

# OTTERPOOL PARK

Environmental Statement (ES) Appendix: 7.9 Great Crested Newt Survey Report – Update to Include 2020 and 2021 Survey Data

MARCH 2022



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## Executive summary

Arcadis Consulting (UK) Limited has been commissioned on behalf of Otterpool Park LLP to undertake surveys for great crested newt (*Triturus cristatus*) to inform an Environmental Impact Assessment (EIA) for the proposed Development and accompany an amended outline planning application. The proposed Development is 'Otterpool Park', a garden settlement located within Folkestone, Kent. The development area has been identified as an 'area of search'; hereafter, the area of search is referred to as "the site".

The site is located within the administrative boundary of Folkestone and Hythe District Council (F&HDC) and spans a large area located immediately south of Junction 11 of the M20. The site is largely agricultural in nature comprising arable and pasture fields, a disused horseracing course with an artificial lake ('Folkestone Racecourse Lake'), areas modified from historical use (airfields), existing historic settlements and relatively new industrial areas. The site area encompasses the proposed Otterpool Park Area Development application site (presented in red on all figures) and is 589 ha in area.

Targeted great crested newt surveys were initially conducted between 10 April and 31 May 2017. A total of 21 ponds were surveyed throughout the site based on a previous assessment of their habitat suitability (HSI Habitat Suitability Index score). Three separate survey methods were used per survey. These included a combination of torch surveying, bottle trapping, netting and/or egg searching, depending on pond conditions at the time of survey. Four surveys were conducted on all 21 ponds to determine the presence / likely absence of GCN, with a further two surveys being conducted on eight of those ponds that were found to contain GCN to determine the population size.

In April and May 2020 ponds that were accessible were resurveyed using a HSI for their suitability for GCN. In total 17 ponds were revisited. Of these, eight ponds were deemed suitable for GCN. EDNA samples of these identified two ponds with a positive eDNA result, both located in the vicinity of the Westenhanger Castle, within 50m of a pond previously identified as supporting GCN.

In June 2021 two additional ponds were surveyed, of which only one was considered suitable for GCN, but provided a negative eDNA test result.

In total, during the surveys 2017 - 2021, ten ponds had confirmed GCN presence (seven on site and three adjacent to the site in Harringe Brooks Woods / Otterpool Manor). The highest peak adult count on any one night of survey was 11, found on the 15 April at Barrow Hill Farm. This qualifies as a medium population under Natural England Guidelines. The remaining ponds are classified as low populations (<10 peak count) and are located at Champneys Farm, Harringe Brooks Wood, Westenhanger Castle, Hillhurst Farm and north of Folks Wood Way/ Lympe Village.

It is likely that the majority of these ponds are isolated populations, many of them remnants of previous populations. The medium population found at Barrow Hill Farm is likely to be an isolated population as surrounding ponds were classified as unsuitable / dry habitat and are also separated from Barrow Hill Farm by the Ashford Road, which would act as a potential barrier to newt migration.

However, it is likely that the ponds within Champneys Farm and surrounding Harringe Brooks Wood form a metapopulation structure. This means that smaller populations within separate ponds can migrate between ponds when conditions fluctuate providing greater stability and resilience within the overall population. This is dependent on good connectivity between suitable ponds/habitat. Champneys Farm is well linked to Harringe Brooks Wood with no/limited barriers to newt migration. The newts found in these ponds are likely to be part of one large overall population in and around Harringe Brooks Wood.

Other amphibian species were found in the majority of ponds on site, these included smooth newt (*Lissotriton vulgaris*), palmate newt (*Lissotriton helveticus*), common frog/frog tadpoles (*Rana temporaria*) and common toad/toad tadpoles (*Bufo bufo*). An assessment of the conservation status of these species and proposed mitigation is presented in ES Chapter 7: Biodiversity.

Within the proposed Development, many areas of value for great crested newts will be retained and enhanced. Only one pond which supports GCN will be directly lost to the proposed Development, which is pond 27 located in the east of the site. It was not possible to preserve this pond with sufficient terrestrial habitat to support a GCN population. This pond supports an isolated, small population of GCN therefore an alternative mitigation approach to retention was deemed more appropriate. There will however be a loss of terrestrial habitat associated with the ponds and additional mitigation will be required to safeguard GCN populations. A Code of Construction Practice (CoCP) will be produced to include best practice construction mitigation. The requirement for licensing is to be determined with Natural England. The requirement for further survey at later stages of the planning process will be determined by the details of the planning tiers in relation to the development, and the mitigation approach determined for each tier.

# 1 Introduction

## 1.1 Overview

1.1.1 Arcadis Consulting (UK) Limited has been commissioned on behalf of Otterpool Park LLP to undertake surveys for great crested newt (GCN) (*Triturus cristatus*) to inform an EIA for the proposed Development and accompany an amended outline planning application. The proposed Development is 'Otterpool Park, a garden settlement located within Folkestone, Kent. The development area has been identified as an 'area of search'; hereafter, the area of search is referred to as "the site". This report presents the results of GCN surveys conducted between 2017 and 2021.

## 1.2 Site Location and Setting

- 1.2.1 The site is located within Folkestone, Kent within the administrative boundary of Folkestone and Hythe District Council (F&HDC) and spans a large area located immediately south of Junction 11 of the M20. The site is largely agricultural in nature with the majority of the site comprising arable and pasture fields, a disused horseracing course with an artificial lake ('Folkestone Racecourse Lake'), areas modified from historical use (airfields), existing historic settlements and relatively new industrial areas.
- 1.2.2 The M20 motorway, Channel Tunnel Rail Link and Westenhanger Station are located to the north of the site, beyond which lie the villages of Stanford and Postling within a largely rural setting including the Kent Downs Area of Outstanding Natural Beauty (AONB). This AONB extends to the east, beyond which lies the town of Hythe, and to the south where it includes Lympe village. The site also includes the settlements of Barrowhill, Sellindge, Westenhanger and Newingreen. Lympe Industrial Park and some areas of woodland are located immediately south of the site. In addition, East Stour River flows through the site in a north-east to west direction. The site is centred on BNG TR 111 363.
- 1.2.3 An aerial image illustrating the site surveyed is presented in Image 1. Photographs of the site can be found in Appendix G- Photographs.



Image 1 Aerial imagery of the site

## 1.3 Proposed Development

1.3.1 The proposed Otterpool Park Development is located on approximately 589 ha of land within the wider study area as shown in Figure 1. The planning application seeks permission for a new garden settlement accommodating up to 8,500 homes (Use Classes C2 and C3) and Use Class E, F, B2, C1, Sui Generis development, including use of retained buildings as identified, with related infrastructure, highway works, green and blue infrastructure, with access, appearance, landscaping, layout and scale matters to be reserved. A summary of the maximum floorspace areas for each land use type is provided in Chapter 4: The Site and the Proposed Development of the Environmental Statement (ES).

## 1.4 Great Crested Newt Biology

1.4.1 Newts are the largest member of the Pleurodelinae subfamily, with an adult length range of 90 -170mm (Mckinnel *et al.* 2012). They are found widely throughout Europe but numbers have significantly declined in the past century predominantly due to agricultural intensification. Although afforded protection in the UK as a European Protected Species, GCN are widespread throughout much of England and Wales. They occur only sparsely in south-west England, mid Wales and Scotland and are absent from Northern Ireland (JNCC, accessed 2017). A national report published in 2013 estimated the population to be approximately 75,000 (European Union, 2013).

1.4.2 Great Crested Newts spend the majority of time in areas of rough grassland, scrub and woodland but breeding takes place in small to medium sized ponds. They emerge from hibernation in March-April and commute to neighbouring ponds/waterbodies to breed from April-June, providing the night temperature is above 5°C. Females can lay between 200 and 400 eggs per breeding season, which are hidden in the folds of aquatic vegetation (Mckinnel *et al.* 2012).

1.4.3 GCN are thought to have a metapopulation structure, whereby the overall population of newts within a given area of suitable habitat is made up of smaller populations (metapopulations). This allows for stability within the overall population despite stochasticity (fluctuations in population size due to random chance events) within metapopulations. However, this method of sustaining the population is dependent on habitat connectivity between metapopulations allowing emigration/immigration to occur (Hanski, 1998).

1.4.4 Despite the population evolving methods to avoid extinction, there has still been a noticeable decline in numbers within the last century. The main reasons for this are thought to be as follows:

- Agricultural intensification: Ponds within agricultural land are often now deliberately destroyed to increase the amount of space available for crop growth. If ponds remain there is often a lack of available surrounding vegetation due to intensive ploughing and pesticide use (Langton *et al.*, 2001).
- Habitat fragmentation: As mentioned above, for metapopulation structures to succeed, habitat connectivity is vital. In the last century, agricultural intensification and urbanisation has led to increased loss of suitable newt habitat, preventing newt migration and therefore inhibiting population stability.
- Introduction of fish: there has been evidence to suggest that the introduction of fish for fishing or ornamental purposes has contributed to newt decline in some cases, due to the fact that fish prey on newt eggs and larvae (Mckinnel *et al.* 2012).

## 1.5 Legislation and Conservation Status

- 1.5.1 The great crested newt is protected under two key pieces of national legislation. It qualifies as a European Protected Species (EPS) and is listed under Schedule 5 of the Wildlife and Countryside Act 1981 (as amended) (WCA, 1981).
- 1.5.2 Under the WCA it is an offence to:
- Intentionally or \*recklessly disturb a great crested newt whilst it is occupying a structure or place which it uses for shelter or protection;
  - Intentionally or \*recklessly obstruct access to any structure or place used for shelter or protection by a great crested newt;
  - Sell, offer or expose for sale, or to possess or transport for sale alive or dead otter or any part of or anything derived from a great crested newt.
- \*The term “recklessly” was added as an amendment to the WCA 1981 as a result of the Countryside and Rights of Way Act (CROW Act, 2000).
- 1.5.3 GCN are also included on Schedule 2 of the Conservation of Habitats and Species Regulations (2017) which makes it an offence to:
- Deliberately capture or kill a great crested newt;
  - Deliberately disturb a great crested newt (where disturbance is likely to impair their ability to survive, breed or reproduce, rear or nurture their young; or to hibernate or migrate; or to affect significantly the local distribution or abundance of great crested newts).
  - Damage or destroy a breeding site or resting place of a great crested newt; and
  - Be in possession of, control, transport, sell or exchange, or offer for sale or exchange any live or dead wild great crested newt or any part of a wild great crested newt or anything derived from a great crested newt.
- 1.5.4 Derogation licences may be granted by Natural England under Regulation 53 of the Conservation of Habitats and Species Regulations (2017) for certain purposes affecting great crested newts, including development works. Regulation 53 (2)(e) states that such licences can be granted for the purpose of “*preserving public health or public safety or other imperative reasons of overriding public interest including those of a social or economic nature and beneficial consequences of primary importance for the environment*”. Those activities listed under Schedule 2 (see above) would not constitute an offence if carried out in accordance with the terms of such a licence. Such licenses must be supported by a method statement outlining mitigation/compensation measures to provide for the continued favourable conservation status of the species locally (i.e. a Development Mitigation License); or (where available) compensatory payments into an overarching landscape scale mitigation strategy (i.e. District Level Licensing).

## 2 Methodology

### 2.1 Desk Study

- 2.1.1 The purpose of the desk study is to review existing information available in the public domain and to obtain information held by statutory and non-statutory consultees. Information was requested for great crested newt within a 2km radius of the site as recommended in the Institute of Environmental Assessment's 'Guidelines for Baseline Ecological Assessment' (1997).
- 2.1.2 Desk study information was collected from a number of sources, including ecological appraisals from previous planning applications on site and protected species information from Kent and Medway Biological Record Centre (KMBRC) in March 2018. An updated information request for GCN records was requested in April 2020 from KMBRC. Results are presented and discussed and can be seen in Section 3 and Table 3.

### 2.2 Field Surveys Overview

- 2.2.1 Between 2016 and 2021 a range of surveys and assessments have been conducted, including:
- Habitat Suitability Index (HSI) Assessments (general details of this assessment type in Appendix H);
  - Population Surveys (Details in Section 2.9); and
  - eDNA Surveys (general details of this assessment type in Appendix D).
- 2.2.2 the sections below outline the surveys conducted on site, with a summary of the complete survey set conducted on each water body presented in Table 2

### 2.3 Habitat Suitability Index (HSI) Assessments 2017

- 2.3.1 Using a 1:25,000 Ordnance Survey (OS) map of the site and information collected during the Phase 1 surveys, all water bodies were identified within the site. In addition to this water bodies were identified within 500m of the survey boundary where there were no migration barriers (such as major road crossings and rivers) to the movement of newts between the water bodies and the site. This is defined as the survey area.
- 2.3.2 The water bodies identified were visited on 15, 16, 21 and 22 March 2017 by Aline Brodzinski (Senior Ecologist, MCIEEM) and Ewan Gibson (Ecologist, GradCIEEM) during a pond scoping exercise. All water bodies were assessed for their potential to support amphibians, including great crested newts, using the HSI assessment tool for great crested newts initially developed by Oldham et al. (2000). The HSI assessment used ten pond characteristics to provide a numerical assessment of the habitat value of a water body for GCN, the details of this approach are presented in Appendix H. The value of terrestrial habitat within the Survey Area for use by foraging and hibernating amphibians was also assessed during the pond scoping survey. The locations of the water bodies which were assessed in 2017 are presented on Figure 1.
- 2.3.3 The results of the HSI assessments (which value a pond's suitability for GCN from 'Poor' to 'Excellent') were reviewed using professional judgement of the ponds location and connectivity to site to determine which water bodies required further surveys. In line with applicable guidance (OPDM 2005) this is considered a robust approach as it identifies features with a 'reasonable likelihood' of supporting GCN. This approach was agreed with the LPA as outlined in ES Appendix 7.2.

## 2.4 HSI Assessments 2020

2.4.1 In April and May 2020 accessible ponds were re-assessed using the HSI assessment tool (Oldham et al., 2000) to update the validity of the 2017 HSI surveys. The surveys were undertaken by Brandon Murray (Principal Ecologist, MCIEEM) and Ewan Gibson (Ecologist, ACIEEM). In total, 17 ponds were visited and of these, 14 were subject to HSI assessment (three ponds dry at time of survey and could not be assessed using HSI techniques). This also included the HSI assessment of one new pond not previously surveyed (dry in previous years). The location of the ponds surveyed in 2020 are shown on Figure 2.

## 2.5 HSI Assessments 2021

2.5.1 In June 2021 two additional ponds (not previously surveyed due to site boundary change) were assessed using the HSI assessment tool Oldham et al. (2000). The surveys were undertaken by Joel Cronin (Ecologist ACIEEM) on 30 June 2021. The location of the ponds surveyed in 2021 are shown on Figure 2.

## 2.6 eDNA Surveys 2017 and 2018

2.6.1 An environmental DNA (eDNA) survey is a technique whereby water samples are taken from a pond and sent to a laboratory for testing to determine the presence or absence of GCN DNA within the water. These surveys need to be conducted in a certain manner according to the prescriptions of a Natural England Technical Advice Note (Biggs et al. 2014). The protocol followed was according to the testing lap instructions, shown in Appendix D, which meets the protocol set by Natural England. The testing was conducted by eDNA testing company ADAS.

2.6.2 During the masterplanning process, the project area was revised on several occasions, bringing additional ponds into the ZOI (zone of influence) of the development following the initial 2016 and 2017 assessments. None of these ponds were on the site, all ponds were over 100m from the site boundary and were isolated from the site by roads of varying widths. However, it was considered that understanding the presence /absence of GCN within these water bodies would assist with understanding the population distribution of GCN beyond the site, and feed into the impact assessment of the site. A summary of the surveys conducted on each pond is presented in Table 2.

2.6.3 As population data was not required, it was determined that eDNA surveys would provide a sufficient resolution of data required for the EIA submission (presence / absence data). As a result, six ponds were surveyed using eDNA techniques in Spring 2018 by Ewan Gibson and Brandon Murray (Associate Technical Director). The locations of these ponds is presented on Figure 1. Surveyor pen portraits are presented in Appendix F.

## 2.7 eDNA Surveys 2020

2.7.1 In April and May 2020 eight ponds were surveyed using eDNA techniques. The surveys were undertaken by Brandon Murray and Ewan Gibson. The ponds surveyed were those that were accessible during the time of the survey, considered suitable for GCN and had no prior recorded presence of GCN. This included one additional pond (27a) that had not been previously surveyed. The location of the ponds surveyed in 2020 are shown on Figure 4 and Figure 6.

## 2.8 eDNA Surveys 2021

2.8.1 In June 2021 one suitable pond near Stone Street to the east of the site (previously not surveyed) was surveyed using eDNA techniques. The survey was undertaken by Joel

Cronin. The location of the ponds surveyed in 2021 are shown on on Figure 4 and Figure 6.

## 2.9 Population Surveys 2017

- 2.9.1 Based on HSI results from surveys in 2017, ponds requiring further survey were selected. Subsequent great crested newt surveys were undertaken in 2017 by Brandon Murray, Aline Brodzinski, Ellen Poppleton (Assistant Ecologist, GradCIEEM) and Ewan Gibson. The locations of the ponds surveyed is presented on Figure 1.
- 2.9.2 Traditional presence/absence surveys were undertaken at all potentially suitable water bodies to determine (as far as reasonably possible) the presence or likely absence of this species. Surveys were carried out according to the most up-to-date survey guidance for this species (English Nature, 2001). Up to four visits were undertaken in suitable weather conditions, and a combination of survey techniques was used, as outlined below. Reasons for not undertaking the survey methods at a particular water body are given in the results tables in section 3. The four visits took place on the following dates: week beginning 10 April, week beginning the 18 April, week beginning the 2 May and week beginning 9 May. If GCN presence was confirmed during one or more of these visits, then two further surveys were undertaken during the week beginning the 15 May and the week beginning the 31 May, to provide a total of six surveys per-pond, on which to base a population estimate, as recommended within the GCN Mitigation Guidelines (Natural England, 2001).
- 2.9.3 A combination of three of four available survey techniques was adopted. Which techniques were selected was dependant on pond conditions at the time of survey. Survey techniques were taken from GCN Mitigation Guidelines (Natural England, 2001) and consisted of:
- Torching – after nightfall, ponds were torched using Clue Lights (1,000,000+ Candle Power). Any GCN or other amphibians observed were recorded. This technique was dependant on pond visibility.
  - Egg searching – suitable vegetation within/on the verges of ponds was searched for characteristic “folds” indicative of vegetation selected for egg deposition. If folded vegetation was identified then this was slowly dissected to see if it contained an egg and to distinguish if the egg belonged to great crested newt or other newt species. It was important that once eggs were found within the pond, no further egg searches were carried out as the pond has already been identified as a breeding site and further searches would only disturb the eggs. This technique was dependant on the presence of suitable vegetation.
  - Netting – ponds were “sweep netted”, skimming the pond floor so as to collect any GCN residing there. Nets were then carried over to the pond edge, sifted through, contents recorded and then released back into the pond. Netting by nature is disruptive therefore is only used if necessary/ if other techniques are not available.
  - Bottle trapping – bottles were created from empty 2 litre plastic bottles with inverted tops, creating a funnel-like structure. These were then attached to bamboo canes, allowing them to be secured to the pond floor or bank, so that the trap was suspended within the pond. Bottles were left in the pond overnight and then collected in the morning. Any newts captured were recorded and then released immediately back into the pond. This was dependant on pond depth and temperature (below 5°C there is a risk to newt survival within the traps).

