by the dispersal of individuals. In the case of GCN, this usually applies to clusters of ponds located within 250m of each other, which is considered a typical travel distance for GCN across terrestrial habitat (English Nature, 2001).
- 2.2.7 All the identified ponds were assessed to determine their suitability for GCN. Those ponds unsuitable for GCN were 'scoped out' of further surveys. Water bodies such as rivers, large lakes and canals were scoped out due to their large size and/or presence of flowing water creating unsuitable breeding conditions for GCN (Langton et al., 2001). Water bodies identified as fishing ponds or fishing lakes were also excluded from further assessment, as fish predate GCN larvae and greatly reduce the likelihood of GCN presence (English Nature, 2001). Water bodies with major physical barriers including motorways, A-roads, major railways or extensive areas of sub-optimal terrestrial habitat between them and the project area, were scoped out. GCN are unlikely to cross such barriers and are therefore unlikely to be affected by construction work associated with the project.
- 2.2.8 All the remaining water bodies were 'scoped in' and subject to field surveys, where land access permitted. Ponds closest to the Order Limits (<50m) were designated as 'priority ponds' to highlight the potential core GCN habitat that is most likely to be impacted by construction of the project (Annex A).



### 2.3 Field Surveys

2.3.1 During field surveys in 2018, any additional water bodies not previously identified from existing maps or aerial imagery (e.g. ponds obscured from aerial imagery by woodland) were mapped using a GIS application on a tablet. Each newly identified pond was subjected to the same assessments and surveys as all other ponds. Conversely, some ponds identified from desk studies were found to no longer exist or exist in a different state (e.g. one original pond that had since split into two separate ponds).

#### **Habitat Suitability Index (HSI) Assessment**

2.3.2 Where land access permitted, all ponds 'scoped in' to field surveys were subject to a HSI assessment in accordance with the methodology described by Oldham *et al.* (2000). A HSI is a numerical index between 0 and 1, where 0 indicates unsuitable habitat and 1 represents optimal habitat for breeding GCN. A score is calculated based on the results of ten suitability indices, all of which are factors thought to affect GCN presence. The resulting score categorises the pond based on its 'suitability' to support GCN and can be used to influence the decision of whether further detailed survey work is required, as shown by Table 2.1.

Table 2.1: Habitat Suitability Index Scoring System (Oldham et al., 2000)

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

- 2.3.3 In accordance with HSI methodology the following features were assessed:
  - geographical location;
  - pond area;
  - pond permanence;
  - water quality;
  - pond shading;
  - number of waterfowl;
  - occurrence of fish;
  - pond density/other ponds within 1km;
  - terrestrial habitat quality; and
  - macrophyte cover.
- 2.3.4 The results of the HSI survey were used to inform the decision of whether to undertake presence/likely absence surveys. In general, ponds that scored greater than 0.5 were scoped in for further surveys (i.e. 'poor' suitability ponds were generally scoped out). However, it is recognised that GCN are often found in ponds



with 'poor' suitability and so professional judgement was used to decide whether to undertake further surveys of these. For example, if a pond with poor suitability to support GCN is located within close proximity to ponds with higher suitability for GCN, consideration was given to scoping in the pond; or, if the pond scored more than 0.5 but supported features that would likely preclude the presence of breeding GCN (such as a high population of fish), consideration was given to scoping out the pond.

2.3.5 A record of all HSI scores and justification for scoping a pond in or out of further surveys was made using survey sheets on an iPad, the results of which can be seen in Annex A.

#### **Environmental DNA (eDNA) Surveys**

- 2.3.6 Where land access permitted, all ponds within the 250m buffer considered to have potential to support GCN were subject to eDNA surveys, to confirm the presence or likely absence of the species. These surveys were undertaken by a licensed and appropriately trained ecologist between 15 April and 30 June 2018, as per Natural England guidelines (Natural England, 2015).
- 2.3.7 The survey protocol was conducted in accordance with the guidance set out by Biggs *et al.* (2014). Twenty water samples were taken from around the perimeter of each pond using a 40ml ladle, focusing on areas most likely to be used by GCN. The water samples were then transferred to a Whirl-Pak bag. The Whirl-Pak bag was gently shaken to mix any eDNA across the whole water sample. A pipette was then used to transfer 15ml of water from the Whirl-Pak bag into each of six conical tubes containing a preserving fluid. Each conical tube was shaken vigorously for 10 seconds to mix the water sample and preservative. The six conical tubes were labelled and sent to a Nature Metrics laboratory for analysis. Nature Metrics are approved by Natural England to conduct such analysis and provide reliable results.
- 2.3.8 All samples were analysed by scientists at a Nature Metrics laboratory. The laboratory creates a section of DNA known as a primer which is specific to GCN. The primer binds to the GCN ribonucleic acid (RNA) (which acts as a messenger for carrying instructions from DNA for controlling the synthesis of proteins) and initiates the replication process. The polymerase chain reaction (PCR) is used to separate the strands of RNA, allowing primer bonding to occur. The DNA is then amplified in the chain reaction and analysed for GCN DNA presence. If GCN DNA is present, the primer reacts with it and produces readable levels of GCN DNA.
- 2.3.9 Conclusive results of this one survey would confirm the presence or likely absence of GCN in any given pond (Natural England, 2015).

#### **Population Estimate Surveys**

2.3.10 Due to the temporary, reversible and short-duration nature of the project (including the embedded design and good practice measures described in Chapter 4 Design Evolution and Chapter 16 Environmental Management and Mitigation) it is considered that in most instances all impacts to GCN can be confidently predicted and appropriate mitigation can be implemented without the need to estimate population size.



- 2.3.11 Population estimate surveys were undertaken where a high impact is predicted to a metapopulation of GCN, or if installation was to affect an area that has potential to support 'medium' or 'high' populations of GCN. This typically only applied to locations where construction activity would affect 'core' GCN habitat. Core habitat is typically considered to be within 50m of a GCN pond, and to be of high value to a GCN for foraging or hibernating purposes. Areas within 50m of GCN ponds but separated by dispersal barriers or sub-optimal habitats, such as extensive areas of hardstanding or arable fields, were typically not subject to population estimate surveys. However, as large GCN populations have been recorded in apparently sub-optimal conditions, this assessment was made on a case-by-case basis and took account of all baseline information and advice from Natural England. The precautionary approach was adopted, as required.
- 2.3.12 The above approach is broadly consistent with that described in Natural England's survey guidance table (Natural England, 2015a) and aligns with Natural England's European Protected Species Licensing Policy number 4: *Appropriate and relevant surveys where the impacts of development can be confidently predicted.*
- 2.3.13 Where land access and conditions on site permitted, ponds that required population estimate surveys were subject to six survey visits between mid-May and mid-June. The surveys were undertaken in suitable weather conditions. During each visit, bottle trapping and torching survey techniques were used where applicable, with netting used only if one of these methods was not appropriate to a particular pond. All surveys were undertaken by appropriately licensed ecologists in accordance with guidelines provided by English Nature (2001).
- 2.3.14 The results of these surveys have informed a population estimate using guidance provided by English Nature (2001). These results and corresponding population estimates can be found in Table 3.3 and Annex A.

### 2.4 Survey Constraints

#### Field Surveys

- 2.4.1 Field surveys were limited in some locations as land access permission was not available at the time of survey.
- 2.4.2 In some instances, all or some survey types were restricted or considered unreasonable due to health and safety concerns associated with the water body or surrounding area.
- 2.4.3 Due to the iterative design changes to the Order Limits throughout 2018, some ponds were not surveyed because they were outside the 250m buffer until after the GCN survey season had ended e.g. Pond 130a.
- 2.4.4 Pond 223a was not identified until October 2018, by which time it was too late to carry out eDNA or population surveys during the 2018 field season, although an HSI assessment was undertaken.
- 2.4.5 In total, 7% of the 205 ponds identified in the study area were not subject to field surveys due to the reasons above.



- 2.4.6 Hot weather and low rainfall experienced throughout summer 2018 resulted in water levels dropping across many of the water bodies visited. In numerous cases, HSI assessments and eDNA surveys could not be carried out due to a pond being completely dry. For four of the ponds that were population surveyed, water levels dropped too low to enable the completion of the six surveys typically required for a population estimate.
- 2.4.7 The above constraints are not considered likely to affect the baseline information collected. However, pre-construction surveys would be completed if existing baseline survey data need to be updated or supplemented (measure G33 in the Register of Environmental Actions and Commitments, as detailed in Chapter 16 Environmental Management and Mitigation).

#### eDNA Surveys

2.4.8 A single inconclusive eDNA result was returned for Pond 93b. This was likely due to inhibition resulting from sediment in the sample, owing to the shallow nature of the water body. Despite this result, the pond was scoped out of further survey as it was a very shallow area of slow-flowing water in a wet woodland that was considered sub-optimal for GCN (Sheet 21 Figure 7.10.1). As further justification for scoping this pond out, all ponds within 250m that were successfully sampled, returned negative eDNA results.

#### **Population Estimate Surveys**

- 2.4.9 Population surveys were carried out towards the end of the GCN survey season due to time constraints associated with obtaining land access permission for the large number of ponds being assessed. However, all survey visits did fall within the appropriate months for GCN surveys (Natural England, 2015).
- 2.4.10 Four ponds subject to population surveys dried up before all six surveys could be completed: Pond 127a, Pond 129a, Pond 194a and Pond 201 (Figure 7.10.2). Pond 194a dried up after the third survey; Pond 129a dried up after the fourth survey; and Pond 127a and Pond 201 dried up after the fifth survey.

### 3 Results

3.1.1 A total of 205 ponds were assessed by the study. Eighty-eight of these were identified as 'priority ponds', being located within 50m of the Order Limits (Figure 7.10.1; Annex A).

### 3.2 Desk Study

- 3.2.1 Data received from HBIC comprised a total of 15 GCN records within the study area through Hampshire, including records within 250m of the Order Limits. Several of these records relate directly to ponds 'scoped in'. Where possible these ponds were still eDNA surveyed to confirm their continuing presence but were not population surveyed as population class size could be assumed from the existing records (Table 3.1).
- 3.2.2 Data requests have been made to SBIC, but at the time of writing this report, no results have been received.



- 3.2.3 Data provided by SARG comprised of two points where GCN had previously been recorded within the study area. These records coincided with ponds that returned positive eDNA results during field surveys. These ponds are located beyond the 250m buffer surrounding the Order Limits.
- 3.2.4 No records of GCN existed within the data provided by GiGL.
- The peak counts from relevant records are noted in Annex A. The location of all GCN records provided by HBIC and SARG can be seen in Figure 7.10.1.

### 3.3 Field Study

#### **HSI Assessments**

- 3.3.1 During field surveys, numerous ponds were immediately scoped out of further survey for a variety of reasons; for example, the pond had dried up, contained a large population of fish, or was chemically-treated (Annex A). Ponds that were not immediately excluded were subject to HSI assessments. A total of 153 HSI assessments were carried out within the study area. A summary of the HSI results is provided in Table 3.1.
- 3.3.2 The numerical index value and corresponding GCN habitat suitability rating of each individual pond can be seen in Annex A.

Table 3.1: Total Number of Ponds Given Each Suitability Rating From HSI Assessments

Suitability for GCN	Number of Ponds					
Poor	16					
Below average	24					
Average	28					
Good	65					
Excellent	20					

#### eDNA Surveys

- 3.3.3 In total, 121 ponds were eDNA surveyed within the 250m buffer. Of these surveys, there were 15 positive results, 105 negative results and one inconclusive result. The eDNA result of every pond surveyed can be seen in Annexes A and B. All ponds with positive results in the study area are in Table 3.2 and their location in relation to the Order Limits can be seen in Figure 7.10.2. Table 3.2 also shows those ponds where GCN have been confirmed or assumed by other methods.
- 3.3.4 Annex B displays a greater number of ponds surveyed and a greater number of positive results than those described above. This is due to surveys originally being based on the pipeline corridor options in early 2018 and then the preferred corridor, and the survey area being subsequently reduced and revised as the route and Order Limits were finalised. Any results in Annex B that are not mentioned elsewhere in this report or shown in Figures 7.10.1 relate to ponds no longer within the 250m buffer.
- 3.3.5 GCN presence has been assumed for Pond 223a despite no eDNA survey having been undertaken. This pond was not identified until after the end of the survey



season. However, it has good habitat suitability for GCN and is located within 100m of Pond 223, which returned a positive eDNA survey result.

Table 3.2: Ponds Within 250m of the Order Limits with Confirmed/Assumed GCN Presence

Pond ID	GCN Presence Confirmed By
Pond 11	eDNA survey
Pond 39	eDNA and population surveys
Pond 41	eDNA survey
Pond 50	eDNA survey
Pond 55	eDNA and population survey
Pond 56	HBIC records
Pond 57	eDNA and population survey
Pond 57a	eDNA survey
Pond 63	GCN egg found
Pond 65a	eDNA survey
Pond 71	HBIC records
Pond 71a	HBIC records
Pond 73	eDNA survey
Pond 127	Population survey
Pond 127a	Population survey
Pond 128	Population survey
Pond 129a	eDNA and population survey
Pond 180	eDNA survey
Pond 194a	eDNA and population surveys
Pond 194c	eDNA and population surveys
Pond 201	eDNA and population surveys
Pond 223	eDNA survey
Pond 223a	Assuming presence due to proximity (<100m) to Pond 223 and suitable connecting habitat.

#### **Population Estimate Surveys**

- 3.3.6 A total of 10 ponds were surveyed to estimate population class size due to their proximity to the Order Limits and potential to contribute to metapopulations. Individually, the findings of the population surveys suggested two ponds with medium breeding populations and eight with small breeding populations (Table 3.3).
- 3.3.7 Of the population estimate surveys (Table 3.3), only Pond 39 is considered unlikely to form part of a metapopulation due to its isolation. Only one pond, Pond 39a, is within 500m and that was considered unsuitable to support GCN due to its low HSI score and as it was dry (Table A1, Annex A). All other ponds subject to population surveys are considered to form part of metapopulations. For these ponds, a population size class has been determined using the maximum count obtained from all ponds during any one survey, as described.



#### <u>Upper Froyle Metapopulation (Ponds 55, 56, 57, 57a)</u>

The peak count for a single night of survey was nine, which indicates a small population. However, HBIC desk study information suggests that Pond 56 and Pond 57 (approximately 230m and 200m from the Order Limits, respectively) support medium populations (Annex A). As such, it is considered that the Upper Froyle metapopulation area would likely support at least a medium breeding population (Sheet 4 of Figure 7.10.2).

#### Windlemere Golf Course Metapopulation (Ponds 127, 127a, 128, 129a)

- 3.3.9 The peak count on a single night using a single method to avoid double counting was 41, which suggests at least a medium population of GCN across the former Windlemere Golf Course area (Sheet 6 of Figure 7.10.2).
- 3.3.10 Pond 129 at the former Windlemere Golf Course returned a negative result for GCN eDNA and therefore GCN are considered absent in this pond despite the nearby positive results. Pond 129 was much deeper and with less vegetation than the other ponds on the golf course.

#### Foxhills Golf Course Metapopulation (Ponds 194a, 194c, 201)

- 3.3.11 The peak count for a single night of surveys at ponds with a positive eDNA result on Foxhills Golf Course was six, suggesting at least a small breeding metapopulation across the Foxhills Golf Course area (Sheet 8 of Figure 7.10.2).
- 3.3.12 Pond 201 falls within the boundary of Foxhills Golf Course and is likely to contribute to the same wider metapopulation as Ponds 194a and 194c. However, as it is more than 800m from the nearest GCN pond on the golf course (Pond 187, outside of the survey area) and more than 1km from Ponds 194a and 194c, regular interchange of GCN between these ponds is unlikely.
- 3.3.13 Peak counts and population size classes for individual ponds can be seen in Table 3.3. Full population survey data can be provided on request.

**Table 3.3: Population Survey Results Summary** 

Metapopulation Name	Pond ID	Peak Count	Individual Population Class Size	Metapopulation Population Class Size	
N/A	39	2	Small	N/A	
Upper Froyle	55	6	Small	Medium	
	57a	3	Small		
Windlemere Golf	127	20	Medium	Medium	
Course	127a	14	Medium		
	128	7	Small		
	129a	1 Small			
Foxhills Golf	194a	0	Likely small	Small	
Course	194c	6	Small		
	201	0	Likely small		



### 4 Discussion

- 4.1.1 The GCN populations identified within the 250m buffer of the Order Limits likely represent a small proportion of the overall GCN populations in the counties of Surrey and Hampshire, where GCN have recently been recorded in most 10km squares (ARC Trust, 2018). All ponds with confirmed or assumed GCN presence are in Table 3.2 and Figure 7.10.2.
- 4.1.2 The GCN Species Action Plan for Hampshire states that approximately 45 breeding populations are known within Hampshire, and that these are concentrated along the south coast and eastern border of the county. However, it also states that comprehensive surveys of ponds across Hampshire have not been undertaken but that populations in the north of the county are thought to be strong (Hampshire Biodiversity Partnership, 2003).
- 4.1.3 Known GCN sites in Surrey are scattered across the county, though are more concentrated in rural areas of the Wealdon Clay and where pond density is high (SARG, 2018).
- 4.1.4 The results of the surveys show that GCN are present within 250m of the Order Limits at several locations. Construction activity has the potential to kill or injure these animals, or damage, destroy or fragment their terrestrial habitat.
- 4.1.5 As such, construction works likely to affect GCN would be subject to mitigation secured under a EPS licence. Further information with respect to GCN mitigation is provided in Appendix 7.15 Draft Great Crested Newt EPS Licence Application.
- 4.1.6 Pre-construction surveys would be completed if existing baseline survey data need to be updated or supplemented. These surveys would be undertaken in accordance with the relevant guidance provided by Natural England at that time.