## 2.10 Population Assessment 2017

- 2.10.1 A rapid population size class assessment was undertaken for ponds utilising the methodology presented within the Great Crested Newt Mitigation Guidelines 2001 (English

Nature, 2001). The table below (Table 1) outlines the sized class parameters used within this report.

- 2.10.2 In line with the methodology presented within the Great Crested Newt Mitigation Guidelines 2001 (English Nature, 2001) the population size is based on a spring survey of adult numbers, as egg, larval and juvenile counts can give a misleading indication of overall population size without complex interpretation. Examining the results of the survey, the maximum adult count per pond per night gained through torch survey or bottle-trapping is utilised as the count figure. For water bodies where there is reasonable certainty that there is regular interchange of animals between ponds (typically, within 250m and with an absence of barriers to dispersal), counts are summed across ponds (for counts obtained on the same visit).

Table 1: Population size class assessment

Peak count of 'population'	Size Class
Up to 10	Low
Counts between 11 – 100	Medium
Counts over 100	High

## 2.11 Summary of Surveys Conducted 2016 - 2021

- 2.11.1 This section outlines the rationale behind the surveys and assessments conducted on each pond between 2016 and 2021. The relevant information is presented in Table 2.

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Table 2: Summary of assessments on each pond conducted 2016 – 2021

Pond identifier	HSI 2017	Population Survey 2017	eDNA Survey 2017 / 2018	HSI Assessment 2020	eDNA survey 2020	HSI Assessment 2021	eDNA survey 2021
1	Yes	Yes – suitability identified	No- 2017 population survey confirmed absence so this was not required	No – No access permitted			
2	Yes	Yes – suitability identified	No- 2017 population survey confirmed absence so this was not required	No – No access permitted			
3	Yes	Yes – suitability identified	No- 2017 population survey confirmed absence so this was not required	No – No access permitted			
4	Yes	Yes – suitability identified	No- 2017 population survey confirmed absence so this was not required	No – No access permitted			
5	Yes	Yes – population present	No- 2017 population survey confirmed absence so this was not required	No – No access permitted			
6	Yes	No – Poor HSI and stocked with fish	No – not required	No – No access permitted			

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Pond identifier	HSI 2017	Population Survey 2017	eDNA Survey 2017 / 2018	HSI Assessment 2020	eDNA survey 2020	HSI Assessment 2021	eDNA survey 2021
7	Yes	No – outside of the 500m survey area	Yes- Negative (completed for avoidance of doubt and to cover future changes to the site boundary)	No – outside of the 500m survey area	No – outside of the 500m survey area	No – outside of the 500m survey area	No – outside of the 500m survey area
8	Yes	Yes – population absent	No – pond excavated and stocked with fish not required to survey	No – No access permitted	No – No access permitted	No – No access permitted	No – No access permitted
9	Yes	Yes – GCN present	No- 2017 population survey confirmed absence so this was not required	No – No access permitted	No – No access permitted	No – No access permitted	No – No access permitted
10	Yes	No pond was dry when surveys were conducted-	No – pond dry	No – No access permitted	No – No access permitted	No – No access permitted	No – No access permitted
11	Yes	Yes – GCN present	No – presence confirmed	No – No access permitted	No – No access permitted	No – No access permitted	No – No access permitted
12	Yes	Yes – GCN present	No – presence confirmed	No – No access permitted	No – No access permitted	No – No access permitted	No – No access permitted
13a	Yes	Yes – population absent	No – absence confirmed	Yes – update required	Yes - Negative	No – not required – results considered sufficient to inform the ES	No – not required – results considered sufficient to inform the ES
13b	Yes	Yes – population absent	No – absence confirmed	Yes – update required	Yes - Negative	No – not required – results considered	No – not required – results considered

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Pond identifier	HSI 2017	Population Survey 2017	eDNA Survey 2017 / 2018	HSI Assessment 2020	eDNA survey 2020	HSI Assessment 2021	eDNA survey 2021
						sufficient to inform the ES	sufficient to inform the ES
14	Yes	Yes – population absent	No – absence confirmed	No – pond dry	No – pond dry	No – not required – results considered sufficient to inform the ES	No – not required – results considered sufficient to inform the ES
15a/b	Yes	Yes – GCN present	No – presence confirmed	No – no change in status and population already confirmed	No – no change in status and population already confirmed	No – not required – results considered sufficient to inform the ES	No – not required – results considered sufficient to inform the ES
16	Yes	Yes – population absent	No – absence confirmed	Yes – update required	No – HSI confirmed very poor status (stocked with fish)	No – not required – results considered sufficient to inform the ES	No – not required – results considered sufficient to inform the ES
17	Yes	Yes – GCN present	No – presence confirmed	Yes – update required	No – no change in status and population already confirmed	No – not required – results considered sufficient to inform the ES	No – not required – results considered sufficient to inform the ES
18	Yes	No – poor HSI and considered very unlikely to support GCN	No – unsuitable for GCN	No – unsuitable for GCN	No – unsuitable for GCN	No – not required – results considered sufficient to inform the ES	No – not required – results considered sufficient to inform the ES
19	Yes	Yes – population absent	No – absence confirmed	Yes – update required	No – no change in status and population already confirmed	No – not required – results considered sufficient to inform the ES	No – not required – results considered sufficient to inform the ES
20	Yes	Yes – population absent	No – absence confirmed	Yes – update required	Yes - Negative	No – not required – results considered sufficient to inform the ES	No – not required – results considered sufficient to inform the ES

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Pond identifier	HSI 2017	Population Survey 2017	eDNA Survey 2017 / 2018	HSI Assessment 2020	eDNA survey 2020	HSI Assessment 2021	eDNA survey 2021
21a	Yes	Yes – population absent	No – absence confirmed	Yes – update required	Connected to pond 20 with floodwater - Negative	No – not required – results considered sufficient to inform the ES	No – not required – results considered sufficient to inform the ES
21b	Yes	Yes – population absent	No – absence confirmed	Yes – update required	Connected to pond 20 with floodwater - Negative	No – not required – results considered sufficient to inform the ES	No – not required – results considered sufficient to inform the ES
21c	No – pond not present	No – pond not present	No – pond not present	Yes	No – pond dry	No – not required – results considered sufficient to inform the ES	No – not required – results considered sufficient to inform the ES
22	Yes	Yes – population absent	No – population survey completed	Yes – update required	Yes -positive	No – not required – results considered sufficient to inform the ES	No – not required – results considered sufficient to inform the ES
23	Yes	Yes – GCN present	No – population survey completed	Yes – update required	Yes -positive	No – not required – results considered sufficient to inform the ES	No – not required – results considered sufficient to inform the ES
23a	Yes	No – pond dry	No – pond dry	Yes – update required	Yes -positive	No – not required – results considered sufficient to inform the ES	No – not required – results considered sufficient to inform the ES
24	Yes	No – Ornamental Pond with raised sides and fish	No – pond unsuitable	Yes – update required	Yes - Negative	No – not required – results considered sufficient to inform the ES	No – not required – results considered sufficient to inform the ES
25	Yes	No – pond dry	No – pond dry	Yes – update required	No – pond dry	No – not required – results considered	No – not required – results considered

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Pond identifier	HSI 2017	Population Survey 2017	eDNA Survey 2017 / 2018	HSI Assessment 2020	eDNA survey 2020	HSI Assessment 2021	eDNA survey 2021
						sufficient to inform the ES	sufficient to inform the ES
26	Yes	No – pond dry	No – pond dry	Yes – update required	No – pond dry	No – not required – results considered sufficient to inform the ES	No – not required – results considered sufficient to inform the ES
27	Yes	Yes – GCN present	No – presence / absence survey conducted	Yes – update required	No – no change in status and population already confirmed	No – not required – results considered sufficient to inform the ES	No – not required – results considered sufficient to inform the ES
27A	No – pond not present	No – pond not present	No – pond not present	Yes	Yes - Negative	No – not required – results considered sufficient to inform the ES	No – not required – results considered sufficient to inform the ES
28	Yes	Yes – population absent	No – presence / absence survey conducted	Yes – update required	Yes - Negative	No – not required – results considered sufficient to inform the ES	No – not required – results considered sufficient to inform the ES
29	Yes	No - Poor quality for GCN and isolated from the site by the A20	Yes - Negative	Yes – update required	No – no change in status and population absence already confirmed	No – not required – results considered sufficient to inform the ES	No – not required – results considered sufficient to inform the ES
30	Yes	No – Ornamental Pond with raised sides	No – not required – results considered sufficient to inform the ES	No – not required – results considered sufficient to inform the ES	No – not required – results considered sufficient to inform the ES	No – not required – results considered sufficient to inform the ES	No – not required – results considered sufficient to inform the ES
31	No – no access. Negative	No – no access. Negative eDNA provided by landowner	No – not required – results considered	Yes – update required	No – not required – results considered sufficient to inform the ES	No – not required – results considered sufficient to inform the ES	No – not required – results considered sufficient to inform the ES

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Pond identifier	HSI 2017	Population Survey 2017	eDNA Survey 2017 / 2018	HSI Assessment 2020	eDNA survey 2020	HSI Assessment 2021	eDNA survey 2021
	eDNA provided by landowner		sufficient to inform the ES				
32	No – no access	No – no access	No – no access	No – no access	No – no access	No – no access	No – no access
33	Yes	No -outside of the survey area as identified in 2017 (site redline was smaller)	Yes - Negative	No – not required – results considered sufficient to inform the ES considering pond status and isolation form the site.	No – not required – results considered sufficient to inform the ES considering pond status and isolation form the site.	No – not required – results considered sufficient to inform the ES considering pond status and isolation form the site.	No – not required – results considered sufficient to inform the ES considering pond status and isolation form the site.
34	Yes	No -outside of the survey area as identified in 2017 (site redline was smaller)	Yes - Negative	No – not required – results considered sufficient to inform the ES considering pond status and isolation form the site.	No – not required – results considered sufficient to inform the ES considering pond status and isolation form the site.	No – not required – results considered sufficient to inform the ES considering pond status and isolation form the site.	No – not required – results considered sufficient to inform the ES considering pond status and isolation form the site.
35	Yes	No -outside of the survey area as identified in 2017 (site redline was smaller)	Yes - Negative	No – not required – results considered sufficient to inform the ES considering pond status and isolation form the site.	No – not required – results considered sufficient to inform the ES considering pond status and isolation form the site.	No – not required – results considered sufficient to inform the ES considering pond status and isolation form the site.	No – not required – results considered sufficient to inform the ES considering pond status and isolation form the site.
36	Yes	No -outside of the survey area as identified in 2017 (site redline was smaller)	Yes - Negative	No – not required – results considered sufficient to inform the ES considering pond status and isolation form the site.	No – not required – results considered sufficient to inform the ES considering pond status and isolation form the site.	No – not required – results considered sufficient to inform the ES considering pond status and isolation form the site.	No – not required – results considered sufficient to inform the ES considering pond status and isolation form the site.

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Pond identifier	HSI 2017	Population Survey 2017	eDNA Survey 2017 / 2018	HSI Assessment 2020	eDNA survey 2020	HSI Assessment 2021	eDNA survey 2021
37	No – within bear enclosure (but concrete ponds so negligible suitability)	No -outside of the survey area as identified in 2017 (site redline was smaller)	No – within bear enclosure	No – not required – results considered sufficient to inform the ES considering pond status and isolation form the site.	No – not required – results considered sufficient to inform the ES considering pond status and isolation form the site.	No – not required – results considered sufficient to inform the ES considering pond status and isolation form the site.	No – not required – results considered sufficient to inform the ES considering pond status and isolation form the site.
38	No – within bear enclosure (but concrete ponds so negligible suitability)	No -outside of the survey area as identified in 2017 (site redline was smaller)	No – within bear enclosure	No – not required – results considered sufficient to inform the ES.	No – not required – results considered sufficient to inform the ES	No – not required – results considered sufficient to inform the ES	No – not required – results considered sufficient to inform the ES
39	No -outside of the survey area as identified in 2017 (site redline was smaller)	No -outside of the survey area as identified in 2017 (site redline was smaller)	No – connected to pond 35 so not required	No – not required – results considered sufficient to inform the ES	No – not required – results considered sufficient to inform the ES	No – not required – results considered sufficient to inform the ES	No – not required – results considered sufficient to inform the ES
40	Yes	No – Very poor suitability and isolated from the site by Harringe Lane	No – pond very low suitability	Yes – update required as pond status had changed	Yes - negative	No – not required – results considered sufficient to inform the ES	No – not required – results considered sufficient to inform the ES
41	Yes	No – pond dry	No – pond dry	No – pond dry	No – pond dry	No – pond dry	No – pond dry
42	No – raised fish pond with zero suitability	No – raised fish pond with zero suitability	No – raised fish pond with zero suitability	No – raised fish pond with zero suitability	No – raised fish pond with zero suitability	No – raised fish pond with zero suitability	No – raised fish pond with zero suitability
43	No – No access	No – No access	No – No access	No – No access	No – No access	Yes	Yes – Negative

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Pond identifier	HSI 2017	Population Survey 2017	eDNA Survey 2017 / 2018	HSI Assessment 2020	eDNA survey 2020	HSI Assessment 2021	eDNA survey 2021
44	No – No access	No – No access	No – No access	No – No access	No – No access	Yes	No – poor HSI and a stocked fish pond

## 2.12 Survey Limitations

- 2.12.1 Access to some areas of the ponds was difficult due to dense vegetation, thick mud or pond depth. This prevented maximum coverage in certain ponds during the population surveys (i.e. during torch and bottle trapping), but coverage was considered sufficient for the purpose of this survey.
- 2.12.2 Water levels within ponds fluctuated. By the end of the 2017 surveys, nine ponds (of the 22 initially surveyed) had completely dried or the number of bottle traps surveyors were able to set had declined. This made it difficult to replicate previous survey methodology each time the same pond was visited. However where possible, if bottle traps could not continue to be used an appropriate alternative method was employed
- 2.12.3 Great crested newts are a mobile species and their presence/absence within ponds on site can vary over a breeding season. It is possible that some of the ponds where no GCN were found may contain GCN at any given time between April – June, particularly if they are in close proximity to a pond where GCN presence is confirmed.
- 2.12.4 One of the six ponds surveyed using eDNA techniques in 2018 was found to have very poor water quality at the time of the survey. As a result, the eDNA test was inconclusive. However, considering the very low suitability of the pond for GCN, and the lack of GCN in the surrounding ponds, it is considered that this will not have adversely impacted the ability to utilise the results for the impact assessment (i.e, the results of the HSI assessment are considered sufficient to inform the ES).
- 2.12.5 Due to the outbreak of the COVID-19 virus in 2020, survey scope was greatly impacted and had to be altered to what was safe and practical to achieve. As such, the 2020 surveys endeavoured to collect the information intrinsic to ensuring the submission is founded on robust survey data, whilst acknowledging that the surveys needed to be proportionate in light of the additional risks to Arcadis employees and members of the public. Modifications to As a result, the following changes were made to the survey scopes:
- For the update surveys, access was not requested to parcels of land where members of the public were likely to be at increased risk of coming into contact with Arcadis employees.
  - Access to private homes and businesses (excluding farms) was not requested, both to reduce exposure risk and to avoid potential for negative reactions to interaction with Arcadis staff.
- 2.12.6 These changes were made in line with the CIEEM guidance (CIEEM 2019) which was applicable at the time of the survey scoping and were agreed with the LPA as presented in ES Appendix 7.2.
- 2.12.7 Despite these survey limitations, survey effort was considered suitable for this assessment.
- 2.12.8 It should be noted that pond conditions and the environment are subject to change over time and this survey is only reflective of the status of great crested newts on site at the time of survey.

## 3 Results

### 3.1 Desk Study

- 3.1.1 Desktop information was received from the organisations below in Table 3. Information from the organisations listed below was either taken from previous ecological appraisals for planning applications within the site or from record centres.

Table 3: Desk study results

Organisation	Data Received
Kent and Medway Biological Records Centre (Records post 2000 listed), Data Search Conducted March 2018 and Updated April 2020	Two records of GCN were recorded in 2014 at Folkestone Racecourse (TR122369) on the 5 May and the 6 June.  The updated information request from KMBRC in April 2020 did not return any new records of GCN.
CSa Environmental Planning	As part of the Link Park Employment Land Phase 2, great crested newt surveys were undertaken on seven waterbodies, between March-May 2007. Surveys identified a single male GCN at Upper Otterpool.
K B Ecology	K B Ecology undertook GCN surveys on five ponds between March-May 2011. This confirmed a low population of great crested newts in Harringe Brooks Wood and a medium population by Otterpool Manor.
Ecology Solutions Ltd	Ecology Solutions Ltd were instructed in January 2013 to undertake an Ecological Assessment of an area of land south of Sellindge/M20 motorway. Six ponds were surveyed during May-June 2013. Great crested newts were confirmed in six waterbodies with a peak adult count of 12 (medium population).
Bramley associates	A planning report associated with developments at the Holiday Extras site was received. This confirmed that eDNA surveys were undertaken in 2016 on a pond in this area (pond 31) in 2016 and that GCN were confirmed to be absent.

### 3.2 HSI Assessments 2017 and Third Party eDNA Results

- 3.2.1 In 2017 and 2018, thirty-four ponds were considered for survey due to their location within or adjacent to (providing no major barriers intersect) the boundary. Of these 21 were considered suitable for survey based on their Habitat Suitability Index assessment scores. The full results of the HSI assessments are shown in Appendix A and on Figure 1 and Figure 3.
- 3.2.2 Pond 14 was initially surveyed but surveys were abandoned as it declined in quality and was deemed unsuitable for GCN.
- 3.2.3 Pond 31 was not selected for survey based on negative 2016 eDNA results (Bramley Associates 2016).
- 3.2.4 Access to pond 32 was not permitted in 2018 therefore this was not surveyed however in 2020 an HSI assessment could be carried out from a distance.

### 3.3 HSI Assessments 2020

- 3.3.1 In 2020, ponds that were accessible were surveyed for their current suitability for GCN on 30 April and 1 May. In total 17 ponds were visited; of these ponds, three were dry and could not be surveyed and one was a new pond that had not been surveyed before. One pond that could not be assessed in 2018 (pond 32) had an HSI assessment performed

from a distance on 16 June 2020, showing a poor HSI score. The location of the ponds surveyed in 2020 are shown on Figure 2 and the full results of the HSI assessments are shown in Appendix A.