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# **Annex A – Summary of All Pond Survey Results**

Table A1: Summary of All Ponds within 250m of the Order Limits

Pond ID	Scoped In/Out?	Justification for Scoping Out	Priority Pond	HSI Score	Pond Suitability	eDNA Results	Population Surveyed	Population Estimate	GCN Present	Notes
1	In	N/A	No	N/A	N/A	N/A	N/A	N/A	Unknown	The pond was not surveyed as landowner permission was refused. Pond is approximately 150m from the Order Limits and is separated by buildings and hard standing. Only one other pond is located within 500m, but that pond is outside the study area as it is beyond the 250m buffer. Due to its isolation, there is considered to be a low risk of Pond 1 supporting a medium/large breeding population (if GCN are present). Given its distance and separation from the Order Limits, there is a low risk of major impacts to GCN due to installation.
5	In	N/A	No	0.77	Good	Negative	N/A	N/A	No	
5a	Out	Poor HSI score. A very shallow scrape. Closest pond is approx. 230m away (Pond 5) and was negative for GCN eDNA.	Yes	0.42	Poor	N/A	N/A	N/A	No	
6	In	N/A	No	0.78	Good	Negative	N/A	N/A	No	
6a	In	N/A	No	0.76	Good	Negative	N/A	N/A	No	
8	In	N/A	Yes	0.67	Average	Negative	N/A	N/A	No	



Pond ID	Scoped In/Out?	Justification for Scoping Out	Priority Pond	HSI Score	Pond Suitability	eDNA Results	Population Surveyed	Population Estimate	GCN Present	Notes
9	In	N/A	No	0.56	Below average	Negative	N/A	N/A	No	
10	Out	Fishing pond not suitable for GCN	N/A	0.35	Poor	N/A	N/A	N/A	No	
11	In	N/A	No	0.76	Good	Positive	No - core GCN habitat would not be impacted	N/A	Yes	Pond is over 100m from Order Limits.
11a	In	N/A	No	0.58	Below average	Negative	N/A	N/A	No	
12	Out	Pond was dry and appears to have been so for a long time	N/A	N/A	N/A	N/A	N/A	N/A	No	
12a	In	N/A	Yes	0.67	Average	Negative	N/A	N/A	No	
12b	Out	Poor HSI score - a shallow scrape that dries annually	Yes	0.45	Poor	N/A	N/A	N/A	No	
13	In	N/A	No	0.78	Good	Negative	N/A	N/A	No	
14	In	N/A	No	0.45	Poor	Negative	N/A	N/A	No	
15	In	N/A	No	0.57	Below average	Negative	N/A	N/A	No	
16	In	N/A	No	0.70	Good	Negative	N/A	N/A	No	
17	In	N/A	No	N/A	N/A	N/A	N/A	N/A	Unknown	The pond was not surveyed as landowner permission was refused. Pond is approximately 240m from the Order Limits. Only one other pond is located within 500m and that is Pond 18, which is approximately 320m away and returned a negative result for GCN



Pond ID	Scoped In/Out?	Justification for Scoping Out	Priority Pond	HSI Score	Pond Suitability	eDNA Results	Population Surveyed	Population Estimate	GCN Present	Notes
										eDNA. Due to its isolation, there is considered to be a low risk of Pond 17 supporting a medium/large breeding population (if GCN are present). Given its distance from the Order Limits, there is a low risk of major impacts to GCN due to installation.
18	In	N/A	Yes	0.73	Good	Negative	N/A	N/A	No	
19	Out	Pond was dry	N/A	N/A	N/A	N/A	N/A	N/A	No	
20	In	N/A	Yes	0.60	Average	Negative	N/A	N/A	No	
21	Out	Pond no longer exists - land being developed	N/A	N/A	N/A	N/A	N/A	N/A	No	
22	In	N/A	No	0.68	Average	Negative	N/A	N/A	No	
22a	In	N/A	No	0.57	Below average	Negative	N/A	N/A	No	
23	In	N/A	No	0.56	Below average	Negative	N/A	N/A	No	
24	In	N/A	Yes	0.55	Below average	Negative	N/A	N/A	No	
25	In	N/A	No	0.61	Average	Negative	N/A	N/A	No	
26	In	N/A	No	N/A	N/A	N/A	N/A	N/A	Unknown	The pond was not surveyed as landowner permission was refused. Pond is approximately 130m from the Order Limits. Only one other pond is located within 500m and that is Pond 27, approximately 490m away and returned a negative result for GCN eDNA. Due to its isolation, there is considered to be a low risk of Pond 26 supporting a medium/large breeding population (if



Pond ID	Scoped In/Out?	Justification for Scoping Out	Priority Pond	HSI Score	Pond Suitability	eDNA Results	Population Surveyed	Population Estimate	GCN Present	Notes
										GCN are present). Given its distance from the Order Limits, there is a low risk of major impacts to GCN due to installation.
27	In	N/A	No	0.69	Average	Negative	N/A	N/A	No	
27a	In	N/A	No	0.61	Average	Negative	N/A	N/A	No	
27b	In	N/A	No	0.56	Below average	Negative	N/A	N/A	No	
27c	In	N/A	No	0.65	Average	Negative	N/A	N/A	No	
28	In	N/A	No	0.75	Good	Negative	N/A	N/A	No	
32	Out	Pond no longer exists.	No	N/A	N/A	N/A	N/A	N/A	No	
33	In	N/A	No	0.69	Average	Negative	N/A	N/A	No	
37	Out	Poor HSI score - was drying out, no macrophytes and heavy poaching of the banks by sheep. Closest pond is approx. 100m away (Pond 38) and was negative for GCN eDNA.	N/A	0.43	Poor	N/A	N/A	N/A	No	
38	In	N/A	Yes	0.53	Below average	Negative	N/A	N/A	No	
39	In	N/A	Yes	0.68	Good	Positive	Yes	Small	Yes	
39a	Out	Poor HSI score and pond was	N/A	0.43	Poor	N/A	N/A	N/A	No	



Pond ID	Scoped In/Out?	Justification for Scoping Out	Priority Pond	HSI Score	Pond Suitability	eDNA Results	Population Surveyed	Population Estimate	GCN Present	Notes
		almost completely dry								
39b	Out	Not a pond - shallow dry depression in a field that may occasionally collect water	N/A	N/A	N/A	N/A	N/A	N/A	No	
41	In	N/A	No	0.73	Good	Positive	No - core GCN habitat would not be impacted	N/A	Yes	Core GCN habitat would not be impacted as the pond is at least 180m from the nearest point of the Order Limits.
42	In	N/A	No	0.72	Good	Negative	N/A	N/A	No	
43	Out	Pond was dry – a shallow scrape that dries regularly and has been dry for some time. Closest pond is approx. 90m away (Pond 44) and was negative for GCN eDNA.	N/A	N/A	N/A	N/A	N/A	N/A	No	
44	In	N/A	Yes	0.73	Good	Negative	N/A	N/A	No	
49	In	N/A	No	0.76	Good	Negative	N/A	N/A	No	
50	In	N/A	Yes	0.85	Excellent	Positive	No - HBIC data already confirm presence and indicative	Medium	Yes	HBIC data suggest a maximum count of 59 GCN was made here in 2013.



Pond ID	Scoped In/Out?	Justification for Scoping Out	Priority Pond	HSI Score	Pond Suitability	eDNA Results	Population Surveyed	Population Estimate	GCN Present	Notes
							population estimate.			
51	In	N/A	No	0.73	Good	Negative	N/A	N/A	No	
52	In	N/A	No	0.54	Below average	Negative	N/A	N/A	No	
54	Out	Pond is located 240m from the Order Limits on the other side of the A31 dual carriageway – total barrier to dispersal.	N/A	N/A	N/A	N/A	N/A	N/A	N/A	
55	In	N/A	Yes	0.70	Good	Positive	Yes	Small	Yes	
56	In	N/A	No	N/A	N/A	N/A	No - HBIC data already confirm presence	Medium	Yes	The pond was not surveyed as it was outside the 250m buffer during the survey season. Following changes to the Order Limits after the GCN survey season, the pond is now on the edge of the 250m buffer. However, HBIC data were used to confirm GCN presence. These data suggest a maximum count of 14 GCN was made here in 2013.
57	In	N/A	No	0.84	Excellent	Positive	No - HBIC data already confirm presence	Medium	Yes	HBIC data suggest a maximum count of 21 GCN was made here in 2003.
57a	In	N/A	Yes	0.76	Good	Positive	Yes	Small	Yes	
59	In	N/A	Yes	0.50	Below average	Negative	N/A	N/A	No	
61	Out	Pond no longer exists	N/A	N/A	N/A	N/A	N/A	N/A	No	



Pond ID	Scoped In/Out?	Justification for Scoping Out	Priority Pond	HSI Score	Pond Suitability	eDNA Results	Population Surveyed	Population Estimate	GCN Present	Notes
63	In	N/A	No	0.72	Good	Negative	No - core GCN habitat would not be impacted	N/A	Yes	Despite a negative eDNA result, a GCN egg was found at the time of the survey. This suggests a very low GCN population below the detectability of eDNA surveys.
64	In	N/A	No	0.71	Good	Negative	N/A	N/A	No	
65	In	N/A	No	0.86	Excellent	Negative	N/A	N/A	No	
65a	In	N/A	No	0.75	Good	Positive	No - core GCN habitat would not be impacted	N/A	Yes	
65b	In	N/A	No	0.86	Excellent	Negative	N/A	N/A	No	
68	In	N/A	No	N/A	N/A	N/A	N/A	N/A	No	Not surveyed as originally outside of survey area.
69	In	N/A	No	0.64	Average	Negative	N/A	N/A	No	
70	In	N/A	No	N/A	N/A	N/A	N/A	N/A	Unknown	The pond was not surveyed as landowner permission was refused. Pond is approximately 115m from the Order Limits. GCN presence confirmed in Pond 71 approximately 340m to the north.
71	In	N/A	No	N/A	N/A	N/A	No - HBIC data already confirm presence	Small	Yes	The pond was not surveyed as landowner permission was refused. HBIC data suggest a maximum count of 4 GCN was made here in 2014.
71a	In	N/A	Yes	N/A	N/A	N/A	No - HBIC data already confirm presence	Small	Yes	The pond was not surveyed as landowner permission was refused. HBIC data suggest a maximum count of 1 GCN was made here in 2014.
73	In	N/A	No	0.68	Average	Positive	No - core GCN habitat would	N/A	Yes	



Pond ID	Scoped In/Out?	Justification for Scoping Out	Priority Pond	HSI Score	Pond Suitability	eDNA Results	Population Surveyed	Population Estimate	GCN Present	Notes
							not be impacted			
74	In	N/A	Yes	N/A	N/A	N/A	N/A	N/A	Unknown	The pond was not surveyed as landowner permission was refused. Pond is approximately 50m from the closest above ground construction works area within the Order Limits. Ponds 71 and 73 are both within 500m (approximately 320m south and 420m southwest, respectively) and were positive for GCN eDNA; however, these ponds are separated by the busy A287 road. Pond 76 is approximately 350m to the northwest and was negative for GCN eDNA. The presence of GCN in Pond 74 cannot be ruled out.
75	Out	Multiple aerial imagery sources suggest the pond no longer exists.	Yes	N/A	N/A	N/A	N/A	N/A	No	The pond was not field surveyed as landowner permission was not available at the time. However, multiple sources of recent aerial imagery suggest the pond no longer exists.
76	In	N/A	Yes	0.73	Good	Negative	N/A	N/A	No	
77	In	N/A	Yes	0.62	Average	Negative	N/A	N/A	No	
78	In	N/A	Yes	0.69	Average	Negative	N/A	N/A	No	
78a	Out	Pond was dry	Yes	0.75	Good	N/A	N/A	N/A	No	
78b	In	N/A	Yes	0.77	Good	Negative	N/A	N/A	No	
78c	Out	Poor HSI score – shallow woodland scrape that was drying out. No macrophytes.	Yes	0.46	Poor	N/A	N/A	N/A	No	



Pond ID	Scoped In/Out?	Justification for Scoping Out	Priority Pond	HSI Score	Pond Suitability	eDNA Results	Population Surveyed	Population Estimate	GCN Present	Notes
		Pond 78b is approximately 50m to the west and was negative for GCN eDNA.								
78d	Out	Shallow woodland scrape that was dry. Pond 78b is approximately 50m to the west and was negative for GCN eDNA.	Yes	0.48	Poor	N/A	N/A	N/A	No	
80	Out	Not a pond - area flooded with a hose pipe for a horse water-jump	No	0.63	Average	N/A	N/A	N/A	No	
81	In	N/A	No	0.71	Good	Negative	N/A	N/A	No	
82	In	N/A	No	0.76	Good	Negative	N/A	N/A	No	
83	In	N/A	Yes	0.77	Good	Negative	N/A	N/A	No	
84	In	N/A	No	0.75	Good	Negative	N/A	N/A	No	
85	In	N/A	No	0.70	Good	Negative	N/A	N/A	No	
86	Out	Poor HSI score - lots of waterfowl and carp present	No	0.35	Poor	N/A	N/A	N/A	No	



Pond ID	Scoped In/Out?	Justification for Scoping Out	Priority Pond	HSI Score	Pond Suitability	eDNA Results	Population Surveyed	Population Estimate	GCN Present	Notes
87b	Out	Pond was almost completely dry	No	N/A	N/A	N/A	N/A	N/A	No	
87c	Out	Pond was almost completely dry	No	N/A	N/A	N/A	N/A	N/A	No	
89	In	N/A	No	0.76	Good	Negative	N/A	N/A	No	
90	Out	Poor HSI score - lots of waterfowl and carp present	No	0.35	Poor	N/A	N/A	N/A	No	
92	In	N/A	No	0.52	Below average	Negative	N/A	N/A	No	
93	In	N/A	Yes	0.90	Excellent	Negative	N/A	N/A	No	
93a	Out	A dry woodland scrape	No	0.60	Average	N/A	N/A	N/A	No	
93b	Out	More an area of shallow wet woodland but was eDNA surveyed as surveyors were present to survey other ponds. Inconclusive due to sediment in water samples and considered not suitable for GCN.	Yes	0.50	Below average	Inconclusive (see notes)	N/A	N/A	No	The water was eDNA surveyed despite appearing sub-optimal for GCN, the water was a shallow area of wet woodland with a slow flow and lots of sediment. Lab analysis returned an inconclusive result, but the water body is considered absent of GCN due to the conditions of the water body and negative eDNA results for all ponds within 500m.



Pond ID	Scoped In/Out?	Justification for Scoping Out	Priority Pond	HSI Score	Pond Suitability	eDNA Results	Population Surveyed	Population Estimate	GCN Present	Notes
94a	In	N/A	No	0.85	Excellent	Negative	N/A	N/A	No	When water levels are higher 94a and 94b would connect. However, at time of survey there was 20m dry between them so surveyed separately.
94b	In	N/A	No	0.84	Excellent	Negative	N/A	N/A	No	When water levels are higher 94a and 94b would connect. However, at time of survey there was 20m dry between them so surveyed separately.
95	In	N/A	No	0.77	Good	Negative	N/A	N/A	No	
96	In	N/A	No	0.57	Below average	Negative	N/A	N/A	No	
97	In	N/A	Yes	0.81	Excellent	Negative	N/A	N/A	No	
98	In	N/A	No	0.53	Below average	Negative	N/A	N/A	No	
99	In	N/A	No	0.61	Average	Negative	N/A	N/A	No	
100a	In	N/A	Yes	0.57	Below average	N/A	N/A	N/A	Unknown	This pond was not surveyed during the 2018 survey season. Pond is approximately 10m from the closest above ground construction works area within the Order Limits. There are no other ponds within 500m and outside of the immediate surrounding woodland there are barriers to dispersal, including urban developments and a railway line. The presence of GCN in Pond 100a cannot be ruled out, although there is considered to be a low risk of Pond 100a supporting a medium/large breeding population and so there would be a low risk of major impacts to GCN due to installation.
102	In	N/A	Yes	0.76	Good	Negative	N/A	N/A	No	



Pond ID	Scoped In/Out?	Justification for Scoping Out	Priority Pond	HSI Score	Pond Suitability	eDNA Results	Population Surveyed	Population Estimate	GCN Present	Notes
103	Out	See Notes column.	Yes	N/A	N/A	N/A	N/A	N/A	No	The pond was not surveyed for health and safety reasons. Although the pond could not be accessed, all surrounding water bodies (e.g. Ponds 103a, b, c and 104) were completely unsuitable for GCN. Pond 103 is isolated by the A331 dual carriageway to the west and fishing lakes and railway to the east. The pond is within the floodplain of the River Blackwater and is highly likely to support fish. There is considered to be very low potential for this pond to support GCN.
103a	Out	Fishing lake, not suitable for GCN	Yes	N/A	N/A	N/A	N/A	N/A	No	
103b	Out	Fishing lake, not suitable for GCN	Yes	N/A	N/A	N/A	N/A	N/A	No	
103c	Out	Fishing lake, not suitable for GCN	Yes	N/A	N/A	N/A	N/A	N/A	No	
104	Out	A fish breeding pond for stocking nearby fishing lakes	Yes	N/A	N/A	N/A	N/A	N/A	No	
104a	Out	See Notes column	No	0.59	Below average	N/A	N/A	N/A	No	All surrounding water bodies (e.g. Ponds 103 a, b, c and 104) were completely unsuitable for GCN. Pond 104a is isolated by the A331 dual carriageway to the west and fishing lakes and railway to the east. The pond is within a reedbed and floodplain of the River Blackwater and is highly likely to support fish. There is considered to be very low potential for this pond to support GCN.