### 3.4 HSI Assessment 2021

3.4.1 In June 2021, a HSI assessment of two additional ponds (ponds 43 and 44) in the east of the site near Stone Street identified that only one of the ponds (pond 43) had potential to support GCN. This pond underwent an eDNA survey (results provided in section 3.8). The locations of the ponds are shown on Figure 1 and the full results of the HSI assessments are provided in Appendix A and on Figure 2 and Figure 4.

### 3.5 Population Assessment 2017

3.5.1 Of the 21 suitable ponds surveyed in 2017, GCN presence was confirmed in eight. The maximum adult count on any one night of survey (the peak adult count) was 11. This was recorded in pond 15 on the 11 April 2017. This represents a medium population accorded to Natural England Guidelines (Natural England, 2001). The remaining seven ponds all had peak adult counts below 10 qualifying them as low populations. All ponds surveyed and their peak adult counts are represented in Table 4 below. Individual results tables for waterbodies are presented in Appendix B. These results are also presented on Figure 5.

Table 4: Peak adult counts

Pond Number	Peak adult count in any one visit	Date of Peak Adult Count	Population size
1	0	n/a	n/a
2	Dried after second survey		
3	0	n/a	n/a
4	0	n/a	n/a
5	2	10/04/2017 18/04/2017	Small (up to 10)
6	Poor suitability for GCN – not surveyed		
7	eDNA survey conducted – see below		
8	0	n/a	n/a
9	3	02/05/2017	Small (up to 10)
10	Dry at time of surveys, not surveyed		
11	8	10/04/2017	Both ponds small (up to 10) – Combined peak count of two ponds also small (up to 10 on a single visit)
12	4	31/05/2017	
13a	0	n/a	n/a

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Pond Number	Peak adult count in any one visit	Date of Peak Adult Count	Population size
13b	0	n/a	n/a
14	0	n/a	n/a
15	11	11/04/2017	Medium (11 – 100)
16	0	n/a	n/a
17	1	04/05/2017	Small (up to 10)
18	Poor suitability for newts – not surveyed		
19	0	n/a	n/a
21a	0	n/a	n/a
21b	0	n/a	n/a
22	0	n/a	n/a
23	1	19/04/2017	Small (up to 10)
23a	Dry at time of surveys, not surveyed		
24	Ornamental pond with raised brick sides. Not surveyed		
25	Dry at time of surveys, not surveyed		
26	Dry at time of surveys, not surveyed		
27	8	03/05/2017	Small (up to 10)
28	0	n/a	n/a
29	Poor quality for GCN and isolated from the site by the A20 – eDNA conducted see below		
30	Ornamental pond with raised sides. Not surveyed		
31	eDNA results provided by landowner		
32	Access to pond not permitted - not surveyed.		
33	eDNA survey conducted – see below		
34	eDNA survey conducted – see below		
35	eDNA survey conducted – see below		
36	eDNA survey conducted – see below		
37	Concrete pit in bear enclosure – not suitable for GCN, not surveyed		
38	Concrete pit in bear enclosure – not suitable for GCN, not surveyed		

Pond Number	Peak adult count in any one visit	Date of Peak Adult Count	Population size
39	Connected to 35 – see pond 35		
40	Poor suitability for newts – not surveyed		
41	Dry at time of surveys, not surveyed		

### 3.6 eDNA Results 2018

3.6.1 Following the assessments undertaken in 2016 and 2017, eDNA surveys were conducted on additional ponds within the ZOI in 2018. The table below (Table 5) outlines the results of these surveys. The full results are presented in Appendix E.

Table 5: 2018 eDNA survey results.

Pond	eDNA date	Surveyors	Result	Notes
7	14.06.2018	Brandon Murray, Rebecca Beale	Negative	N/A
29	29.06.2018	Brandon Murray, Katy Smart	Inconclusive due to evidence of degradation or residual inhibition	Pond water quality very poor, highly unlikely to be suitable for GCN.
33	15.06.2018	Brandon Murray, Rebecca Beale	Negative	N/A
34	28.06.2018	Brandon Murray, Katy Smart	Negative	N/A
35	15.06.2018	Brandon Murray, Rebecca Beale	Negative	N/A
36	15.06.2018	Brandon Murray, Rebecca Beale	Negative	N/A

### 3.7 eDNA Results 2020

3.7.1 Two of the eight surveyed ponds returned a positive eDNA result for GCN, summarised in Table 6. This included one additional pond (pond 23a) not previously surveyed (dry in previous years) and one pond that previously did not support GCN (pond 22).

3.7.2 Figure 2 presents the pond locations where eDNA surveys were undertaken in 2020. The full results of the eDNA surveys are presented in Appendix E and on Figure 4.

Table 6 2020 eDNA survey results

Pond	eDNA date	Surveyors	Result	Notes
13a+13b	01.05.2020	Brandon Murray	Negative	N/A
20	30.04.2020	Brandon Murray	Negative	N/A

Pond	eDNA date	Surveyors	Result	Notes
22	30.04.2020	Brandon Murray	Positive	N/A
23a	30.04.2020	Brandon Murray	Positive	N/A
24	30.04.2020	Brandon Murray	Negative	N/A
27a	30.04.2020	Brandon Murray	Negative	N/A
28	30.04.2020	Brandon Murray	Negative	N/A
40	06.05.2020	Brandon Murray	Negative	N/A

### 3.8 eDNA Results 2021

3.8.1 The 30 June 2021 survey of pond 43 to the east of the site, undertaken by Joel Cronin, returned a negative result for GCN DNA. Figure 1 presents the pond location where the eDNA survey was undertaken in 2021. The full results of the eDNA survey are presented in Appendix E and on Figure 5.

### 3.9 Summary of Ponds With GCN presence

3.9.1 The surveys conducted between 2017 and 2021 have confirmed 10 ponds on or adjacent to the site have GCN presence. These are presented in the table below (Table 7), along with the survey in which they were identified and their location (on or adjacent to the site). These ponds are presented on Figure 6.

Table 7: Summary of ponds with confirmed GCN presence

Pond no.	Survey when population was initially identified	Location in relation to the site redline
5	Population survey 2017	Adjacent to the site in Harringe Brooks Wood
9	Population survey 2017	On-site
11	Population survey 2017	On-site
12	Population survey 2017	On-site
15	Population survey 2017	On-site
17	Population survey 2017	On-site

Pond no.	Survey when population was initially identified	Location in relation to the site redline
22	eDNA 2020	On-site
23	Population survey 2017	On-site
23a	eDNA 2020	On-site
27	Population survey 2017	On-site

### 3.10 Location of Ponds With GCN Presence Confirmed

3.10.1 In accordance with the desk study results, GCN were located in close proximity to Harringe Brooks Wood (pond 9 and pond 5) and Westenhanger Castle (pond 22, 23 and 23a). Additionally, they were also found at Hillhurst Farm (pond 27), Champneys Farm (ponds 11 and 12), Barrow Hill Farm (pond 15) and north of Folks Wood Way (pond 17). The GCN status and location of all ponds on site is represented in Figure 5. Table 8 shows the proximity of each pond where GCN is confirmed in relation to other ponds with confirmed GCN. A 250m buffer around each of these ponds is presented on Figure 6 to indicate the potential/likelihood of metapopulations.

Table 8: Location of ponds with confirmed GCN presence in relation to other confirmed occupied ponds

Pond no.	Within 50m	Within 250m	Within 500m	Within 1000m	No. of ponds potentially connected within 250m
5	None	None	None	Pond 9	0
9	None	None	None	Pond 5 Pond 11 Pond 12 Pond 15 (isolated by the A20)	0
11	None	Pond 12	Pond 12	Pond 5 Pond 9 Pond 12 Pond 15 (isolated by the A20)	1
12	None	Pond 11	Pond 11	Pond 5 Pond 9 Pond 11 Pond 15 (isolated by the A20)	1
15	None	None	None	Pond 9 (isolated by the A20) Pond 11 (isolated by the A20) Pond 12 (isolated by the A20)	0

Pond no.	Within 50m	Within 250m	Within 500m	Within 1000m	No. of ponds potentially connected within 250m
17	None	None	None	None	0
22	Pond 23 Pond 23A	Pond 23 Pond 23a	Pond 23 Pond 23a	Pond 23 Pond 23a Pond 27 (isolated by Stone Street)	2
23	Pond 22 Pond 23A	Pond 22 Pond 23A	Pond 22 Pond 23A	Pond 23 Pond 23a Pond 27 (isolated by Stone Street)	2
23a	Pond 22 Pond 23	Pond 22 Pond 23	Pond 22 Pond 23	Pond 23 Pond 23a Pond 27 (isolated by Stone Street)	2
27	None	None	None	Pond 23 (isolated by Stone Street)	0

3.10.2 The table above shows that the majority of ponds and pond clusters which support GCN are isolated (i.e. populations of GCN associated with each of the ponds / pond clusters are likely to belong to a single pond or pond cluster, with minimal regular movement of GCN between these areas). The exceptions to this are ponds 11 and 12, which are connected and within 250m of each other, and ponds 22, 23, and 23A which are all within 50m of each other.

3.10.3 As a result, for ponds 11 and 12, the findings of the surveys of these populations were combined to identify the combined peak count of these two ponds (i.e. the population size of the GCN population associated with the two ponds). The combined peak count of the two ponds was still 8 adults, therefore it was concluded that a small population was present associated with the two ponds.

3.10.4 With regards to ponds 22, 23 and 23A, during the 2017 surveys no GCN were found in ponds 22 and 23A, but the eDNA confirmed that these ponds are utilised. As no GCN were recorded in the population surveys, and pond 22 has suboptimal GCN habitat and pond 23 dries regularly, the populations in these ponds are unlikely to be larger than a small population, even with the three ponds combined into a metapopulation. As such a small population is presumed.

### 3.11 Incidental Results 2021

3.11.1 On 2 September 2021 a single GCN was observed within the edge of an arable field (at approximately TR 12545 36263) <50m south-west of Pond 31. It is therefore considered that Pond 31 is likely to be colonised by GCN in the future, despite previous surveys concluding that GCN were absent.

### 3.12 Other Species

- 3.12.1 Whilst undertaking the surveys in 2017, other amphibian species were identified within the ponds surveyed. These included smooth newt (*Lissotriton vulgaris*), palmate newt (*Lissotriton helveticus*), common frog/frog tadpoles (*Rana temporaria*) and common toad/toad tadpoles (*Bufo bufo*). In addition, the non-native marsh frog (*Pelophylax ridibundus*) was observed in multiple ponds on site, with particularly large populations observed in ponds 9 and 19, 20 and 21. An assessment of the value of these ecological features and appropriate mitigation is presented in the ES Chapter 7: Biodiversity.
- 3.12.2 Smooth and palmate newts were found in the majority of ponds on site, with a significantly large population found in pond 5 (42 smooth and 44 palmate newts) on 10 April 2017. Further information is provided in Appendix B.

## 4 Discussion

### 4.1 Discussion of Results

- 4.1.1 The ponds with GCN presence confirmed are spread throughout the site at the following locations: Harringe Brooks Wood, Champneys Farm, Westenhanger Castle, Hilhurst farm, Barrow Hill Farm and north of Folks Wood Way. A summary of the impact radii around these ponds is shown in Figure 6.
- 4.1.2 Pond 15 (Barrow Hill Farm) contained the highest peak adult count of 11, qualifying it as a medium population (Natural England, 2015). This pond is not within 500m of any other pond with GCN presence confirmed but is within 500m of ponds 10 and 41. These were ruled out as unsuitable for GCN and therefore were not surveyed. They are also separated from pond 15 by a Ashford Road, which presents a notable potential barrier to the movement of GCN. This is likely to be an isolated population.
- 4.1.3 Pond 22 (positive eDNA result in 2020), pond 23 (peak adult count 1 in 2017) and pond 23a (positive eDNA result in 2020) are in close association (Westenhanger Castle). The ponds are within 250m of five other ponds, two of which were unsuitable and three of which were surveyed but no GCN presence was confirmed (19, 20 and 21). Given the likely absence of GCN in the surrounding ponds it is likely that ponds 22, 23 and 23a support a small, isolated population across all three ponds. Pond 22 was extensively overgrown and appeared to be deteriorating in quality. It is possible but considered unlikely that newts could migrate to these ponds from pond 15 using the connecting watercourses running throughout the racecourse. It is also possible that they could migrate from pond 27 as there is suitable hedgerow connectivity, but again this is unlikely as it is located over 500m away and would involve crossing a busy road.
- 4.1.4 Pond 27 is located at Hilhurst Farm, this contained a peak adult count of eight, qualifying it as a low population. It is within 250m of pond 28 which was surveyed but not found to have any GCN present. It is also within 500m of pond 25 which was considered unsuitable for GCN. This population can therefore be treated as an isolated population. It is likely that without significant maintenance this pond will become redundant, as the water levels were very low in this pond, and it was becoming silted up and overgrown with emergent vegetation.
- 4.1.5 Pond 17 is located north of Folks Wood Way. This had a peak adult count of 1 and is likely to be a remnant population. Ponds 16 and 18 are within 500m of this pond but 18 was unsuitable for GCN and pond 16 was surveyed and GCN were found to be absent. Although pond 16 has good watercourse connectivity to pond 17, it was noted on survey that it also contained large fish. Pond 32 is also within 500m of pond 17. Survey access to pond 32 was denied in 2018 so therefore suitability/presence of newts could not be determined. In June 2020 an HSI assessment was performed on pond 32 from a distance and showed that the pond had a poor HSI score and was unlikely to harbour GCN.
- 4.1.6 All ponds with GCN confirmed (5, 9, 11 and 12) within the land south of Ashford Road and west of Otterpool Lane (Champneys Farm and Harringe Brooks Wood) are likely to form one metapopulation (*a group of associated populations - a metapopulation is made up from newts which breed in, and live around, a cluster of ponds with some interchange of newts between ponds, even though most adults consistently return to the same pond to breed*) (Langton *et al* 2001). Of these, pond 11 contained the highest peak adult count of eight, with similar numbers of GCN being recorded each time the pond was surveyed. Ponds 3, 4 and 8 are within 500m of the metapopulation but no GCN were found to be present in these ponds. Ponds 6, 10, 41 and 42 were assessed as unsuitable for GCN. Ponds 11 and 12 are within 500m of ponds 13 and 14 located at Upper Otterpool. According to the desk study one male great crested newt was found during surveys in

2007 (CSa, 2015). Both ponds 13 and 14 were surveyed but no GCN presence was confirmed. Otterpool Lane is a B road which presents a likely obstruction to the movement of GCN between the Harringe Brook Wood metapopulation and these ponds, reducing the likelihood of newt migration.

- 4.1.7 Although Pond 31 had been surveyed and GCN were previously found to be absent, in 2021 an incidental record of a GCN within 50m of the pond suggests that it may be colonised in the future.
- 4.1.8 Other amphibian species were also recorded during the surveys including smooth newt, palmate newt, common frog/frog tadpoles and common toad/toad tadpoles. Smooth and palmate newts were found in the majority of ponds surveyed. Tadpoles/ toadpoles, frog and toad tended to be found in the larger more established ponds such as pond 16 and pond 19. Marsh frog, which are non-native and invasive were found within some of the ponds on site.

## 5 Mitigation Recommendations and Further Work

### 5.1 Introduction

- 5.1.1 This section of the report outlines the high level mitigation proposed to provide for the favourable conservation status of Great Crested Newts within and associated with the site being maintained. Full details of the GCN mitigation is provided in ES Appendix 7.18: GCN Mitigation Strategy and will continue to evolve during detailed design. The mitigation hierarchy is applied throughout (i.e. avoid, mitigate, compensate, enhance).
- 5.1.2 In the absence of mitigation, the proposals would contravene associated wildlife legislation and therefore an EPS mitigation license will be required to permit the development. Further details of the licencing procedure appropriate for each phase of the development will need to be secured at Tier 2 and 3 of the planning process.

### 5.2 Design Mitigation

#### Avoidance of impacts to GCN populations (design)

- 5.2.1 In line with the mitigation hierarchy, the first step of the proposed mitigation for impacts to GCN will be avoidance. Within the development, many areas of value for GCN will be retained and enhanced. This section provides a summary of the avoidance approaches with full details in the ES Chapter 7: Biodiversity and ES Appendix 7.18.
- Pond 5, which supported a small population of GCN is to be retained adjacent to the development. This will be immediately surrounded by excellent woodland habitat associated with Harringe Brooks Woods and the surrounding area. In addition, enhancement for GCN around the north and east of the woodland is proposed.
  - Pond 9; which supports a small population of GCN, is to be retained. Connectivity between this pond and the woodland to the south (Harringe Brooks Woods), beyond which lies pond 5 is to be retained. Connectivity to ponds 11 and 12 to the east is also to be retained. As with pond 5, the conservation status of the population associated will be enhanced through the creation of new ponds and habitats around the north and east of Harringe Brooks Woods.
  - Ponds 11 and 12, which support a small GCN population, are to be retained adjacent to the site. Connectivity between these ponds and ponds 5 and 9 to the west will be maintained. Connectivity to Terrestrial habitat to the east will also be enhanced, and new terrestrial habitat will be formed within the SSSI to the east.
  - Pond 15, which supports a medium GCN population will be retained within the development. Habitat to the east adjacent to the East Stour River will be enhanced to provide terrestrial habitat for these species.
  - Pond 17, which supports a low population of GCN is to be retained. Terrestrial habitat to the southeast of the site is to be enhanced.
  - Ponds 22, 23 and 23a; which support a small GCN population is to be retained within the development. The country park south of the castle and retained habitats around these ponds will provide terrestrial habitat for the species associated with these ponds.
- 5.2.2 Only one pond which supports GCN will be directly lost to the development, which is pond 27 located in the east of the site. It was not possible to preserve this pond with sufficient terrestrial habitat to support a GCN population. This pond supports an isolated, small population of GCN therefore an alternative mitigation approach to retention was deemed more appropriate.

5.2.3 There will however be a loss of terrestrial habitat associated with the ponds and additional mitigation will be required to safeguard GCN populations.

5.2.4 A summary of the impacts to GCN populations on and around the site as a result of the proposed development is shown in Table 9 below.