Pond ID	Scoped In/Out?	Justification for Scoping Out	Priority Pond	HSI Score	Pond Suitability	eDNA Results	Population Surveyed	Population Estimate	GCN Present	Notes
104b	Out	Fishing lake, not suitable for GCN	Yes	N/A	N/A	N/A	N/A	N/A	No	
105	Out	Fishing lake, not suitable for GCN	Yes	N/A	N/A	N/A	N/A	N/A	No	
106	Out	Fishing lake, not suitable for GCN	Yes	N/A	N/A	N/A	N/A	N/A	No	
107	Out	Fishing lake, not suitable for GCN	Yes	N/A	N/A	N/A	N/A	N/A	No	
108	Out	Fishing lake, not suitable for GCN	Yes	N/A	N/A	N/A	N/A	N/A	No	
108a	Out	Completely isolated from project by busy roads on all sides	No	N/A	N/A	N/A	N/A	N/A	N/A	
108b	Out	Fishing lake, not suitable for GCN	Yes	N/A	N/A	N/A	N/A	N/A	No	
108c	Out	Fishing lake, not suitable for GCN	Yes	N/A	N/A	N/A	N/A	N/A	No	
108d	Out	Fishing lake, not suitable for GCN	Yes	N/A	N/A	N/A	N/A	N/A	No	
109	In	N/A	Yes	0.68	Average	Negative	N/A	N/A	No	
110	Out	A raised, chemically- treated pond with a water fountain	No	N/A	N/A	N/A	N/A	N/A	No	
111	Out	A raised, chemically- treated pond	No	N/A	N/A	N/A	N/A	N/A	No	



Pond ID	Scoped In/Out?	Justification for Scoping Out	Priority Pond	HSI Score	Pond Suitability	eDNA Results	Population Surveyed	Population Estimate	GCN Present	Notes
		with a water fountain								
112	Out	A raised, chemically- treated pond with a water fountain	No	N/A	N/A	N/A	N/A	N/A	No	
113	Out	A raised, chemically- treated pond with a water fountain	No	N/A	N/A	N/A	N/A	N/A	No	
114	Out	Too shallow to HSI assess or eDNA survey	Yes	N/A	N/A	N/A	N/A	N/A	No	
115	Out	Stocked with fish	No	0.47	Poor	N/A	N/A	N/A	No	
115a	In	N/A	Yes	0.70	Good	Negative	N/A	N/A	No	
118	In	N/A	Yes	0.77	Good	Negative	N/A	N/A	No	
118a	Out	Not a pond but a temporary area of flooded woodland	Yes	N/A	N/A	N/A	N/A	N/A	No	
124a	Out	Completely separated from the Order Limits by urban development and the B311.	No	N/A	N/A	N/A	N/A	N/A	No	
125	In	N/A	Yes	0.76	Good	Negative	N/A	N/A	No	



Pond ID	Scoped In/Out?	Justification for Scoping Out	Priority Pond	HSI Score	Pond Suitability	eDNA Results	Population Surveyed	Population Estimate	GCN Present	Notes
126	Out	Construction works within 250m would be within, and/or separated by, the carriageway of the busy B311.	No	N/A	N/A	N/A	N/A	N/A	N/A	
127	In	N/A	No	0.73	Good	N/A	Yes	Medium	Yes	Pond was population surveyed in the evening and due to be eDNA surveyed the following morning. GCN presence was confirmed by the first population survey and so an eDNA survey was not required.
127a	In	N/A	No	0.82	Excellent	N/A	Yes	Medium (from five population surveys, one dry)	Yes	Pond was population surveyed in the evening and due to be eDNA surveyed the following morning. GCN presence was confirmed by the first population survey and so an eDNA survey was not required. Pond dried up after the fifth population survey and so a sixth survey could not be carried out.
128	In	N/A	Yes	0.88	Excellent	N/A	Yes	Small	Yes	Pond was population surveyed in the evening and due to be eDNA surveyed the following morning. GCN presence was confirmed by the first population survey and so an eDNA survey was not required.
129	In	N/A	No	0.81	Excellent	Negative	Yes (may have formed part of metapopulation with nearby	Absent	No	



Pond ID	Scoped In/Out?	Justification for Scoping Out	Priority Pond	HSI Score	Pond Suitability	eDNA Results	Population Surveyed	Population Estimate	GCN Present	Notes
							eDNA positive ponds)			
129a	In	N/A	Yes	0.85	Excellent	Positive	Yes	Small (from four population surveys, two dry)	Yes	Pond dried up after the fourth population survey and so the remaining two surveys could not be carried out.
130	In	N/A	No	0.75	Good	Negative	N/A	N/A	No	
130a	In	N/A	Yes	0.66	Average	N/A	N/A	N/A	Unknown	This ditch was not surveyed during the 2018 survey season. The ditch is approximately 470m from the Windlemere Golf Course metapopulation and connected by suitable terrestrial habitat. As such, GCN presence cannot be ruled out.
130b	In	N/A	No	0.42	Poor	N/A	N/A	N/A	Unknown	This ditch was not surveyed during the 2018 survey season. The ditch is approximately 620m from the Windlemere Golf Course metapopulation but is connected to Pond 130a and connected to Windlemere Golf Course by suitable terrestrial habitat. As such, GCN presence cannot be ruled out.
130c	Out	Poor HSI score - a flooded pit of approximately 1.5m x 1.5m. Unsuitable for GCN.	Yes	0.40	Poor	N/A	N/A	N/A	No	
134	In	N/A	No	0.75	Good	Negative	N/A	N/A	No	
136	In	N/A		0.69	Average	Negative	N/A	N/A	No	



Pond ID	Scoped In/Out?	Justification for Scoping Out	Priority Pond	HSI Score	Pond Suitability	eDNA Results	Population Surveyed	Population Estimate	GCN Present	Notes
141	In	N/A		0.51	Below average	Negative	N/A	N/A	No	
142a	Out	Pond was dry at eDNA survey	Yes	0.70	Good	N/A	N/A	N/A	No	Pond is a roadside drainage ditch approximately 20m from the Order Limits. There are no other ponds within 500m. Due to its isolation and propensity for drying out, there is considered to be a low risk of Pond 142a supporting GCN.
144	Out	Poor HSI score with major fish presence	No	0.43	Poor	N/A	N/A	N/A	No	
145	Out	Pond no longer exists	No	N/A	N/A	N/A	N/A	N/A	No	
146	Out	Pond no longer exists	No	N/A	N/A	N/A	N/A	N/A	No	
151	In	N/A	No	0.75	Good	Negative	N/A	N/A	No	
151a	In	N/A	No	0.63	Average	Negative	N/A	N/A	No	
151b	In	N/A	No	0.76	Good	Negative	N/A	N/A	No	
153	In	N/A	Yes	0.80	Excellent	Negative	N/A	N/A	No	
155	In	N/A	Yes	0.78	Good	Negative	N/A	N/A	No	
155a	Out	See Notes column	Yes	0.64	Average	N/A	N/A	N/A	No	Water body was identified after the 2018 eDNA survey season ended. The water body is an ephemeral area of wet heathland on acid soils. Twelve ponds within 500m were surveyed and confirmed absent of GCN eDNA. There are no confirmed GCN ponds within 500m. The pond is considered to have negligible potential to support GCN.