Table 9: Summary of impacts to GCN populations on the site

GCN population	Impacts to ponds and mitigation	Impacts to terrestrial habitats and Mitigation
Small population associated with Pond 5	No direct impacts Fragmentation from pond 9	Terrestrial habitat loss >50m from the pond
	Mitigated via tunnel creation and new pond creation around Harringe Brooks Wood.	Mitigation will be in the form of enhanced habitat around Harringe Brooks Wood.
Small population associated with Pond 9	No direct impacts Fragmentation from ponds 11, 12 and 5	Extensive terrestrial habitats loss
	Mitigated via tunnel creation and new pond creation around Harringe Brooks Wood.	Mitigation will be in the form of habitat creation and enhancement including greater connectivity around Harringe Brooks Wood
Small population associated with ponds 11 and 12	No direct impacts Fragmentation from ponds 5 and 9	Terrestrial habitat loss >50m from the pond
	Mitigated via tunnel creation and new pond creation around Harringe Brooks Wood.	Mitigation will be in the form of habitat creation and enhancement including greater connectivity around Harringe Brooks Wood and within the SSSI east of the ponds (enhanced connectivity across Otterpool Lane)
Pond 15	No direct impacts	Terrestrial habitat loss >50m from the pond
	Additional ponds will be created around the East Stour River corridor, particularly to the north.	Mitigation will be in the form of habitat creation and enhancement around the East Stour River corridor and particularly to the north of pond 15, associated with a SuDS area.
Pond 17	No direct impacts	Terrestrial habitat loss >50m from the pond
	Additional ponds will be created around the East Stour River corridor	Mitigation will be in the form habitat creation and enhancement to the west of Lymyne village
Pond 22	No direct impacts	Some impacts to terrestrial habitats (>50m from the pond).
	Additional ponds will be created around the East Stour River corridor	Mitigation will be in the form habitat creation and enhancement around the East Stour River corridor, and within the park between Westenhanger Castle and the retained racecourse lake.
Pond 23	No direct impacts	Some impacts to terrestrial habitats (>50m from the pond).

GCN population	Impacts to ponds and mitigation	Impacts to terrestrial habitats and Mitigation
	Additional ponds will be created around the East Stour River corridor	Mitigation will be in the form habitat creation and enhancement around the East Stour River corridor, and within the park between Westenhanger Castle and the retained racecourse lake.
Pond 23a	No direct impacts	Some impacts to terrestrial habitats (>50m from the pond).
	Additional ponds will be created around the East Stour River corridor	Mitigation will be in the form habitat creation and enhancement around the East Stour River corridor, and within the park between Westenhanger Castle and the retained racecourse lake.
Pond 27	Pond removed	All terrestrial habitat lost
	Additional ponds will be created around Harringe Brooks Wood and in the north west of the site	Mitigation will be in the form habitat creation and enhancement in the area around Harringe Brooks Wood and in the north west of the site.

## Habitat enhancement and creation

- 5.2.5 Within the development, there will be embedded design measures to make sure that GCN can utilise areas of the site and move through the site. This will include retention and enhancement buffers of rough grassland around retained habitat features including hedgerows and between retained areas of habitats. In addition, SuDS areas, where appropriate, will be designed to provide GCN habitats with the provision of rough grassland, ponds and ephemeral waterbodies and hibernacula.
- 5.2.6 Elsewhere within the site, areas designed specifically to provide habitat for GCN will be created, including a large area (approximately 14ha) in the north west of the site, which will be a dedicated nature area, and will include dedicated enhancement for GCN, including ponds and hibernacula. This is shown in more detail in the mitigation strategy (ES Appendix 7.18).
- 5.2.7 An area of terrestrial habitat enhancement will also be located adjacent to Harringe Brooks woods, which will contain ponds and terrestrial habitats.
- 5.2.8 In order to enhance the connectivity between new and retained ponds on the site, tunnels for GCN will be created beneath roads where key connectivity is identified.
- 5.2.9 The parcels of the development will also be designed to safeguard GCN, with permeable garden barriers (hedges) where appropriate and offset gully pots, where practicable.
- 5.2.10 It is likely that there will need to be a suite of enhancement conducted to make sure that areas identified for GCN mitigation and compensation are created prior to certain construction milestones within the development phasing. Details of the proposed management of all created and retained habitats is also likely to be required.

## 5.3 Construction Avoidance and Mitigation

- 5.3.1 A Code of Construction Practice (CoCP) (ES Appendix 4.2) will be produced to include best practice construction mitigation. This will avoid and/or reduce impacts to areas that are not within the development area which will reduce direct mortality. It will also layout the areas which would operate under an EPS licence and which areas require trapping and

translocation. A BAP (Biodiversity Action Plan) will also outline how GCN will be safeguarded during the construction process.

## 5.4 Additional Mitigation

5.4.1 In addition to the design and construction mitigation above, during detailed design and construction of the development, it is likely that additional actions may be required to safeguard GCN. These actions may include:

- Habitat creation plans to be evolved with the detailed design and tiers of the development (i.e. outlining the habitats within the development parcels) to create and enhance habitats;
- Habitat manipulation to displace great crested newts into retained habitats adjacent to habitats to be removed;
- Tool box talks to be created and provided to on site staff to inform them of the protected status of GCN;
- Licensed capture and translocation of GCN from areas to be lost into retained/enhanced habitats may be required, this will need to be determined in liaison with Natural England. There is potential that a small number of GCN may be moved from the pond to be lost to the newly created area in the north west, to 'seed' this area with a population of GCN, which will have connectivity to the metapopulation in the west of the site (around pond 5, 9,11 and 12).

## 5.5 Requirement for Licensing

5.5.1 The requirement and approach to licensing is to be determined with Natural England. Multiple approaches may be undertaken, these are outlined broadly below, it is beyond the scope of this document to fully outline these approaches and when they may be appropriate.

- Traditional licensed methods (involving trapping and translocation) from GCN from development areas;
- A 'site wide licence' the appropriateness of this will depend upon the buildout timescales;
- Licensing using the 'new licensing policies' which may not require any trapping and translocation, but necessitate an increased area of enhancement;
- Licensing using a District Level Licensing approach.

5.5.2 Alternatively, a combination of these three approaches may be used across the multiple parcels of the development.

## 5.6 Operational Mitigation

### Safeguarding habitats

5.6.1 In order to minimise operational impacts to retained and enhanced GCN populations, likely to be predominantly through human disturbance and impacts from domestic animals, the following approaches would be implemented:

- Green infrastructure would be designed to limit human accessibility to the most sensitive areas;
- Buffers will be created and maintained around retained and created GCN areas to limit impacts from humans and domestic animals;
- Newly created habitats, particularly the area in the north west will be positioned away from development where possible to minimise impacts from humans.

## **Maintenance and monitoring**

- 5.6.2 Maintenance and monitoring will be required of any retained or created habitats. An outline of the desired outcomes for the monitoring and maintenance is provided within a site BAP (ES Appendix 7.20). As each planning tier progresses, detailed strategies will be required for creation, management and maintenance of the habitats created (this is beyond the remit of this document).
- 5.6.3 A broad outline of the locations of proposed habitat creation is provided within the mitigation strategies (ES Appendix 7.18).

## **5.7 Further Survey**

- 5.7.1 The requirement for further survey at later stages of the planning process will be determined by the details of the planning tiers in relation to the development, and the mitigation approach determined for each tier. If an individual licence approach (or site wide licence) is determined to be the most appropriate mitigation strategy for a given parcel, updated population surveys may be required but should be considered in line with NE's relatively new planning policy implementation approach which allows more holistic decisions to be undertaken.

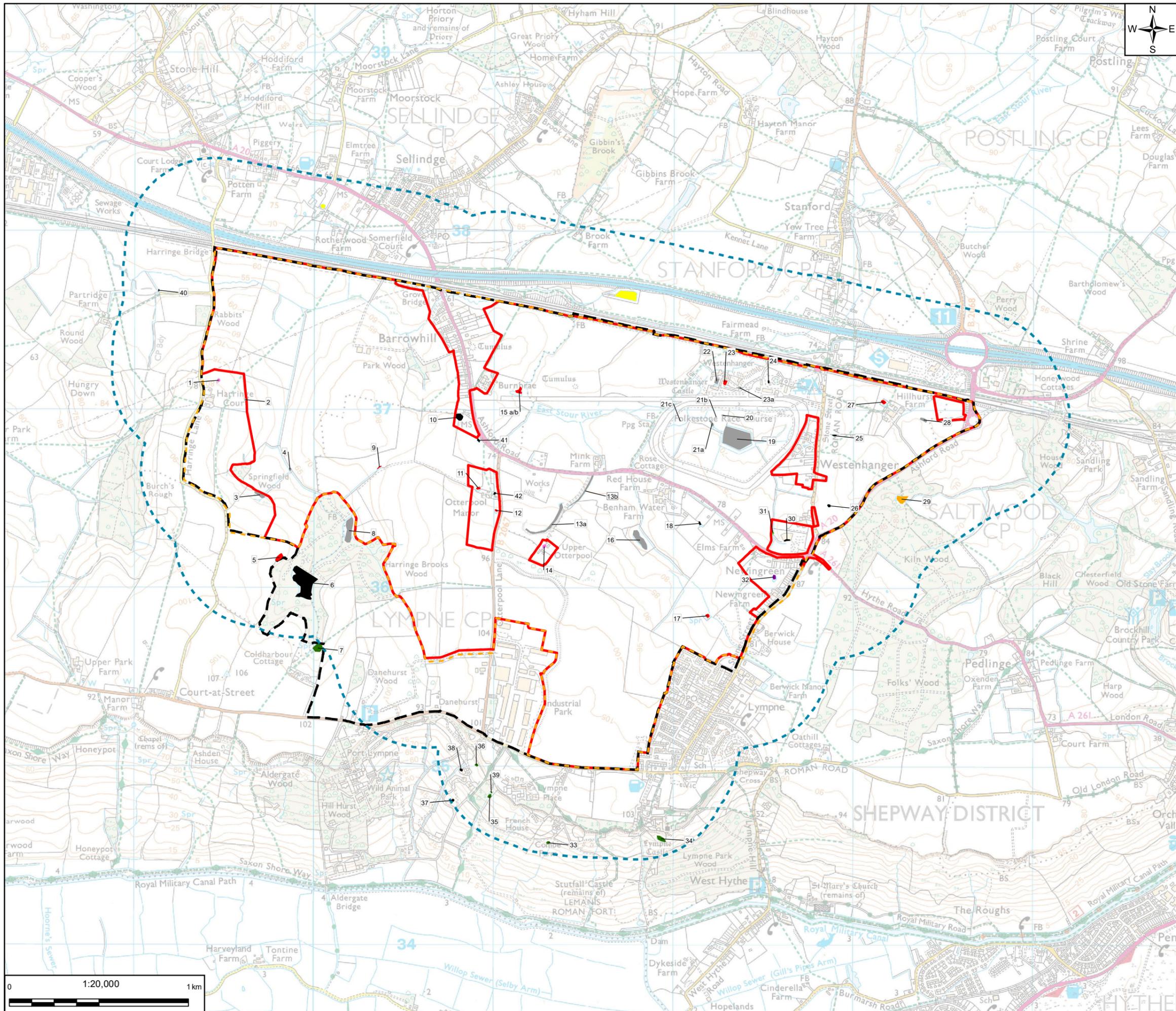
## 6 Conclusions

- 6.1.1 Habitat Suitability Index (HSI) assessments and targeted great crested newt surveys were undertaken between March and May 2017. A total of 21 ponds were surveyed based on previous Habitat Suitability Index Scores. In eight of these ponds GCN presence was confirmed, with one pond (pond 15) containing a medium great crested newt population and the remaining ponds containing a low great crested newt population according to Natural England Guidelines. To update the validity of the survey in April-May 2020, Habitat Suitability Index (HSI) assessments were undertaken for the 17 ponds that were accessible at the time of the surveys and eDNA surveys were undertaken where applicable. One additional pond (pond 23a) and one pond that previously did not support GCN (pond 22) were found to contain GCN based on the results of the eDNA surveys. HSI assessments of two further ponds (ponds 43 and 44) and an additional eDNA survey of one of these ponds was undertaken in 2021, which returned a negative result.
- 6.1.2 Pond 15 (Barrow Hill Farm) is likely to be an isolated population. Ponds 22, 23 and 23a (Westenhanger Castle), 27 (Hillhurst Farm) and 17 (north of Folks Wood Way) are also likely to be isolated metapopulations. These are likely to be remnant populations due to isolation and/or degrading pond conditions.
- 6.1.3 The small GCN populations at ponds 5, 9, 11 and 12 are likely to form a metapopulation. Although some of these ponds are located 500m away from one another, there is good connectivity between them with minimal barriers to dispersal. This supports previous survey results in 2011 which suggested that Harringe Brooks Wood and its surrounding environment supports a population of great crested newts.
- 6.1.4 Other amphibian species were also recorded in the majority of the ponds on site including smooth newt, palmate newt, common frog/frog tadpoles and common toad/toad tadpoles. The non-native invasive species marsh frog was also found within the site. The valuation and mitigation for these features is presented in the ES Chapter 7: Biodiversity.
- 6.1.5 Within the development, many areas of value for great crested newts will be retained and enhanced. Only one breeding pond is to be lost within the development.

## 7 References

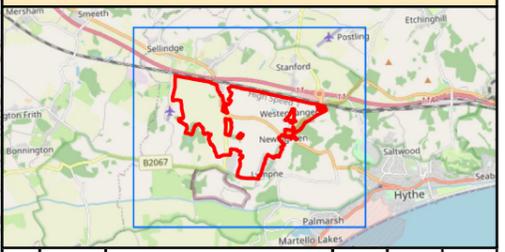
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Ref 1	Anon (2000) Countryside and Rights of Way Act. HMSO, London, [online] ( <a href="http://www.legislation.gov.uk/ukpga/2000/37/introduction">http://www.legislation.gov.uk/ukpga/2000/37/introduction</a> )
Ref 2	Anon (2017) The Conservation of Habitats and Species Regulations. HMSO, London, [online] ( <a href="http://www.legislation.gov.uk/uksi/2017/1012/contents/made">http://www.legislation.gov.uk/uksi/2017/1012/contents/made</a> ).
Ref 3	Anon (2013) Third Report by the United Kingdom under Article 17 on the implementation of the Directive: S1166 - Great crested newt ( <i>Triturus cristatus</i> ). European Community Directive on the Conservation of Natural Habitats and of Wild Fauna and Flora, UK.
Ref 4	Anon (Accessed 2017) Great crested newts: surveys and mitigation for development projects. Natural England, London,[online]( <a href="https://www.gov.uk/guidance/great-crested-newts-surveys-and-mitigation-for-development-projects">https://www.gov.uk/guidance/great-crested-newts-surveys-and-mitigation-for-development-projects</a> )
Ref 5	Bramley Associates 2016, An Ecological Scoping Report for Holiday Extras, Ashford Road, Newingreen, Kent
Ref 6	CIEEM (2013). Guidelines for Preliminary Ecological Appraisal. Chartered Institute of Ecology and Environmental Management, UK.
Ref 7	CIEEM (2016). Guidelines for Ecological Impact Assessment in the UK and Ireland. Chartered Institute of Ecology and Environmental Management, UK.
Ref 8	CIEEM (2019) Ecology surveying and COVID 19 available at <a href="#">Coronavirus (COVID-19)   CIEEM</a>
Ref 9	Hanski, I. (1998). Metapopulation dynamics. <i>Nature</i> 396: 41–49.
Ref 10	Joint Nature Conservation Committee (no date): Great crested newt species. [online] Available at: Great crested newt ( <i>Triturus cristatus</i> ) - Special Areas of Conservation ( <a href="http://jncc.gov.uk">jncc.gov.uk</a> ) [Accessed June 2017].
Ref 11	Langton, T., Beckett, C., Foster, J. (2001). Great Crested Newt Conservation Handbook. Froglife, Suffolk.
Ref 12	Mckinnell, J. (2012). Great Crested Newt: Species Management in Scotland. Species Action Framework, UK.
Ref 13	Natural England (2001) Great crested newt mitigation guidelines ISBN 1 85716 568 3
Ref 14	ODPM (2005) <a href="#">odpm-circ-0605.qxd (publishing.service.gov.uk)</a>

**Figure 1: Pond Scoping survey and overview 2017 – 2018**



- Legend**
- Outline Planning Application Boundary
  - Framework Masterplan Boundary
  - Study Area
  - 500 m Buffer (ZoI)
  - Pond Surveyed 2017 GCN Present
  - Pond Surveyed 2017 No GCN Present
  - Pond Not Surveyed (isolated from site / over 500m from site)
  - No GCN Present (2016 eDNA)
  - No GCN Present (2018 eDNA)
  - eDNA Result Inconclusive (2018 eDNA)
  - Pond Not Fully Surveyed (dried / not considered / No GCN present)
  - Pond Not Surveyed (Access Denied)
  - Pond Not Surveyed (HSI result was unsuitable / pond was dry)

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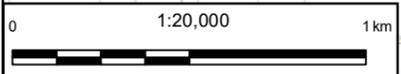
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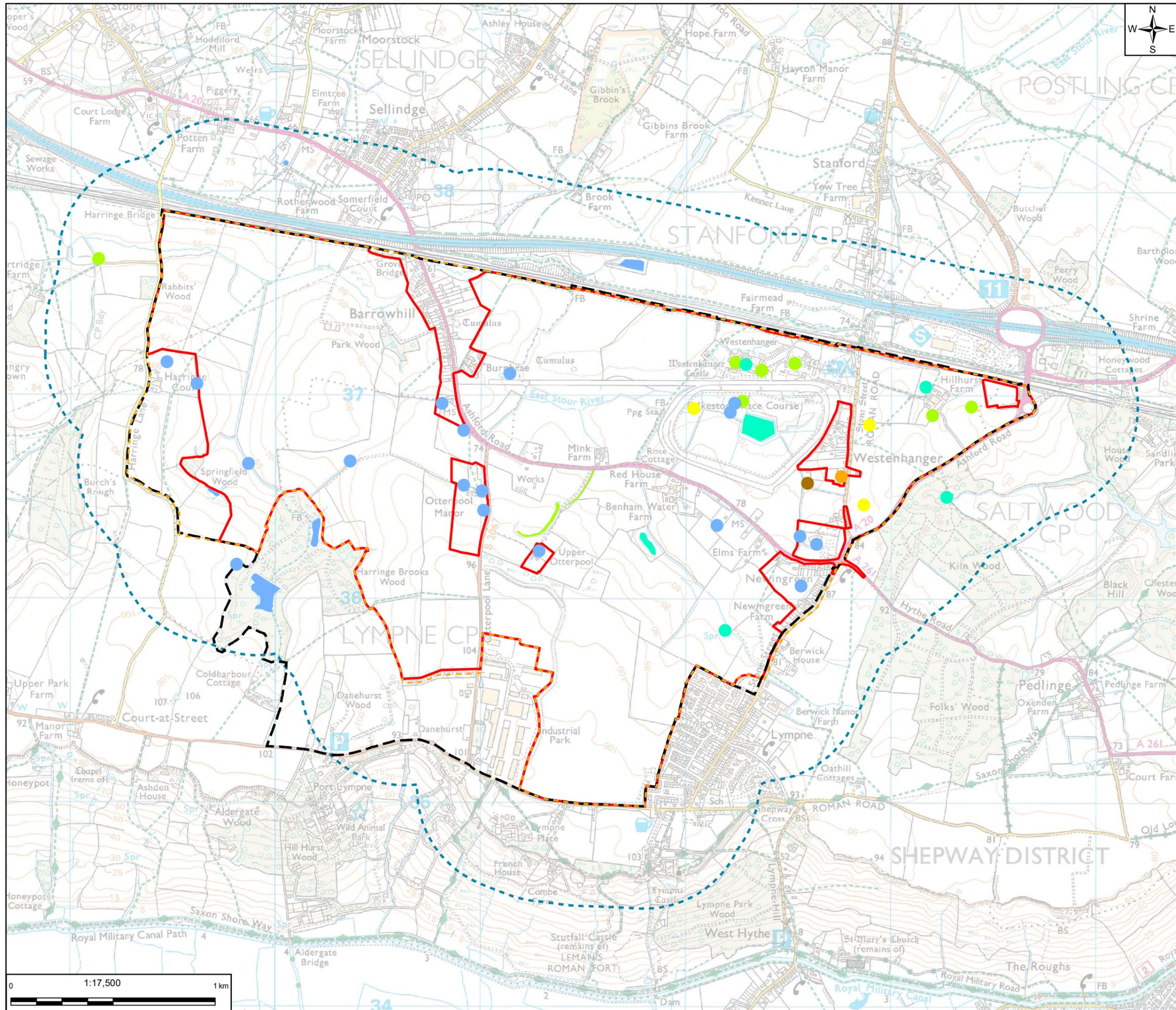
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**Figure 1**  
Pond Scoping and Survey Overview 2017 and 2018

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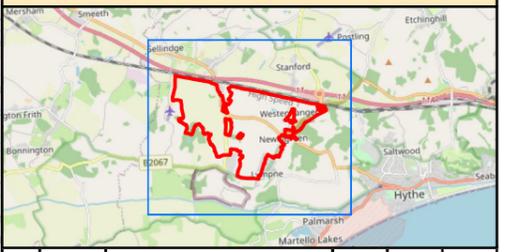


**Figure 2: Pond Scoping survey and overview 2020 – 2021**



- Legend**
- Outline Planning Application Boundary
  - Framework Masterplan Boundary
  - Study Area
  - 500 m Buffer (Zol)
  - Ponds Not Surveyed in 2020 (due to access limitations)
  - Ponds Surveyed in 2020 (HSI)
  - Ponds Surveyed in 2020 (HSI and eDNA)
  - Ponds Dry in 2020
  - Ponds Surveyed in 2018
  - Pond surveyed in 2021 (HSI and eDNA)
  - Pond surveyed in 2021 (HSI)

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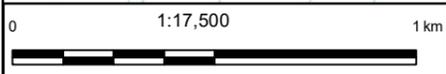
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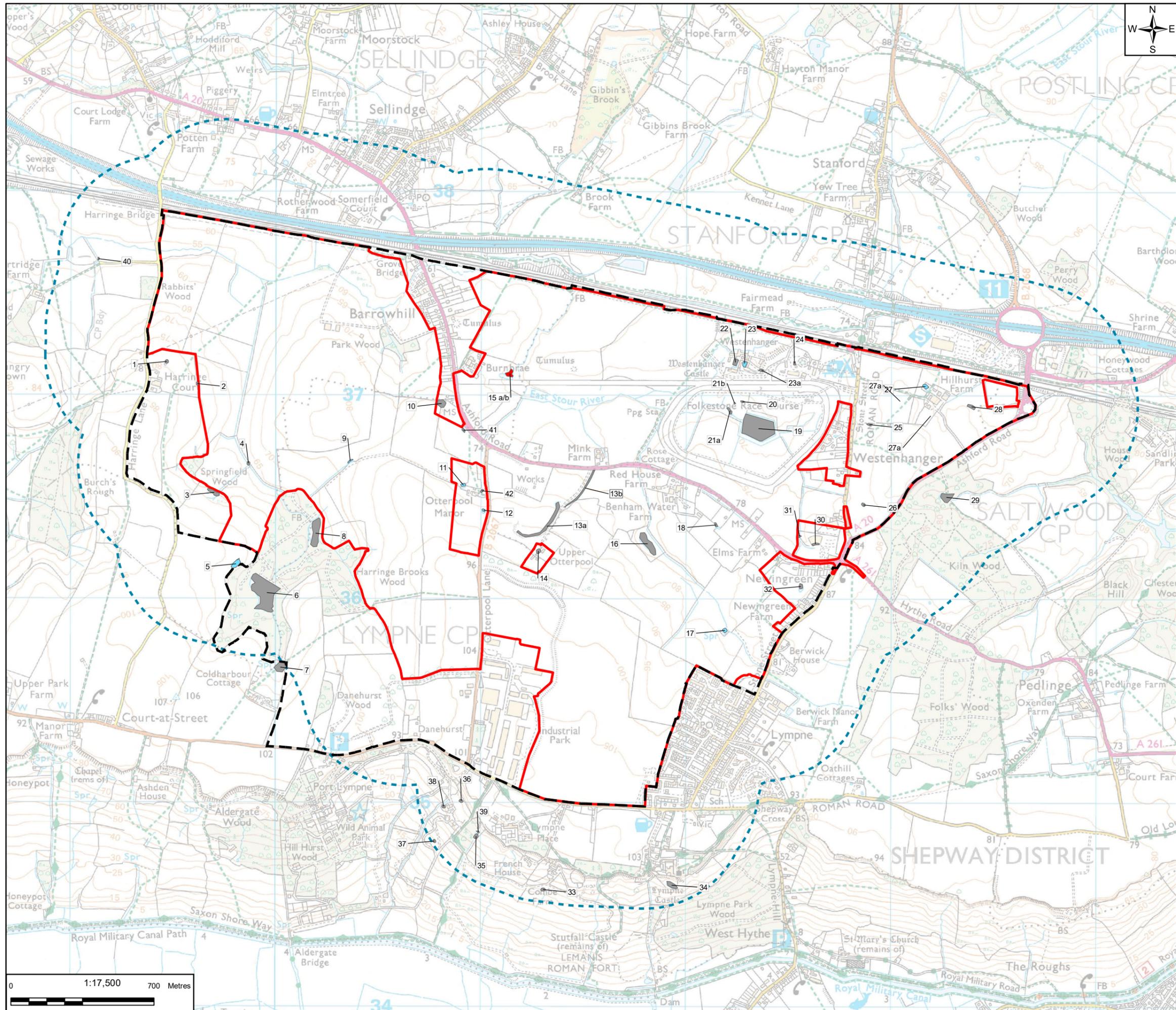
  
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**Figure 2**  
**Pond Scoping Survey and Overview 2020 and 2021**

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### **Figure 3: Overview of 2017 / 2018 GCN survey results**



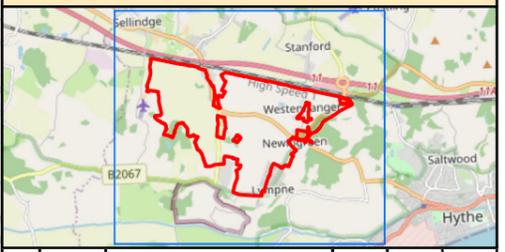
**Legend**

- Outline Planning Application Boundary
- Framework Masterplan Boundary
- 500 m Buffer (Zol)

**Great Crested Newt Population Size**

- Medium
- Low
- Absent

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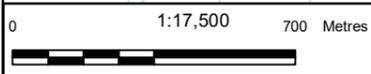
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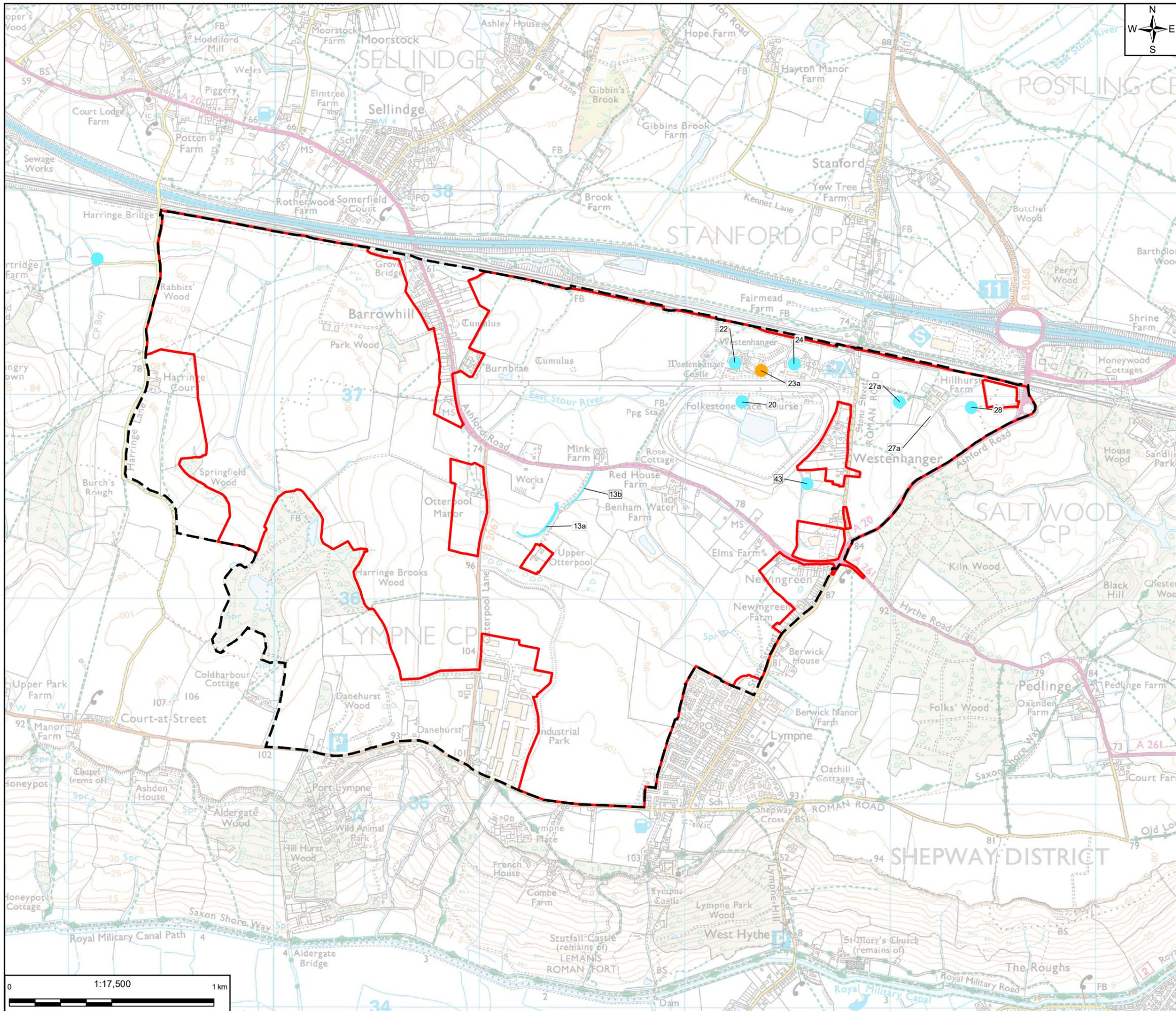
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**Figure 3**  
Overview of 2017 and 2018 GCN Survey Results



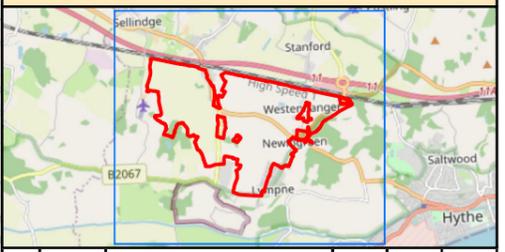
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## Figure 4: 2020 and 2021 eDNA survey results



- Legend**
- Outline Planning Application Boundary
  - Framework Masterplan Boundary
  - Negative
  - Positive

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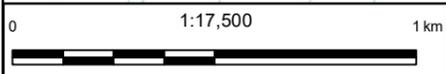


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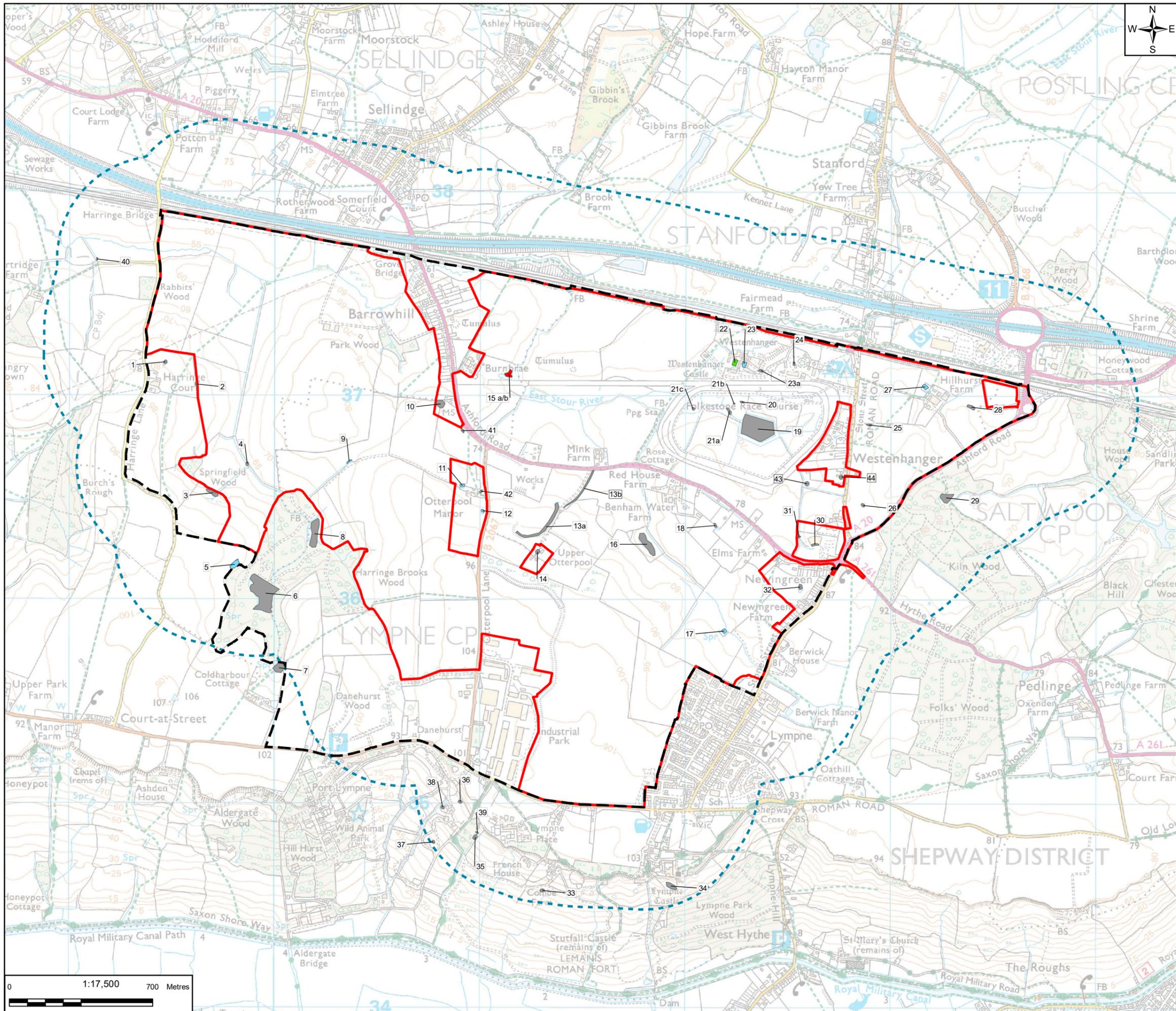
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**Figure 4**  
 2020 and 2021 eDNA results



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## Figure 5: Overview of GCN survey results 2017 – 2021



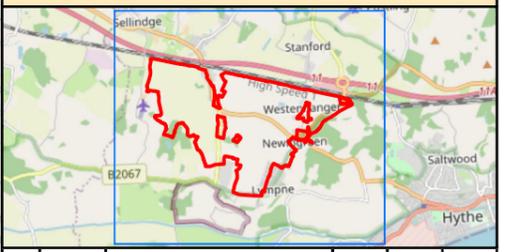
**Legend**

- Outline Planning Application Boundary
- Framework Masterplan Boundary
- 500 m Buffer (Zol)

**Great Crested Newt Population Size**

- Present (eDNA)
- Medium
- Low
- Absent

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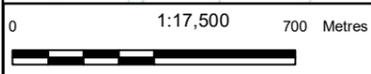
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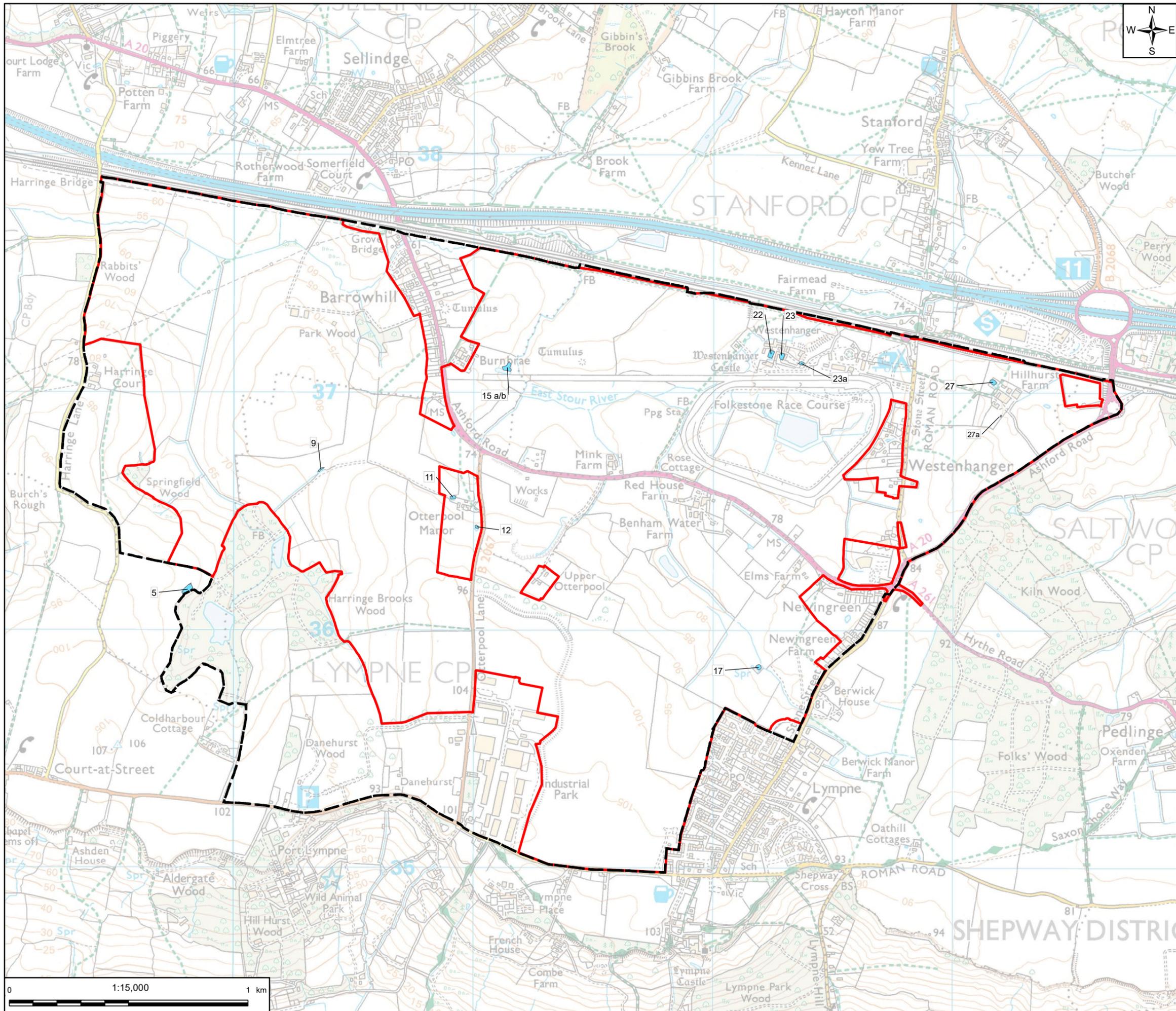
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**Figure 5**  
Overview of GCN  
Survey Results 2017-2021

scale	original size	datum	grid
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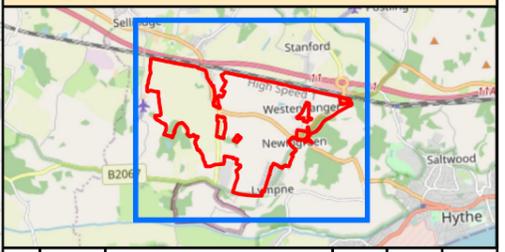