Pond ID	Scoped In/Out?	Justification for Scoping Out	Priority Pond	HSI Score	Pond Suitability	eDNA Results	Population Surveyed	Population Estimate	GCN Present	Notes
156	In	N/A	No	0.72	Good	Negative	N/A	N/A	No	
156a	Out	See Notes column	Yes	0.69	Average	N/A	N/A	N/A	No	Water body was identified after the 2018 eDNA survey ended. The water body is an ephemeral area of wet heathland on acid soils. Twelve ponds within 500m were surveyed and confirmed absent of GCN eDNA. There are no confirmed GCN ponds within 500m. The pond is considered to have negligible potential to support GCN.
158	In	N/A	Yes	0.76	Good	Negative	N/A	N/A	No	
159	In	N/A	No	0.76	Good	Negative	N/A	N/A	No	
160	In	N/A	No	0.80	Excellent	Negative	N/A	N/A	No	
161	In	N/A	No	0.70	Good	Negative	N/A	N/A	No	
164	In	N/A	No	N/A	N/A	N/A	N/A	N/A	Unknown	The pond was not surveyed as landowner permission was refused. The pond is approximately 250m from the Order Limits situated on heathland acid soils. All other eDNA surveyed heathland ponds within 250m have confirmed absence of GCN. The pond is considered to have negligible potential to support GCN.
166	In	N/A	No	0.81	Excellent	Negative	N/A	N/A	No	
166a	In	N/A	Yes	0.74	Good	Negative	N/A	N/A	No	
167	In	N/A	No	0.69	Average	Negative	N/A	N/A	No	
168	In	N/A	No	0.81	Excellent	Negative	N/A	N/A	No	
169	In	N/A	Yes	0.73	Good	Negative	N/A	N/A	No	
178	In	N/A	Yes	0.82	Excellent	Negative	N/A	N/A	No	



Pond ID	Scoped In/Out?	Justification for Scoping Out	Priority Pond	HSI Score	Pond Suitability	eDNA Results	Population Surveyed	Population Estimate	GCN Present	Notes
178a	In	N/A	Yes	0.73	Good	N/A	N/A	N/A	Unknown	This pond was not eDNA or population surveyed during the 2018 survey season as it was not identified until after the survey season had ended. This pond is within the Order Limits but would be avoided by construction activity as pipelaying at this location would be achieved through the use of trenchless techniques. The pond is approximately 160m from Pond 180, a confirmed GCN pond. Despite being on acid soils and frequently disturbed by dogs, the presence of GCN in this pond cannot be ruled out.
180	In	N/A		0.79	Good	Positive	No - access refused for population surveys in 2018	N/A	Yes	Landowner approval to access the pond was refused following the eDNA survey. The pond is approximately 60m from the Order Limits and connected to the proposed construction working area by broadleaved woodland. There is one other pond within 500m with confirmed GCN presence, outside of the 250m buffer. The pond is likely to be acidic due to the underlying geology and proximity to Chobham Common heathland. As such, there is considered to be a low risk of Pond 180 supporting a medium/large breeding population and so there would be a low risk of major impacts to GCN due to installation.
180a	Out	Pond was dry at eDNA survey	Yes	0.51	Below average	N/A	N/A	N/A	No	
183	In	N/A	No	0.74	Good	Negative	N/A	N/A	No	



Pond ID	Scoped In/Out?	Justification for Scoping Out	Priority Pond	HSI Score	Pond Suitability	eDNA Results	Population Surveyed	Population Estimate	GCN Present	Notes
186	In	N/A	No	0.71	Good	Negative	N/A	N/A	No	
190	In	N/A	No	0.69	Average	Negative	N/A	N/A	No	
191	In	N/A	Yes	0.84	Excellent	Negative	N/A	N/A	No	
191a	Out	Scoped out as is approximately 200m from the Order Limits and is separated by extensive areas of hardstanding.	No	N/A	N/A	N/A	N/A	N/A	N/A	
192	In	N/A	No	0.53	Below average	Negative	N/A	N/A	No	
193	In	N/A	No	0.64	Average	Negative	N/A	N/A	No	
194	In	N/A	Yes	0.57	Below average	Negative	N/A	N/A	No	
194a	In	N/A	Yes	0.68	Average	Positive	Yes	Likely small (from three population surveys, three dry)	Yes	Pond dried up after the third population survey and so the remaining three surveys could not be carried out.
194b	In	N/A	No	0.71	Good	Negative	N/A	N/A	No	
194c	In	N/A	No	0.77	Good	Positive	Yes	Small	Yes	
194d	Out	Pond was dry at eDNA survey	No	0.50	Below average	N/A	N/A	N/A	No	
195	In	N/A	Yes	0.45	Poor	Negative	N/A	N/A	No	
196	In	N/A	Yes	0.78	Good	Negative	N/A	N/A	No	
197	In	N/A	Yes	0.71	Good	Negative	N/A	N/A	No	



Pond ID	Scoped In/Out?	Justification for Scoping Out	Priority Pond	HSI Score	Pond Suitability	eDNA Results	Population Surveyed	Population Estimate	GCN Present	Notes
198	In	N/A	Yes	0.54	Below average	Negative	N/A	N/A	No	
199	In	N/A	Yes	0.76	Good	Negative	N/A	N/A	No	
200	In	N/A	Yes	0.75	Good	Negative	N/A	N/A	No	
201	In	N/A	Yes	0.72	Good	Positive	Yes	Likely very small (from five population surveys, one dry)	Yes	Pond dried up after the fifth population survey and so a sixth survey could not be carried out.
202	In	N/A	Yes	0.77	Good	Negative	N/A	N/A	No	
202a	In	N/A	Yes	0.76	Good	Negative	N/A	N/A	No	
203	In	N/A	Yes	0.53	Below average	Negative	N/A	N/A	No	
204	In	N/A	No	0.78	Good	Negative	N/A	N/A	No	
204a	In	N/A	Yes	0.79	Excellent	Negative	N/A	N/A	No	
206	In	N/A	Yes	0.70	Good	Negative	N/A	N/A	No	
206a	In	N/A	Yes	0.79	Good	Negative	N/A	N/A	No	
208	In	N/A	No	0.76	Good	Negative	N/A	N/A	No	
208a	In	N/A	No	0.67	Average	Negative	N/A	N/A	No	
210	In	N/A	Yes	N/A	N/A	N/A	No	N/A	Unknown	Pond was not surveyed as it was not within the 250m study area at the time that the 2018 surveys were undertaken. The pond was scoped in following subsequent design changes to the Order Limits. The pond is within approximately 30m of the Order Limits and the potential presence of GCN cannot be discounted.



Pond ID	Scoped In/Out?	Justification for Scoping Out	Priority Pond	HSI Score	Pond Suitability	eDNA Results	Population Surveyed	Population Estimate	GCN Present	Notes
211	Out	Too shallow to HSI or eDNA	No	0.51	Below Average	N/A	No	N/A	No	
212	In	N/A	No	N/A	N/A	N/A	No	N/A	Unknown	Pond was not surveyed as it was not within the 250m study area at the time that the 2018 surveys were undertaken. The pond was scoped in following subsequent design changes to the Order Limits. The pond is within approximately 130m of the Order Limits and the potential presence of GCN cannot be discounted.
213	In	N/A	Yes	0.73	Good	N/A	N/A	N/A	Unknown	Pond perimeter was heavily overgrown and so surveyors were unable to reach the water to survey. The closest construction works affecting terrestrial habitat would be approximately 100m to the northwest of Pond 213, in an area of pasture to the west of Old Littleton Lane. This area is separated from the pond by two roads: Littleton Lane (a main road) and Old Littleton Lane (a minor access road). There are no suitable water bodies to the west of the Order Limits that GCN would migrate to, further reducing the risk of GCN being within the works area. Impacts to terrestrial habitat to the south would be avoided through the use of trenchless construction techniques associated with the crossing of the River Thames. Although the potential presence of GCN in Pond 213 cannot be discounted, major impacts to GCN would be unlikely given the distance and separation of the



Pond ID	Scoped In/Out?	Justification for Scoping Out	Priority Pond	HSI Score	Pond Suitability	eDNA Results	Population Surveyed	Population Estimate	GCN Present	Notes
										pond from the proposed construction working area, an absence of suitable ponds on the opposite side of the Order Limits, and the quality of the terrestrial habitat that would be affected.
214	In	N/A	Yes	0.59	Below average	N/A	N/A	N/A	Unknown	Pond perimeter was heavily overgrown and so surveyors were unable to reach the water to survey. The closest construction works affecting terrestrial habitat would be approximately 40m to the west of Pond 214, in an area of pasture to the west of Old Littleton Lane. This area is separated from the pond by Old Littleton Lane (a minor access road). There are no suitable water bodies to the west of the Order Limits that GCN would migrate to, this further reduces the risk of GCN being within the works area. Impacts to terrestrial habitat to the south would be avoided through the use of trenchless construction techniques associated with the crossing of the River Thames. Although the potential presence of GCN in Pond 214 cannot be discounted, major impacts to GCN would be unlikely given the quality of terrestrial habitat that would be affected and an absence of suitable ponds on the opposite side of the Order Limits that any GCN present might migrate to.
215	In	N/A	No	N/A	N/A	N/A	N/A	N/A	Unknown	Pond perimeter was heavily overgrown and so surveyors were unable to see or reach the water to survey. The closest construction works affecting terrestrial



Pond ID	Scoped In/Out?	Justification for Scoping Out	Priority Pond	HSI Score	Pond Suitability	eDNA Results	Population Surveyed	Population Estimate	GCN Present	Notes
										habitat would be approximately 140m to the west of Pond 215, in an area of pasture to the west of Old Littleton Lane. This area is separated from the pond by Littleton Lane (a main road), an area of hardstanding (a traveller settlement) and Old Littleton Lane (a minor access road). Pond 215 is surrounded by dense/continuous scrub and broadleaved woodland and there are no suitable water bodies to the west of the Order Limits that GCN would migrate to; this further reduces the risk of GCN being within the works area. Although the potential presence of GCN in Pond 215 cannot be discounted, major impacts to GCN would be unlikely given the distance and separation of the pond from the proposed construction working area, the quality of terrestrial habitat in the immediate vicinity of the pond and the absence of suitable ponds on the opposite side of the Order Limits (making it less likely that GCN would disperse long distances) and the quality of the terrestrial habitat that would be affected.
218	In	N/A	No	N/A	N/A	N/A	N/A	N/A	Unknown	The pond was not surveyed as landowner permission was refused. Pond is approximately 160m from the Order Limits and connected to the proposed working area by arable fields. The dominant land use within 500m is arable. There is one other pond within 500m although this was not surveyed as



Pond ID	Scoped In/Out?	Justification for Scoping Out	Priority Pond		Pond Suitability	eDNA Results	Population Surveyed	Population Estimate	GCN Present	Notes
										it is outside the project's 250m study area. Although the potential presence of GCN in Pond 218 cannot be discounted, major impacts to GCN would be unlikely given the pond's isolation, the quality of the surrounding/affected terrestrial habitat, and the distance from the proposed construction works area.
220	Out	Pond no longer exists		N/A	N/A	N/A	N/A	N/A	No	
221	Out	Pond no longer exists	Yes	N/A	N/A	N/A	N/A	N/A	No	
223	In	N/A	Yes	0.80	Excellent	Positive	No	N/A	Yes	Positive eDNA result but pond was full of stickleback suggesting that this is not a breeding pond but instead used by low numbers of foraging GCN.
223a	In	N/A	Yes	0.78	Good	N/A	N/A	N/A	Yes – assumed	Pond was only identified in October 2018 after the end of the survey season and so couldn't be eDNA surveyed. The pond is approximately 100m to the southeast of Pond 223 (a confirmed GCN pond) and connected to it by woodland and pasture. As such, GCN presence is assumed in Pond 223a.
224	In	N/A	Yes	0.46	Poor	Negative	N/A	N/A	No	



# Annex B - Nature Metrics eDNA Results for All Ponds Surveyed



18296-JA-EJ-1 Order number: JAC-11/12/27/35/36/37/38/42-EJ

## **Great Crested Newt eDNA Results**

Company: Jacobs UK Ltd

Address: 1180 Eskdale Road, Winnersh, Wokingham, RG41 5TU

Contact: Emma James

Project code | Task code: B2325300 | A.P1.PI.WS1

Date of Report: 27 July 2018

Number of samples: 155

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

DNA was precipitated via centrifugation at 14,000 x g and then extracted using Qiagen Blood and Tissue extraction kits.

qPCR amplification was carried out in 12 replicates per sample, using the primers and probe described by Biggs et al. (2014), in the presence of both positive and negative controls.

Results indicate GCN presence in 23 samples ('11', '36', '39', '41', '50', '55', '57a', '59', '65a', '72', '73', '129a', '150', '152', '162', '170', '180', '187', '189', '194a', '194c', '201', '223'). Inhibition was detected in 12 samples ('27c', '42', '65', '87', '93b', '125', '135', '151a', '158', '166', '166a', and '206A'), which was overcome in 11 samples ('27c', '42', '65', '87', '125', '135', '151a', '158', '166', '166a', and '206A') by DNA dilution, but not '93b', which is presented as inconclusive. For the remaining samples, no degradation or inhibition was detected, and all controls performed as expected. Conclusive results are therefore presented.

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

Sample	Pond ID	Date arrived	Inhibition	Degradation	eDNA score	GCN status
GCN18-0233	2	24-Apr	No	No	0	Negative
GCN18-0227	4	24-Apr	No	No	0	Negative
GCN18-0230	5	24-Apr	No	No	0	Negative
GCN18-0236	6	26-Apr	No	No	0	Negative
GCN18-0294	6a	09-May	No	No	0	Negative













GCN18-1274	8	09-May	No	No	0	Negative
GCN18-1238	9	09-May	No	No	0	Negative
GCN18-1246	11	09-May	NA	No	2	Positive
GCN18-0352	11a	09-May	No	No	0	Negative
GCN18-0321	12a	15-May	No	No	0	Negative
GCN18-0330	13	05-Jun	No	No	0	Negative
GCN18-1250	14	15-May	No	No	0	Negative
GCN18-1244	15	15-May	No	No	0	Negative
GCN18-1257	16	15-May	No	No	0	Negative
GCN18-0225	18	26-Apr	No	No	0	Negative
GCN18-0226	20	24-Apr	No	No	0	Negative
GCN18-0229	22	24-Apr	No	No	0	Negative
GCN18-1256	22a	15-May	No	No	0	Negative
GCN18-0232	23	24-Apr	No	No	0	Negative
GCN18-1746	24	11-Jun	No	No	0	Negative
GCN18-0322	25	05-Jun	No	No	0	Negative
GCN18-1271	27	15-May	No	No	0	Negative
GCN18-1280	27a	15-May	No	No	0	Negative
GCN18-1259	27b	15-May	No	No	0	Negative
GCN18-0312	27c	15-May	No	No	0	Negative
GCN18-1247	27d	15-May	No	No	0	Negative
GCN18-0279	28	09-May	No	No	0	Negative
GCN18-0234	30	24-Apr	No	No	0	Negative
GCN18-1730	33	22-May	No	No	0	Negative
GCN18-1758	36	22-May	NA	No	2	Positive
GCN18-1279	38	22-May	No	No	0	Negative
GCN18-1265	39	09-May	NA	No	2	Positive
GCN18-0237	40	24-Apr	No	No	0	Negative
GCN18-0235	41	24-Apr	NA	No	1	Positive
GCN18-1270	42	19-Jun	No	No	0	Negative
GCN18-1261	44	03-Jul	No	No	0	Negative
GCN18-1252	45	15-May	No	No	0	Negative
GCN18-1272	49	15-May	No	No	0	Negative
GCN18-0228	50	24-Apr	NA	No	2	Positive
GCN18-0231	51	24-Apr	No	No	0	Negative
GCN18-1734	52	19-Jun	No	No	0	Negative













GCN18-1750	55	05-Jun	NA	No	12	Positive
GCN18-1273	57	09-May	NA	No	12	Positive
GCN18-1755	57a	11-Jun	NA	No	7	Positive
GCN18-0340	59	23-Apr	No	No	0	Negative
GCN18-0326	63	23-Apr	No	No	0	Negative
GCN18-0345	64	24-Apr	No	No	0	Negative
GCN18-0318	65	15-May	No	No	0	Negative
GCN18-0356	65a	15-May	NA	No	1	Positive
GCN18-1269	65b	15-May	No	No	0	Negative
GCN18-0285	66	23-Apr	No	No	0	Negative
GCN18-1268	69	09-May	No	No	0	Negative
GCN18-0280	72	27-Apr	NA	No	1	Positive
GCN18-1262	73	15-May	NA	No	7	Positive
GCN18-1241	76	22-May	No	No	0	Negative
GCN18-0287	77	23-Apr	No	No	0	Negative
GCN18-0338	78	23-Apr	No	No	0	Negative
GCN18-1258	78b	09-May	No	No	0	Negative
GCN18-0288	79	23-Apr	No	No	0	Negative
GCN18-0289	81	27-Apr	No	No	0	Negative
GCN18-1751	82	05-Jun	No	No	0	Negative
GCN18-0278	83	27-Apr	No	No	0	Negative
GCN18-0334	84	27-Apr	No	No	0	Negative
GCN18-0293	85	27-Apr	No	No	0	Negative
GCN18-0346	87	27-Apr	No	No	0	Negative
GCN18-0286	89	27-Apr	No	No	0	Negative
GCN18-0295	89a	27-Apr	No	No	0	Negative
GCN18-0306	91	23-Apr	No	No	0	Negative
GCN18-0355	92	24-Apr	No	No	0	Negative
GCN18-0311	93	23-Apr	No	No	0	Negative
GCN18-1263	93b	09-May	Yes	No	0	Inconclusive
GCN18-0324	94a	23-Apr	No	No	0	Negative
GCN18-0281	94b	23-Apr	No	No	0	Negative
GCN18-0304	95	23-Apr	No	No	0	Negative
GCN18-0290	96	23-Apr	No	No	0	Negative
GCN18-0314	97	23-Apr	No	No	0	Negative
GCN18-0342	98	23-Apr	No	No	0	Negative