**Figure 6: Overview of Ponds with GCN Presence (2017 – 2021)**



- Legend**
- Outline Planning Application Boundary
  - Framework Masterplan Boundary
  - Ponds
  - 250m Radius Buffer

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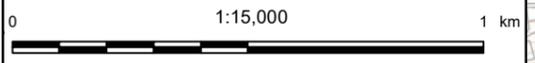
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**Figure 6**  
**Overview of Ponds with**  
**GCN Presence (2017 - 2021)**

scale	original size	datum	grid
1:15,000	A3	Sx	BNG



## APPENDIX A: HSI Results 2017, 2018, 2020 and 2021

Table 10: HSI results for surveyed ponds in 2017 / 2018 (part 1)

		Pond 1	Pond 2	Pond 3	Pond 4	Pond 5	Pond 6	Pond 7	Pond 8	Pond 9	Pond 10	Pond 11	Pond 12	Pond 13	Pond 14	Pond 15a	Pond 15b	Pond 16	Pond 17	Pond 18	Pond 19	Pond 20
SI No	SI Description / Notes	SI Value	Pond dried prior to survey	SI Value	Pond 15a and B largely one waterbody so treated as a single pond	SI Value																
1	Geographic location	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
2	Pond area	0.4	0.05	0.85	0.4	0.8	0.8	0.85	0.6	0.8	0.05	0.6	0.4	0.8	0.8	0.9	0.1	0.8	0.8	0.1	0.85	0.1
3	Pond permanence	0.5	0.1	0.1	0.5	0.1	0.9	0.9	0.5	1	0.1	0.9	0.5	0.1	0.1	0.1	0.1	0.9	0.9	0.1	0.9	0.5
4	Water quality	0.33	0.33	0.33	0.67	0.33	0.67	0.67	0.67	0.67	0.33	0.67	0.67	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.67	0.67
5	Shade	1	1	1	1	0.3	0.5	1	1	1	0.6	1	1	1	1	1	1	1	1	0.4	1	1
6	Water fowl effect	1	1	0.67	1	1	0.67	0.67	0.67	0.67	1	1	1	1	1	1	1	0.67	1	1	0.67	0.67
7	Fish presence	0.67	1	1	1	1	0.01	0.67	1	0.67	1	1	1	1	0.67	0.67	0.67	0.01	0.67	1	0.33	1
8	Pond Density	0.8	0.8	0.85	0.85	0.85	0.85	0.85	0.85	0.95	0.9	0.95	0.95	0.95	0.95	0.9	0.9	1	0.85	0.95	1	1
9	Terrestrial habitat	0.33	0.33	0.33	0.33	1	1	1	1	0.33	0.67	0.67	0.67	0.33	0.33	0.33	0.33	0.33	0.33	0.67	1	1
10	Macrophyte cover	0.3	0.8	0.9	0.7	0.35	0.35	0.35	0.3	0.6	0.9	0.8	0.8	0.85	0.8	1	1	0.35	0.8	0.35	0.35	0.95
HSI Score		0.57	0.45	0.59	0.70	0.55	0.47	0.76	0.71	0.73	0.47	0.84	0.76	0.61	0.58	0.60	0.48	0.42	0.72	0.44	0.72	0.68

Otterpool Park  
 ES Appendix 7.9: Great Crested Newt Survey Report  
 Table 11: HSI results for surveyed ponds in 2017 / 2018 (part 2)

		Pond 21a	Pond 21b	Pond 22	Pond 23	Pond 23a	Pond 24	Pond 25	Pond 26	Pond 27	Pond 28	Pond 29	Pond 30	Pond 31	Pond 32	Pond 33	Pond 34	Pond 35	Pond 36	Pond 37	Pond 38	Pond 40	Pond 41	Pond 42 (garden)
SI No	SI Description / notes	SI Value	SI Value	SI Value	SI Value	Dried out	Ornamental Pond Brick Sides	Pond Dry	Pond Dry	SI Value	SI Value	SI Value	Ornamental Pond, no access for GCN	eDNA information provided by Landowner	Access Denied	SI Value	SI Value	SI Value	SI Value	Concrete bear pond	Concrete bear pond	SI Value	SI Value	SI Value
1	Geographic location	1	1	1	1	1	1	N/A	N/A	1	1	1	N/A	N/A	N/A	1	1	1	1	N/A	N/A	1	1	1
2	Pond area	0.8	0.85	0.95	1	1	0.4	N/A	N/A	1	0.4	0.8	N/A	N/A	N/A	0.6	0.6	0.4	0.2	N/A	N/A	0.05	0.1	0.05
3	Pond permanence	0.1	0.5	0.5	0.5	0.1	0.9	N/A	N/A	1	0.1	0.9	N/A	N/A	N/A	0.9	0.9	0.1	0.5	N/A	N/A	0.1	0.1	0.9
4	Water quality	0.33	0.67	0.67	1	0.67	0.33	N/A	N/A	0.67	0.67	0.33	N/A	N/A	N/A	0.01	0.67	0.01	0.33	N/A	N/A	0.33	0.33	0.33
5	Shade	1	1	0.5	1	0.8	1	N/A	N/A	1	0.6	0.2	N/A	N/A	N/A	1	1	0.2	1	N/A	N/A	1	0.8	1
6	Water fowl effect	1	0.67	1	1	1	1	N/A	N/A	1	1	0.67	N/A	N/A	N/A	0.01	0.67	1	1	N/A	N/A	1	1	1
7	Fish presence	1	1	1	1	1	0.01	N/A	N/A	1	1	0.33	N/A	N/A	N/A	0.67	0.67	1	1	N/A	N/A	1	1	0.01
8	Pond Density	1	1	1	1	1	1	N/A	N/A	1	1	0.6	N/A	N/A	N/A	0.9	0.7	0.9	0.9	N/A	N/A	0.8	0.9	0.95
9	Terrestrial habitat	1	1	1	1	1	0.33	N/A	N/A	0.67	0.67	1	N/A	N/A	N/A	1	1	1	1	N/A	N/A	0.33	0.33	0.67
10	Macrophyte cover	0.85	0.9	0.3	0.9	0.4	0.8	N/A	N/A	1	0.8	0.3	N/A	N/A	N/A	0.35	0.35	0.3	0.3	N/A	N/A	0.85	0.3	0.5
	HSI Score	0.68	0.84	0.74	0.92	0.68	0.45	N/A	N/A	0.92	0.62	0.53	N/A	N/A	N/A	0.32	0.72	0.34	0.62	N/A	N/A	0.45	0.43	0.37

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 Table 12 HSI results for ponds surveyed in 2020

		Pond 13a + 13b	Pond 16	Pond 17	Pond 19	Pond 20	Pond 21c	Pond 22	Pond 23	Pond 23a	Pond 24	Pond 25	Pond 26	Pond 27	Pond 27a	Pond 28	Pond 29	Pond 31	Pond 40
SI No	SI Description / Notes	SI Value	SI Value	SI Value	SI Value	SI Value	Pond dry	SI Value	SI Value	SI Value	SI Value	Pond dry	Pond dry	SI Value					
1	Geographic location	1	1	1	1	1	N/A	1	1	1	1	N/A	N/A	1	1	1	1	1	1
2	Pond area	0.9	0.8	0.7	0.85	0.05	N/A	0.5	0.6	0.1	0.3	N/A	N/A	0.1	0.1	0.2	0.7	0.05	0.2
3	Pond permanence	0.9	0.9	0.9	0.9	0.1	N/A	0.9	0.1	0.1	0.9	N/A	N/A	1	0.1	0.1	0.9	0.9	0.1
4	Water quality	1	0.67	0.33	0.67	0.67	N/A	0.67	0.67	0.67	0.67	N/A	N/A	0.67	0.33	0.33	0.01	0.33	0.33
5	Shade	1	1	1	0.7	1	N/A	0.2	1	1	1	N/A	N/A	1	1	0.7	0.2	1	0.3
6	Water fowl effect	1	0.67	1	0.67	1	N/A	1	1	1	1	N/A	N/A	1	1	1	1	0.67	1
7	Fish presence	0.67	0.01	0.67	0.01	1	N/A	0.67	1	1	0.33	N/A	N/A	1	1	1	0.67	0.3	1
8	Pond Density	0.95	1	0.85	1	1	N/A	1	1	1	1	N/A	N/A	1	1	1	0.6	0.75	0.8
9	Terrestrial habitat	0.67	0.67	0.67	1	1	N/A	0.67	0.67	0.67	0.33	N/A	N/A	0.67	0.67	0.33	1	0.67	1
10	Macrophyte cover	0.9	0.35	0.45	0.35	1	N/A	0.3	1	0.9	0.45	N/A	N/A	0.55	1	0.5	0.3	0.4	1
HSI Score		0.89	0.49	0.72	0.49	0.57	N/A	0.62	0.70	0.58	0.62	N/A	N/A	N/A	0.54	0.49	0.42	0.48	0.52

Table 13 HSI results for ponds surveyed in 2021

		Pond 43	Pond 44
SI No	SI Description / Notes	SI Value	SI Value
1	Geographic location	1	1
2	Pond area	0.05	0.05
3	Pond permanence	1	0.9
4	Water quality	0.67	0.67
5	Shade	0.7	1
6	Water fowl effect	1	1
7	Fish presence	0.67	0.01
8	Pond Density	6	6
9	Terrestrial habitat	0	0.67
10	Macrophyte cover	0.9	0.65
HSI Score		0.64	0.40

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## **APPENDIX B: Pond survey results 2017**





































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**Waterbody 22 - HSI = 0.74 (Good)**

Species	Date	11/04/2017				19/04/2017				03/05/2017				11/05/2017			
	Survey Method	ES	T	BT	N												
Number of bottles				15								14				10	
	Sex / age class																
Great crested newt	♂																
	♀																
Smooth newt	Juvenile																
	♂																
Palmate newt	♀			1													
	♂																
Smooth / palmate newt	♀																
	Gender unknown																
	Juvenile																
EFT																	
Common toad /toad-pole																	
Common frog / tadpole																	
Fish seen?																	

Too cold to trap







## APPENDIX C: Pond surveying conditions 2017

Pond 1			
Brandon Murray (2015-17257-CLS-CLS) Ellen Poppleton			
(1) Date:	Air temp	Veg cover	Turbidity
10/04/2017	11	0	4
(2) Date:	Air temp	Veg cover	Turbidity
18/04/2017	7	0	4
Pond 3			
Brandon Murray (2015-17257-CLS-CLS) Ellen Poppleton			
(1) Date:	Air temp	Veg cover	Turbidity
10/04/2017	8	4	2
(2) Date:	Air temp	Veg cover	Turbidity
18/04/2017	7	4	2
Aline Brodzinski (2015-19142-CLS-CLS) Ellen Poppleton			
(3) Date:	Air temp	Veg cover	Turbidity
02/05/2017	9	4	1
Pond 4			
Brandon Murray (2015-17257-CLS-CLS) Ellen Poppleton			
(1) Date:	Air temp	Veg cover	Turbidity
10/04/2017	8	1	3
(2) Date:	Air temp	Veg cover	Turbidity
18/04/2017	7	1	3
Aline Brodzinski (2015-19142-CLS-CLS) Ellen Poppleton			
(3) Date:	Air temp	Veg cover	Turbidity
02/05/2017	9	1	2
(4) Date:	Air temp	Veg cover	Turbidity

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09/05/2017	7	2	1
<b>Pond 5</b>			
Brandon Murray (2015-17257-CLS-CLS) Ellen Poppleton			
(1) Date:	Air temp	Veg cover	Turbidity
10/04/2018	8	0	1
(2) Date:	Air temp	Veg cover	Turbidity
18/04/2017	7	1	1
Aline Brodzinski (2015-19142-CLS-CLS) Ellen Poppleton			
(3) Date:	Air temp	Veg cover	Turbidity
02/05/2017	12	1	1
(4) Date:	Air temp	Veg cover	Turbidity
08/05/2017	11	0	1
Brandon Murray (2015-17257-CLS-CLS) Ewan Gibson			
(5) Date:	Air temp	Veg cover	Turbidity
15/05/2017	8	4	2
(6) Date:	Air temp	Veg cover	Turbidity
31/05/2017	9	4	1
<b>Pond 8</b>			
Brandon Murray (2015-17257-CLS-CLS) Ellen Poppleton			
(1) Date:	Air temp	Veg cover	Turbidity
10/04/2017	11	0	1
(2) Date:	Air temp	Veg cover	Turbidity
18/04/2017	7	0	1
Aline Brodzinski (2015-19142-CLS-CLS) Ellen Poppleton			
(3) Date:	Air temp	Veg cover	Turbidity
02/05/2017	12	1	1
(4) Date:	Air temp	Veg cover	Turbidity
08/09/2017	11	0	1

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<b>Pond 9</b>			
Brandon Murray (2015-17257-CLS-CLS) Ellen Poppleton			
(1) Date:	Air temp	Veg cover	Turbidity
10/04/2017	11	3	3
(2) Date:	Air temp	Veg cover	Turbidity
18/04/2017	7	3	3
Aline Brodzinski (2015-19142-CLS-CLS) Ellen Poppleton			
(3) Date:	Air temp	Veg cover	Turbidity
02/05/2017	12	2	2
(4) Date:	Air temp	Veg cover	Turbidity
08/05/2017	11	2	1
Brandon Murray (2015-17257-CLS-CLS) Ewan Gibson			
(5) Date:	Air temp	Veg cover	Turbidity
16/05/2017	14	2	2
(6) Date:	Air temp	Veg cover	Turbidity
31/05/2017	13	2	2
<b>Pond 11</b>			
Brandon Murray (2015-17257-CLS-CLS) Ellen Poppleton			
(1) Date:	Air temp	Veg cover	Turbidity
10/04/2017	12	3	2
(2) Date:	Air temp	Veg cover	Turbidity
18/04/2017	7	3	2
Aline Brodzinski (2015-19142-CLS-CLS) Ellen Poppleton			
(3) Date:	Air temp	Veg cover	Turbidity
02/05/2017	13	3	1
(4) Date:	Air temp	Veg cover	Turbidity
08/05/2017	11	2	1
Brandon Murray (2015-17257-CLS-CLS) Ewan Gibson			

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(5) Date:	Air temp	Veg cover	Turbidity
15/05/2017	15	2	1
(6) Date:	Air temp	Veg cover	Turbidity
31/05/2017	13	2	1
<b>Pond 12</b>			
Brandon Murray (2015-17257-CLS-CLS) Ellen Poppleton			
(1) Date:	Air temp	Veg cover	Turbidity
10/04/2017	11	5	3
(2) Date:	Air temp	Veg cover	Turbidity
18/04/2017	7	5	3
Aline Brodzinski (2015-19142-CLS-CLS) Ellen Poppleton			
(3) Date:	Air temp	Veg cover	Turbidity
02/05/2017	12	4	2
(4) Date:	Air temp	Veg cover	Turbidity
08/05/2017	11	4	1
Brandon Murray (2015-17257-CLS-CLS) Ewan Gibson			
(5) Date:	Air temp	Veg cover	Turbidity
15/05/2017	15	4	1
(6) Date:	Air temp	Veg cover	Turbidity
31/05/2017	13	4	1
<b>Pond 13a</b>			
Brandon Murray (2015-17257-CLS-CLS) Ellen Poppleton			
(1) Date:	Air temp	Veg cover	Turbidity
12/04/2017	13	4	1
(2) Date:	Air temp	Veg cover	Turbidity
20/04/2017	9	4	2
Aline Brodzinski (2015-19142-CLS-CLS) Ellen Poppleton			
(3) Date:	Air temp	Veg cover	Turbidity

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04/05/2017	12	4	1
(4) Date:	Air temp	Veg cover	Turbidity
10/05/2017	12	4	1
<b>Pond 13b</b>			
Brandon Murray (2015-17257-CLS-CLS) Ellen Poppleton			
(1) Date:	Air temp	Veg cover	Turbidity
12/04/2017	13	3	1
(2) Date:	Air temp	Veg cover	Turbidity
20/04/2017	11	3	1
Aline Brodzinski (2015-19142-CLS-CLS) Ellen Poppleton			
(3) Date:	Air temp	Veg cover	Turbidity
03/05/2017	11	3	1
(4) Date:	Air temp	Veg cover	Turbidity
10/05/2017	11	3	1
<b>Pond 14</b>			
Brandon Murray (2015-17257-CLS-CLS) Ellen Poppleton			
(1) Date:	Air temp	Veg cover	Turbidity
12/04/2017	13	5	1
<b>Pond 15</b>			
Brandon Murray (2015-17257-CLS-CLS) Ellen Poppleton			
(1) Date:	Air temp	Veg cover	Turbidity
11/04/2017	10	2	2
(2) Date:	Air temp	Veg cover	Turbidity
19/04/2017	6	2	2
Aline Brodzinski (2015-19142-CLS-CLS) Ellen Poppleton			
(3) Date:	Air temp	Veg cover	Turbidity
03/05/2017	11	1	3
(4) Date:	Air temp	Veg cover	Turbidity