GCN18-0344	99	24-Apr	No	No	0	Negative
GCN18-0333	102	23-Apr	No	No	0	Negative
GCN18-0308	109	23-Apr	No	No	0	Negative
GCN18-1245	115a	11-Jun	No	No	0	Negative
GCN18-1255	117	09-May	No	No	0	Negative
GCN18-0300	118	27-Apr	No	No	0	Negative
GCN18-1749	120	05-Jun	No	No	0	Negative
GCN18-1757	121	05-Jun	No	No	0	Negative
GCN18-0282	125	23-Apr	No	No	0	Negative
GCN18-0296	129	11-Jun	No	No	0	Negative
GCN18-1741	129a	11-Jun	NA	No	5	Positive
GCN18-0319	130	27-Apr	No	No	0	Negative
GCN18-1276	132	15-May	No	No	0	Negative
GCN18-1260	132a	15-May	No	No	0	Negative
GCN18-1266	134	15-May	No	No	0	Negative
GCN18-1752	135	19-Jun	No	No	0	Negative
GCN18-1737	136	22-May	No	No	0	Negative
GCN18-1756	141	22-Jun	No	No	0	Negative
GCN18-1240	150	11-Jun	NA	No	11	Positive
GCN18-0305	151	23-Apr	No	No	0	Negative
GCN18-0302	151a	15-May	No	No	0	Negative
GCN18-1242	151b	15-May	No	No	0	Negative
GCN18-0315	152	11-Jun	NA	NI.	10	Docitivo
GCN18-0316		=	IVA	No	10	Positive
	153	23-Apr	No	No	0	Negative
GCN18-0307	153 155					
+		23-Apr	No	No	0	Negative
GCN18-0307	155	23-Apr 23-Apr	No No	No No	0	Negative Negative
GCN18-0307 GCN18-0303	155 156	23-Apr 23-Apr 23-Apr	No No No	No No No	0 0	Negative Negative Negative
GCN18-0307 GCN18-0303 GCN18-0297	155 156 158	23-Apr 23-Apr 23-Apr 23-Apr	No No No	No No No	0 0 0	Negative Negative Negative Negative
GCN18-0307 GCN18-0303 GCN18-0297 GCN18-0358	155 156 158 159	23-Apr 23-Apr 23-Apr 23-Apr 24-Apr	No No No No	No No No No	0 0 0 0	Negative Negative Negative Negative Negative
GCN18-0307 GCN18-0303 GCN18-0297 GCN18-0358 GCN18-0359	155 156 158 159 160	23-Apr 23-Apr 23-Apr 23-Apr 24-Apr 24-Apr	No No No No No	No No No No No	0 0 0 0 0	Negative Negative Negative Negative Negative Negative Negative
GCN18-0307 GCN18-0303 GCN18-0297 GCN18-0358 GCN18-0359 GCN18-0313	155 156 158 159 160 161	23-Apr 23-Apr 23-Apr 23-Apr 24-Apr 24-Apr 23-Apr	No No No No No	No No No No No No	0 0 0 0 0	Negative Negative Negative Negative Negative Negative Negative Negative
GCN18-0307 GCN18-0303 GCN18-0297 GCN18-0358 GCN18-0359 GCN18-0313 GCN18-1731	155 156 158 159 160 161 <b>162</b>	23-Apr 23-Apr 23-Apr 23-Apr 24-Apr 24-Apr 23-Apr	No	No	0 0 0 0 0 0 0	Negative Negative Negative Negative Negative Negative Negative Negative Positive
GCN18-0307 GCN18-0303 GCN18-0297 GCN18-0358 GCN18-0359 GCN18-0313 GCN18-1731 GCN18-0298	155 156 158 159 160 161 <b>162</b> 166	23-Apr 23-Apr 23-Apr 23-Apr 24-Apr 24-Apr 23-Apr 11-Jun 23-Apr	No	No	0 0 0 0 0 0 0 12	Negative Negative Negative Negative Negative Negative Negative Positive Negative
GCN18-0307 GCN18-0303 GCN18-0297 GCN18-0358 GCN18-0359 GCN18-0313 GCN18-1731 GCN18-0298 GCN18-1249	155 156 158 159 160 161 <b>162</b> 166 166a	23-Apr 23-Apr 23-Apr 23-Apr 24-Apr 24-Apr 23-Apr 11-Jun 23-Apr	No N	No	0 0 0 0 0 0 0 12 0	Negative Negative Negative Negative Negative Negative Negative Positive Negative Negative













GCN18-1275	170	11-Jun	NA	No	4	Positive
GCN18-0310	173	23-Apr	No	No	0	Negative
GCN18-0320	174	23-Apr	No	No	0	Negative
GCN18-0299	175	23-Apr	No	No	0	Negative
GCN18-1278	176	11-Jun	No	No	0	Negative
GCN18-1743	177	22-May	No	No	0	Negative
GCN18-0331	178	23-Apr	No	No	0	Negative
GCN18-0301	179	27-Apr	No	No	0	Negative
GCN18-1732	180	22-May	NA	No	1	Positive
GCN18-0332	182	27-Apr	No	No	0	Negative
GCN18-1742	183	22-May	No	No	0	Negative
GCN18-1735	186	22-May	No	No	0	Negative
GCN18-0292	187	23-Apr	NA	No	12	Positive
GCN18-0341	188	23-Apr	No	No	0	Negative
GCN18-1739	189	22-May	NA	No	3	Positive
GCN18-1264	190	09-May	No	No	0	Negative
GCN18-0317	191	23-Apr	No	No	0	Negative
GCN18-0284	192	23-Apr	No	No	0	Negative
GCN18-0283	193	23-Apr	No	No	0	Negative
GCN18-0339	194	23-Apr	No	No	0	Negative
GCN18-0343	194a	24-Apr	NA	No	1	Positive
GCN18-0350	194b	27-Apr	No	No	0	Negative
GCN18-0351	194c	27-Apr	NA	No	1	Positive
GCN18-0357	195	24-Apr	No	No	0	Negative
GCN18-0336	196	23-Apr	No	No	0	Negative
GCN18-0335	197	23-Apr	No	No	0	Negative
GCN18-0323	198	23-Apr	No	No	0	Negative
GCN18-0327	199	23-Apr	No	No	0	Negative
GCN18-0347	200	24-Apr	No	No	0	Negative
GCN18-0291	201	23-Apr	NA	No	4	Positive
GCN18-1738	202	22-May	No	No	0	Negative
GCN18-1733	202a	22-May	No	No	0	Negative
GCN18-0328	203	23-Apr	No	No	0	Negative
GCN18-1748	204	22-May	No	No	0	Negative
GCN18-1754	204a	22-May	No	No	0	Negative
GCN18-1254	205	09-May	No	No	0	Negative













GCN18-1744	206	22-May	No	No	0	Negative
GCN18-1747	206a	22-May	No	No	0	Negative
GCN18-0348	208	24-Apr	No	No	0	Negative
GCN18-0353	208a	15-May	No	No	0	Negative
GCN18-1243	223	19-Jun	NA	No	1	Positive
GCN18-1248	224	11-Jun	No	No	0	Negative

End of report

**Report issued by:** Dr. Cuong Tang

Contact: ct@naturemetrics.co.uk | 01491 829042

#### Understanding your results

**Positive:** GCN DNA has been detected in this sample, meaning that at least one of the

12 replicates has amplified. Remember that this is not a quantitative test, so you should not interpret a high eDNA score (e.g. 12/12) as necessarily indicating a larger population of GCN than a low eDNA score (e.g. 1/12).

**Negative:** No GCN DNA has been detected in this sample, and the internal and external

controls worked as expected. This tells us that if there had been GCN DNA in the sample, we would have detected it, so we can be confident in its absence

from the sample provided.

**Inconclusive:** No GCN DNA was detected in the sample, but the internal controls failed to

amplify as expected. This means that any GCN DNA in the sample might also have failed to amplify properly, so we cannot have confidence in this negative result. Inconclusive results can be caused by degradation of the DNA (when the DNA marker contained in the ethanol in the kits fails to amplify) or by inhibition of the reaction (when the marker added in the lab fails to amplify) caused by certain chemicals or organic compounds that may be present in

the water sample.











## **Figures**

Figure 7.10.1 Desk Study and Field Survey Results

Figure 7.10.2 Positive Pond Locations and Buffers