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09/05/2017	7	1	4
Brandon Murray (2015-17257-CLS-CLS) Ewan Gibson			
(5) Date:	Air temp	Veg cover	Turbidity
15/05/2017	14	1	3
(6) Date:	Air temp	Veg cover	Turbidity
31/05/2017	13	1	3
<b>Pond 16</b>			
Brandon Murray (2015-17257-CLS-CLS) Ellen Poppleton			
(1) Date:	Air temp	Veg cover	Turbidity
12/04/2017	13	0	4
(2) Date:	Air temp	Veg cover	Turbidity
20/04/2017	11	1	4
Aline Brodzinski (2015-19142-CLS-CLS) Ellen Poppleton			
(3) Date:	Air temp	Veg cover	Turbidity
04/05/2017	12	1	4
(4) Date:	Air temp	Veg cover	Turbidity
10/05/2017	12	1	4
<b>Pond 17</b>			
Brandon Murray () Ellen Poppleton			
(1) Date:	Air temp	Veg cover	Turbidity
12/04/2017	13	3	3
(2) Date:	Air temp	Veg cover	Turbidity
20/04/2017	11	4	1
Aline Brodzinski (2015-19142-CLS-CLS) Ellen Poppleton			
(3) Date:	Air temp	Veg cover	Turbidity
04/05/2017	12	4	1
(4) Date:	Air temp	Veg cover	Turbidity
10/05/2017	12	4	1

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Brandon Murray (2015-17257-CLS-CLS) Ewan Gibson			
(5) Date:	Air temp	Veg cover	Turbidity
15/05/2017	15	4	1
(6) Date:	Air temp	Veg cover	Turbidity
31/05/2017	13	4	1
Pond 19			
Brandon Murray (2015-17257-CLS-CLS) Ellen Poppleton			
(1) Date:	Air temp	Veg cover	Turbidity
12/04/2017	13	3	1
(2) Date:	Air temp	Veg cover	Turbidity
20/04/2017	11	3	1
Aline Brodzinski (2015-19142-CLS-CLS) Ellen Poppleton			
(3) Date:	Air temp	Veg cover	Turbidity
03/05/2017	11	3	1
(4) Date:	Air temp	Veg cover	Turbidity
10/05/2017	11	3	1
Pond 21a			
Brandon Murray (2015-17257-CLS-CLS) Ellen Poppleton			
(1) Date:	Air temp	Veg cover	Turbidity
11/04/2017	13	2	1
(2) Date:	Air temp	Veg cover	Turbidity
19/04/2017	6	2	1
Aline Brodzinski (2015-19142-CLS-CLS) Ellen Poppleton			
(3) Date:	Air temp	Veg cover	Turbidity
03/05/2017	11	1	1
(4) Date:	Air temp	Veg cover	Turbidity
11/05/2017	12	1	1
21b			

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Brandon Murray (2015-17257-CLS-CLS) Ellen Poppleton			
(1) Date:	Air temp	Veg cover	Turbidity
11/04/2017	13	1	3
(2) Date:	Air temp	Veg cover	Turbidity
19/04/2017	6	1	3
Aline Brodzinski (2015-19142-CLS-CLS) Ellen Poppleton			
(3) Date:	Air temp	Veg cover	Turbidity
03/05/2017	11	1	3
(4) Date:	Air temp	Veg cover	Turbidity
11/05/2017	12	1	3
Pond 22			
Brandon Murray (2015-17257-CLS-CLS) Ellen Poppleton			
(1) Date:	Air temp	Veg cover	Turbidity
11/04/2017	13	5	1
(2) Date:	Air temp	Veg cover	Turbidity
19/04/2017	6	4	2
Aline Brodzinski (2015-19142-CLS-CLS) Ellen Poppleton			
(3) Date:	Air temp	Veg cover	Turbidity
03/05/2017	11	4	1
(4) Date:	Air temp	Veg cover	Turbidity
11/05/2017	12	4	1
Pond 23			
Brandon Murray (2015-17257-CLS-CLS) Ellen Poppleton			
(1) Date:	Air temp	Veg cover	Turbidity
11/04/2017	13	4	1
(2) Date:	Air temp	Veg cover	Turbidity
19/04/2017	6	4	1
Pond 27			

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Brandon Murray (2015-17257-CLS-CLS) Ellen Poppleton			
(1) Date:	Air temp	Veg cover	Turbidity
11/04/2017	9	3	3
(2) Date:	Air temp	Veg cover	Turbidity
19/04/2017	6	4	3
Aline Brodzinski (2015-19142-CLS-CLS) Ellen Poppleton			
(3) Date:	Air temp	Veg cover	Turbidity
03/05/2017	11	3	2
(4) Date:	Air temp	Veg cover	Turbidity
09/05/2017	11	3	2
Brandon Murray (2015-17257-CLS-CLS) Ewan Gibson			
(5) Date:	Air temp	Veg cover	Turbidity
15/05/2017	14	3	2
(6) Date:	Air temp	Veg cover	Turbidity
31/05/2017	13		
<b>Pond 28</b>			
Brandon Murray (2015-17257-CLS-CLS) Ellen Poppleton			
(1) Date:	Air temp	Veg cover	Turbidity
11/04/2017	9	4	2
(2) Date:	Air temp	Veg cover	Turbidity
19/04/2017	6	4	2

## **APPENDIX D: eDNA survey protocol (ADAS)**

## eDNA Survey Protocol

Kits should be kept at room temperature in an appropriate solvent store, consistent with Home Office regulations.

Kit contents: 1 sterile Whirl-Pak bag; 2 pairs of sterile gloves; 1 sterile 30 mL sampling ladle; a sample box containing 6 x 50 mL sample tubes two thirds full of preserving fluid (contains alcohol); 1 sterile 10 mL pipette; 1 protocol sheet.

Please **keep all packaging** as you will require this for couriered return of samples (see instructions enclosed with your order).

Don't go in the water.

- Collect your eDNA water sample before you do any other surveys at the pond.
- Take the sample whilst standing on the pond bank.
- Don't tread in the pond water itself either before or during collection of the DNA water sample as there is a considerable risk of contaminating your pond sample by bringing in Great Crested Newt DNA in mud and water from other areas on your boots and equipment.

### Walk around the pond, to identify areas where you can take your eDNA samples

Roughly plan where you will collect the 20 water samples from. The aim is to spread the samples out evenly around the pond edge. The samples should be taken from both open water and vegetated areas if present and if possible should avoid water that is less than 10 cm deep. If you cannot access all areas of the pond, spread the samples out as best you can without entering the water. Existing data shows that eDNA can be patchy depending on where the animals have been. Sampling in many areas considerably increases the chance of collecting their eDNA successfully.

NOTE: Before you take each ladle sample, be sure to mix the pond the water column by gently using the ladle to stir the water from the surface to close to the pond bottom **WITHOUT** disturbing the mud in the bottom. DNA 'sinks' and so will often be present in larger amounts close to the pond bottom. **It is important not to collect sediment as this may cause inhibition of the PCR analysis which could lead to an inconclusive result** (please see examples of different sediment levels within sampling tubes at <http://www.adas.uk/Service/edna-analysis-for-great-crested-newt>).

## Sample Collection

- Open your kit and put on a pair of gloves.
- Open the sterile Whirl-Pak bag by tearing off the clear plastic strip along the perforated line, then pull the tabs.

Collect 20 samples of 30 mL of pond water from around the pond (in the areas you have already identified) using the sampling ladle (fill the ladle), and empty each sample into the Whirl-Pak bag.



Dr Helen Rees  
Tel: 01159 516747  
Email: eDNA@adas.co.uk  
www.adas.uk

## Sample Preservation

1. When you have collected your 20 samples, close the bag securely using the top tabs (fold over several times and bend tabs over) and shake the Whirl-Pak bag for 10 seconds. This mixes any DNA across the whole water sample.
2. Put on a fresh pair of gloves to keep the next stage as uncontaminated as possible.
3. Using the clear plastic pipette provided take 15 mL of water from the Whirl-Pak bag, and transfer into one of the six conical tubes containing preserving fluid (i.e. fill tube to the 50 mL mark).
4. **Label the box** containing the six tubes with the date, your name (sampler), the pond name, and grid reference/co-ordinates.

**NOTE: Please do not overfill or under fill the tubes.**

5. Close the tube and ensure the cap is tight - leaky samples could later contaminate the laboratory with DNA.
6. Shake the tube vigorously for 10 seconds to mix the sample and preservative.
7. Repeat for each of the 6 conical tubes in the kit.
8. Double check that the lids are on tightly if they have leaked during shaking please also wipe the tubes.
9. Empty the remaining water from the whirl-Pak bag back into the pond.
10. Place all used gloves, pipettes, rubbish into the sampling bag and dispose.

**If storage of samples is necessary prior to their return please store refrigerated (2-4°C). Samples can be stored in this way for up to 1 month prior to analysis.**

## Returning the kit - Drop off option

Should you wish to return your items directly to us, they can be dropped off at **Vet School Stores, SVMS, Nottingham University, Sutton Bonington Campus, Loughborough, LE12 5RD**. (please note opening times: 8.30am - 4.00pm Monday-Friday) or outside of these times at **Main Reception on College Road**. Please clearly mark your box "FAO Helen Rees: ADAS".

## Booking your DHL Collection

Please email us at [eDNACouriering@adas.co.uk](mailto:eDNACouriering@adas.co.uk) so we can arrange your collection.

**We require the address of where the parcel will be, the number of parcels/number of kits, your contact details and the date of collection.** Wherever possible we will try to book the requested date between 9am-5pm. Once we have booked your return we will email you the DHL collection documents, these will need to be printed off and attached to your parcel before your driver arrives. Please use original packaging wherever possible, if alternative packaging is used you **MUST** attach an **LQ label** (, we send along with your DHL collection documents just in case) and write **UN1170** onto the box or DHL will not transport your parcel. Should you have any problems please call the office on 01159 516747.

## **APPENDIX E: eDNA survey results 2018, 2020, 2021**



Client: Brandon Murray,  
Arcadis,  
34 York Way,  
London,  
N1 9AB

ADAS  
Spring Lodge  
172 Chester Road  
Helsby  
WA6 0AR

Tel: 01159 516747  
Email: Helen.Rees@adas.co.uk

www.adas.uk

Sample ID: 2018-1370                      Condition on Receipt: Good                      Volume: Passed  
Client Identifier: Castle Lypne,  
Otterpool                      Description: pond water samples in preservative  
Date of Receipt: 02/07/2018                      Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control <sup>†</sup>	2 of 2	Real Time PCR	04/07/2018
Degradation Control <sup>§</sup>	Within Limits	Real Time PCR	04/07/2018
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	05/07/2018
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 <sup>-4</sup> ng/μL) <sup>#</sup>	4 of 4	Real Time PCR	As above for GCN

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

Signed:                       Signed: 

Position: Director: Biotechnology                      Position: MD: Biotechnology

Date of preparation: 05/07/2018                      Date of issue: 05/07/2018

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

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

*<sup>†</sup> Recorded as the number of positive replicate reactions at expected C<sub>t</sub> value. If the expected C<sub>t</sub> value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.*

*<sup>§</sup> No degradation is expected within time frame of kit preparation, sample collection and analysis.*

*<sup>#</sup>Additional positive controls (10<sup>-1</sup>, 10<sup>-2</sup>, 10<sup>-3</sup> ng/μL) are also routinely run, results not shown here.*



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Sample ID: 2018-1371                      Condition on Receipt: Medium Sediment                      Volume: Passed  
Client Identifier: D, Otterpool                      Description: pond water samples in preservative  
Date of Receipt: 02/07/2018                      Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control <sup>†</sup>	0 of 2	Real Time PCR	04/07/2018
Degradation Control <sup>§</sup>	Within Limits	Real Time PCR	04/07/2018
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	05/07/2018
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 <sup>-4</sup> ng/μL) <sup>#</sup>	4 of 4	Real Time PCR	As above for GCN

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Position: Director: Biotechnology                      Position: MD: Biotechnology

Date of preparation: 05/07/2018                      Date of issue: 05/07/2018

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

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

*<sup>†</sup> Recorded as the number of positive replicate reactions at expected C<sub>t</sub> value. If the expected C<sub>t</sub> value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.*

*<sup>§</sup> No degradation is expected within time frame of kit preparation, sample collection and analysis.*

*<sup>#</sup> Additional positive controls (10<sup>-1</sup>, 10<sup>-2</sup>, 10<sup>-3</sup> ng/μL) are also routinely run, results not shown here.*



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Sample ID: 2018-1372                      Condition on Receipt: Good                      Volume: Passed  
Client Identifier: E1, Otterpool                      Description: pond water samples in preservative  
Date of Receipt: 02/07/2018                      Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control <sup>†</sup>	2 of 2	Real Time PCR	04/07/2018
Degradation Control <sup>§</sup>	Within Limits	Real Time PCR	04/07/2018
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	05/07/2018
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 <sup>-4</sup> ng/μL) <sup>#</sup>	4 of 4	Real Time PCR	As above for GCN

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

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Position: Director: Biotechnology                      Position: MD: Biotechnology

Date of preparation: 05/07/2018                      Date of issue: 05/07/2018

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

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

*<sup>†</sup> Recorded as the number of positive replicate reactions at expected C<sub>t</sub> value. If the expected C<sub>t</sub> value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.*

*<sup>§</sup> No degradation is expected within time frame of kit preparation, sample collection and analysis.*

*<sup>#</sup>Additional positive controls (10<sup>-1</sup>, 10<sup>-2</sup>, 10<sup>-3</sup> ng/μL) are also routinely run, results not shown here.*



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Sample ID: 2018-1373                      Condition on Receipt: Good                      Volume: Passed  
Client Identifier: Ot-Chamneys South, Otterpool                      Description: pond water samples in preservative  
Date of Receipt: 02/07/2018                      Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control <sup>†</sup>	2 of 2	Real Time PCR	04/07/2018
Degradation Control <sup>§</sup>	Within Limits	Real Time PCR	04/07/2018
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	05/07/2018
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 <sup>-4</sup> ng/μL) <sup>#</sup>	4 of 4	Real Time PCR	As above for GCN

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Position: Director: Biotechnology                      Position: MD: Biotechnology

Date of preparation: 05/07/2018                      Date of issue: 05/07/2018

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

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

*<sup>†</sup> Recorded as the number of positive replicate reactions at expected C<sub>t</sub> value. If the expected C<sub>t</sub> value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.*

*<sup>§</sup> No degradation is expected within time frame of kit preparation, sample collection and analysis.*

*<sup>#</sup>Additional positive controls (10<sup>-1</sup>, 10<sup>-2</sup>, 10<sup>-3</sup> ng/μL) are also routinely run, results not shown here.*



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Sample ID: 2018-1374                      Condition on Receipt: Medium Sediment                      Volume: Passed  
Client Identifier: E, Otterpool                      Description: pond water samples in preservative  
Date of Receipt: 02/07/2018                      Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control <sup>†</sup>	0 of 2	Real Time PCR	04/07/2018
Degradation Control <sup>§</sup>	Within Limits	Real Time PCR	04/07/2018
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	05/07/2018
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 <sup>-4</sup> ng/μL) <sup>#</sup>	4 of 4	Real Time PCR	As above for GCN

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Position: Director: Biotechnology                      Position: MD: Biotechnology

Date of preparation: 05/07/2018                      Date of issue: 05/07/2018

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

*<sup>†</sup> Recorded as the number of positive replicate reactions at expected C<sub>t</sub> value. If the expected C<sub>t</sub> value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.*

*<sup>§</sup> No degradation is expected within time frame of kit preparation, sample collection and analysis.*

*<sup>#</sup>Additional positive controls (10<sup>-1</sup>, 10<sup>-2</sup>, 10<sup>-3</sup> ng/μL) are also routinely run, results not shown here.*

## Appendix 1: Interpretation of results

### Sample Condition

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

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

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

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

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

### What do my results mean?

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

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

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

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

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

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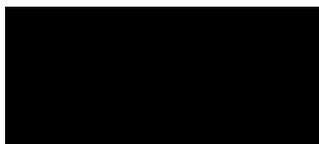
www.adas.uk

Sample ID: 2018-1156      Condition on Receipt: Not Recorded Upon Sample Arrival      Volume: Passed  
Client Identifier: Not Recorded Upon Sample Arrival      Description: pond water samples in preservative  
Date of Receipt: 29/06/2018      Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control <sup>†</sup>	0 of 2	Real Time PCR	03/07/2018
Degradation Control <sup>§</sup>	Evidence of degradation or residual inhibition	Real Time PCR	03/07/2018
Great Crested Newt*	Indeterminate	Real Time PCR	03/07/2018
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 <sup>-4</sup> ng/μL) <sup>#</sup>	4 of 4	Real Time PCR	As above for GCN

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

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Position: Director: Biotechnology      Position: MD: Biotechnology

Date of preparation: 04/07/2018      Date of issue: 04/07/2018

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

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

*<sup>†</sup> Recorded as the number of positive replicate reactions at expected C<sub>t</sub> value. If the expected C<sub>t</sub> value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.*

*<sup>§</sup> No degradation is expected within time frame of kit preparation, sample collection and analysis.*

*<sup>#</sup> Additional positive controls (10<sup>-1</sup>, 10<sup>-2</sup>, 10<sup>-3</sup> ng/μL) are also routinely run, results not shown here.*

## Appendix 1: Interpretation of results

### Sample Condition

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

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

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

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### What do my results mean?

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

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

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

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

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

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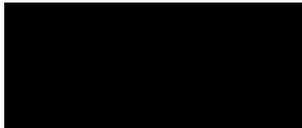
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Sample ID: 2020-0719                      Condition on Receipt: Low Sediment                      Volume: Passed  
Client Identifier: Otterpool 22                      Description: pond water samples in preservative  
Date of Receipt: 14/05/2020                      Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control <sup>†</sup>	2 of 2	Real Time PCR	18/05/2020
Degradation Control <sup>§</sup>	Within Limits	Real Time PCR	18/05/2020
Great Crested Newt*	2 of 12 (GCN positive)	Real Time PCR	18/05/2020
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 <sup>-4</sup> ng/μL) <sup>#</sup>	4 of 4	Real Time PCR	As above for GCN

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

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Position: Director: Biotechnology                      Position: MD: Biotechnology

Date of preparation: 19/05/2020                      Date of issue: 19/05/2020

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

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

*<sup>†</sup> Recorded as the number of positive replicate reactions at expected C<sub>t</sub> value. If the expected C<sub>t</sub> value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.*

*<sup>§</sup> No degradation is expected within time frame of kit preparation, sample collection and analysis.*

*<sup>#</sup> Additional positive controls (10<sup>-1</sup>, 10<sup>-2</sup>, 10<sup>-3</sup> ng/μL) are also routinely run, results not shown here.*

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Sample ID: 2020-0739                      Condition on Receipt: Medium Sediment                      Volume: Passed  
Client Identifier: Otterpool 20                      Description: pond water samples in preservative  
Date of Receipt: 14/05/2020                      Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control <sup>†</sup>	2 of 2	Real Time PCR	18/05/2020
Degradation Control <sup>§</sup>	Within Limits	Real Time PCR	18/05/2020
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	18/05/2020
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 <sup>-4</sup> ng/μL) <sup>#</sup>	4 of 4	Real Time PCR	As above for GCN

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

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Position: Director: Biotechnology                      Position: MD: Biotechnology

Date of preparation: 19/05/2020                      Date of issue: 19/05/2020

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

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

*<sup>†</sup> Recorded as the number of positive replicate reactions at expected C<sub>t</sub> value. If the expected C<sub>t</sub> value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.*

*<sup>§</sup> No degradation is expected within time frame of kit preparation, sample collection and analysis.*

*<sup>#</sup> Additional positive controls (10<sup>-1</sup>, 10<sup>-2</sup>, 10<sup>-3</sup> ng/μL) are also routinely run, results not shown here.*

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Sample ID: 2020-0746                      Condition on Receipt: Low Sediment                      Volume: Passed  
Client Identifier: Otterpool P24                      Description: pond water samples in preservative  
Date of Receipt: 14/05/2020                      Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control <sup>†</sup>	2 of 2	Real Time PCR	18/05/2020
Degradation Control <sup>§</sup>	Within Limits	Real Time PCR	18/05/2020
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	18/05/2020
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 <sup>-4</sup> ng/μL) <sup>#</sup>	4 of 4	Real Time PCR	As above for GCN

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

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Position: Director: Biotechnology                      Position: MD: Biotechnology

Date of preparation: 19/05/2020                      Date of issue: 19/05/2020

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

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*<sup>#</sup>Additional positive controls (10<sup>-1</sup>, 10<sup>-2</sup>, 10<sup>-3</sup> ng/μL) are also routinely run, results not shown here.*

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Sample ID: 2020-0758                      Condition on Receipt: Algae Present                      Volume: Passed  
Client Identifier: 23A Otterpool                      Description: pond water samples in preservative  
Date of Receipt: 14/05/2020                      Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control <sup>†</sup>	2 of 2	Real Time PCR	18/05/2020
Degradation Control <sup>§</sup>	Within Limits	Real Time PCR	18/05/2020
Great Crested Newt*	11 of 12 (GCN positive)	Real Time PCR	18/05/2020
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 <sup>-4</sup> ng/μL) <sup>#</sup>	4 of 4	Real Time PCR	As above for GCN

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

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Position: Director: Biotechnology                      Position: MD: Biotechnology

Date of preparation: 19/05/2020                      Date of issue: 19/05/2020

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

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*<sup>†</sup> Recorded as the number of positive replicate reactions at expected C<sub>t</sub> value. If the expected C<sub>t</sub> value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.*

*<sup>§</sup> No degradation is expected within time frame of kit preparation, sample collection and analysis.*

*<sup>#</sup> Additional positive controls (10<sup>-1</sup>, 10<sup>-2</sup>, 10<sup>-3</sup> ng/μL) are also routinely run, results not shown here.*

## Appendix 1: Interpretation of results

### Sample Condition

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

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

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2. In some cases sediment can cause inhibition of the PCR analysis used to detect GCN eDNA within samples which could lead to an indeterminate result.
3. In some cases sediment can interfere with the DNA extraction procedure resulting in poor recovery of the eDNA which in turn can lead to an indeterminate result.

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

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

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

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

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

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

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

Folio No: E11770  
Report No: 1  
Purchase Order: 50050267  
Client: ARCADIS  
Contact: Joel Cronin

## TECHNICAL REPORT

### ANALYSIS OF ENVIRONMENTAL DNA IN POND WATER FOR THE DETECTION OF GREAT CRESTED NEWTS (*TRITURUS CRISTATUS*)

#### SUMMARY

When great crested newts (GCN), *Triturus cristatus*, inhabit a pond, they continuously release small amounts of their DNA into the environment. By collecting and analysing water samples, we can detect these small traces of environmental DNA (eDNA) to confirm GCN habitation or establish GCN absence.

#### RESULTS

**Date sample received at Laboratory:** 12/07/2021  
**Date Reported:** 23/07/2021  
**Matters Affecting Results:** None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
7807	OTTER POOL POND 1 LYVDEN	TR 12591 36583	Pass	Pass	Pass	Negative	0

If you have any questions regarding results, please contact us: [ForensicEcology@surescreen.com](mailto:ForensicEcology@surescreen.com)

**Reported by:** Chris Troth

**Approved by:** Chris Troth



## **METHODOLOGY**

The samples detailed above have been analysed for the presence of GCN eDNA following the protocol stated in DEFRA WC1067 'Analytical and methodological development for improved surveillance of the Great Crested Newt, Appendix 5.' (Biggs et al. 2014). Each of the 6 sub-sample tubes are first centrifuged and pooled together into a single sample which then undergoes DNA extraction. The extracted sample is then analysed using real time PCR (qPCR), which uses species-specific molecular markers to amplify GCN DNA within a sample. These markers are unique to GCN DNA, meaning that there should be no detection of closely related species.

If GCN DNA is present, the DNA is amplified up to a detectable level, resulting in positive species detection. If GCN DNA is not present then amplification does not occur, and a negative result is recorded.

Analysis of eDNA requires scrupulous attention to detail to prevent risk of contamination. True positive controls, negative controls and spiked synthetic DNA are included in every analysis and these have to be correct before any result is declared and reported. Stages of the DNA analysis are also conducted in different buildings at our premises for added security.

SureScreen Scientifics Ltd is ISO9001 accredited and participate in Natural England's proficiency testing scheme for GCN eDNA testing. We also carry out regular inter-laboratory checks on accuracy of results as part of our quality control procedures.

## **INTERPRETATION OF RESULTS**

**SIC:** **Sample Integrity Check** [Pass/Fail]

When samples are received in the laboratory, they are inspected for any tube leakage, suitability of sample (not too much mud or weed etc.) and absence of any factors that could potentially lead to inconclusive results.

**DC:** **Degradation Check** [Pass/Fail]

Analysis of the spiked DNA marker to see if there has been degradation of the kit or sample between the date it was made to the date of analysis. Degradation of the spiked DNA marker may lead indicate a risk of false negative results.

**IC:** **Inhibition Check** [Pass/Fail]

The presence of inhibitors within a sample are assessed using a DNA marker. If inhibition is detected, samples are purified and re-analysed. Inhibitors cannot always be removed, if the inhibition check fails, the sample should be re-collected.

**Result:** **Presence of GCN eDNA** [Positive/Negative/Inconclusive]

**Positive:** GCN DNA was identified within the sample, indicative of GCN presence within the sampling location at the time the sample was taken or within the recent past at the sampling location.

**Positive Replicates:** Number of positive qPCR replicates out of a series of 12. If one or more of these are found to be positive the pond is declared positive for GCN presence. 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. 0/12 indicates negative GCN presence.

**Negative:** GCN eDNA was not detected or is below the threshold detection level and the test result should be considered as evidence of GCN absence, however, does not exclude the potential for GCN presence below the limit of detection.



## APPENDIX F: Surveyor pen portraits (2017)

Surveyor	Pen Portrait
Brandon Murray (Principal Ecological Consultant) BSc(hons) MCIEEM	Brandon has been a professional ecologist for over eight years. Brandon has been planning, leading and completing GCN surveys for over 6 years. Brandon holds a GCN Class1 Survey licence, and has also held multiple GCN development licences as a named ecologist.
Aline Brodzinski (Senior Ecologist) BSc (hons) MSc MCIEEM	Aline Brodzinski has been a professional ecologist for 7 years, and has a Class 1 GCN survey licence. Aline is proficient in surveying for a range of protected species including bats, great crested newts, badgers, reptiles, water voles and otters.
Ellen Poppleton (Assistant Ecologist) BSc (hons) GradCIEEM	Ellen Poppleton has been an ecologist for over two years. She has experience surveying for reptiles, bats, badgers, amphibians and water voles. Ellen has received internal and on the job training to make sure that she can confidently conduct a range of protected species surveys.
Ewan Gibson, (Assistant Ecologist) BSc (hons) GradCIEEM	Ewan Gibson is a graduate ecologist with a broad range of ecological experience. Ewan has been a professional ecologist for 3 years and has conducted surveys for a range of species, including bats, badger, dormouse, amphibians and reptiles, as well as being licensed to survey for barn owl. Ewan strives to collect and collate data with accuracy and precision. He has received in-house 'on the job' training in order to understand the requirements of these surveys, including the usage of survey equipment and identification of field signs.

## APPENDIX G: Photographs



Photograph 1: Pond 5 – low population size class



Photograph 2: Pond 9 – low population size class



Photograph 3: Pond 11 low population size class



Photograph 4: Pond 15 -low population size class



Photograph 5: Pond 17 low population size class



Photograph 6; Pond 23 low population size class



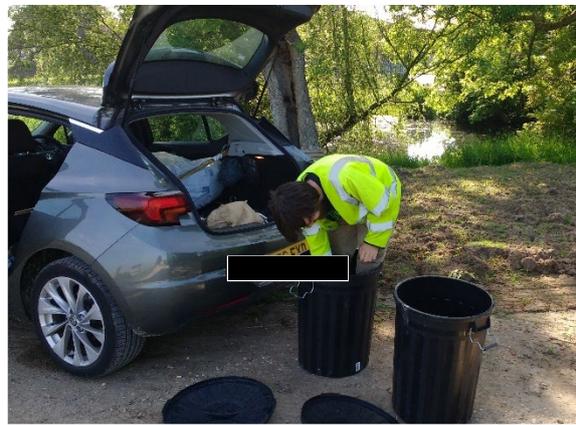
Photograph 7: Pond 27 low population size class



Photograph 8: Pond 20 / 21 ephemeral water bodies – no GCN presence.



Photograph 9: Pond 19 – no GCN presence



Photograph 10: Biocontrol cleaning being undertaken prior to surveys.



Photograph 11: Pond 32 – Poor HSI score

## **APPENDIX H: HSI Methodology**



## Great Crested Newt Habitat Suitability Index

May 2010

### Background

The Habitat Suitability Index (HSI) for the great crested newt was developed by Oldham *et al.* (2000). HSI scoring systems were originally developed by the US Fish and Wildlife Service as a means of evaluating habitat quality and quantity. An HSI is a numerical index, between 0 and 1. Values close to 0 indicate unsuitable habitat, 1 represents optimal habitat. The HSI for the great crested newt incorporates ten suitability indices, all of which are factors known to affect this species. These ten suitability indices are retained in this current Advice Note.

In the HSI system proposed by Oldham *et al.* (2000) one of the suitability indices (SI<sub>1</sub>, terrestrial) involves more lengthy measurement and calculation than the others. In using the HSI system with volunteer surveyors in Kent, Lee Brady has substituted a simpler evaluation of terrestrial habitat quality (a four-point scale), for ease of use.

Several other, local, surveys have utilised the HSI, but incorporating their own variations on the original system. In 2007 a workshop was held at the Herpetofauna Workers' Meeting to evaluate the use of the HSI for the great crested newt, with the aims of:

- Identifying components of the system that may need clarification or refinement
- Agreeing on a standard that can readily be used by volunteers and professionals alike.

The outputs of the workshop and subsequent consultation have been used to formulate the current Advice Note. As far as possible a conservative approach has been adopted in modifying the use of the original HSI suitability indices. However, a major departure is the adoption of Lee Brady's four-point evaluation of terrestrial habitat. This differs from the original HSI in that it has been developed with respect to newt presence/absence at a pond, rather than estimating population size.

### Use and limitations of the HSI

The HSI for great crested newts is a measure of habitat suitability. **It is not a substitute for newt surveys.** In general, ponds with high HSI scores are more likely to support great crested newts than those with low scores. However, the system is not sufficiently precise to conclude that any particular pond with a high score will support newts, or that any pond with a low score will not do so.

There is a positive correlation between HSI scores and the numbers of great crested newts observed. In general, high HSI scores are likely to be associated with greater numbers of great crested newts. The relationship is not sufficiently strong, however, to allow estimations of the numbers of newts in any particular pond.

HSI scoring can be useful in:

- Evaluating the general suitability of a pond, or ponds, for great crested newts
- Comparing general suitability of ponds across different areas
- Evaluating the suitability of receptor ponds in a proposed mitigation scheme
- Identifying habitat management priorities.

### How to collect data and calculate the HSI

The HSI is a geometric mean of ten suitability indices:

$$\text{HSI} = (\text{SI}_1 \times \text{SI}_2 \times \text{SI}_3 \times \text{SI}_4 \times \text{SI}_5 \times \text{SI}_6 \times \text{SI}_7 \times \text{SI}_8 \times \text{SI}_9 \times \text{SI}_{10})^{1/10}$$

- Ten factors are scored for a pond, in the field and from map work (field scores).
- The ten field scores are converted to SI scores, on a scale from 0.01 to 1 (0.01 is used as the lower end of the scale in stead of 0, because multiplying by 0 reduces all other SI scores to 0).
- The ten SI scores are multiplied together.
- The tenth root of this number is calculated ( $x^{1/10}$ ) i.e.  $x$  to the power of 0.1.

The calculated HSI for a pond should score between 1 and close to 0 (the calculations above do not allow the HSI to be exactly 0).

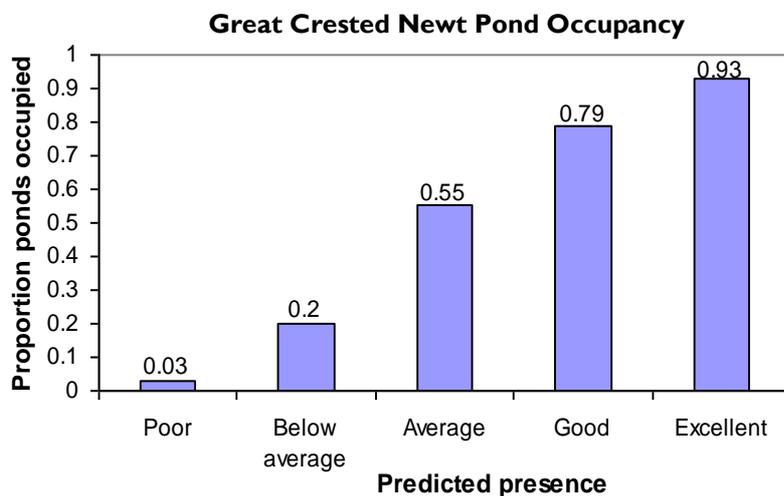
Some of the field scores are categorical, some are numerical. The numerical field scores are converted to SI scores by reading off the values from graphs produced by Oldham *et al.* (2000) reproduced in this Advice Note.

Full details of the scoring system, including descriptions of the criteria used in the categorical scores are given in *Details of suitability indices and definitions of categories* (below). Scores for two of the factors (SI<sub>1</sub> and SI<sub>8</sub>) can be gained as desktop/map exercises and so do not have to be completed in the field. The remaining factors should be recorded as field scores, and later converted to suitability indices, in some cases reading SI scores from the graphs provided. A summary of data to collect is given in the appendix *Summary of scoring system*.

### Categorisation of HSI scores

Lee Brady has developed a system for using HSI scores to define pond suitability for great crested newts on a categorical scale:

HSI	=	Pond suitability
< 0.5	=	poor
0.5-0.59	=	below average
0.6-0.69	=	average
0.7-0.79	=	good
> 0.8	=	excellent



The graph shows occupancy of ponds by great crested newts in south-east England. 248 ponds were surveyed on three to six occasions, using egg-searching, torching and bottle-trapping. As pond suitability increases from 'poor' to 'excellent', so does the proportion of ponds occupied by great crested newts.

### Details of suitability indices and definitions of categories

#### Factor 1. Geographic location (SI<sub>1</sub>)

Sites should be scored according to the zone in which they occur. This scoring can be carried out either in the field, or as part of a desktop exercise.

Zone A, location is optimal, SI = 1

Zone B, location is marginal, SI = 0.5

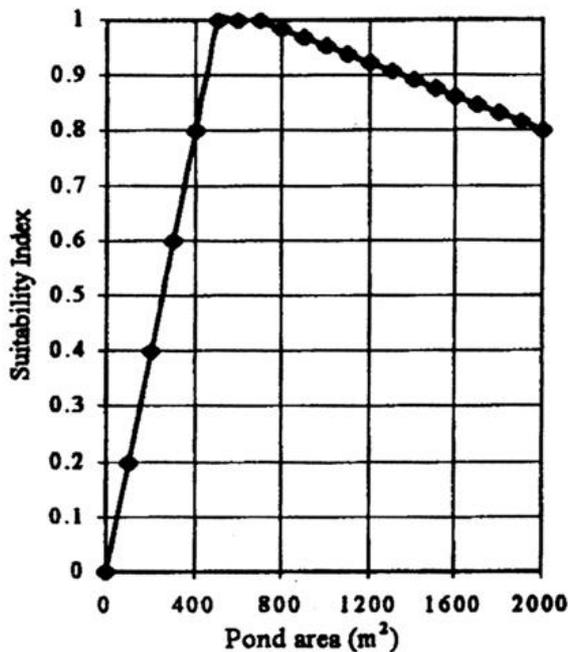
Zone C, location is unsuitable, SI = 0.01.

Some sites will fall on boundary lines between zones. In such cases, select medium-value scores i.e. Zone B.



## Factor 2. Pond area

Pond area is the surface area of the pond when water is at its highest level (excluding flooding events). This is usually in the spring. If the pond is being measured at another time of year, the spring time area should still be evident from vegetation types and evidence of a draw down zone around the pond.



Pond area should be measured as accurately as possible. There are several ways of doing this, for example by measuring axes of regularly shaped ponds, either by pacing out in the field, or taking measurements from a map. Irregularly shaped ponds may have to be treated as a series of geometric shapes, calculating the area for each and adding together.

Since it can be difficult reading off SI scores from the graph, pond area should be rounded to the nearest 50 m<sup>2</sup>.

It can be particularly difficult to read off SI scores for very small ponds. For ponds smaller than 50 m<sup>2</sup> use a score of 0.05.

For ponds larger than 2000 m<sup>2</sup> omit this factor from the HSI calculation (as there are no data for such large ponds).  
i.e.  $HSI = (SI_1 \times SI_3 \times SI_4 \times SI_5 \times SI_6 \times SI_7 \times SI_8 \times SI_9 \times SI_{10})^{1/9}$ .

## Factor 3. Permanence

Pond permanence should be deduced from local knowledge and personal judgement. A landowner may know how often a pond dries. However, if not, the surveyor should make a judgement based on water level at the time of the survey, and taking seasonality into consideration. For example, a pond that is already dry by late spring is likely to dry out every year, etc.

Category	SI	Criteria
Never dries	0.9	Never dries.
Rarely dries	1.0	Dries no more than two years in ten or only in drought.
Sometimes dries	0.5	Dries between three years in ten to most years.
Dries annually	0.1	Dries annually.

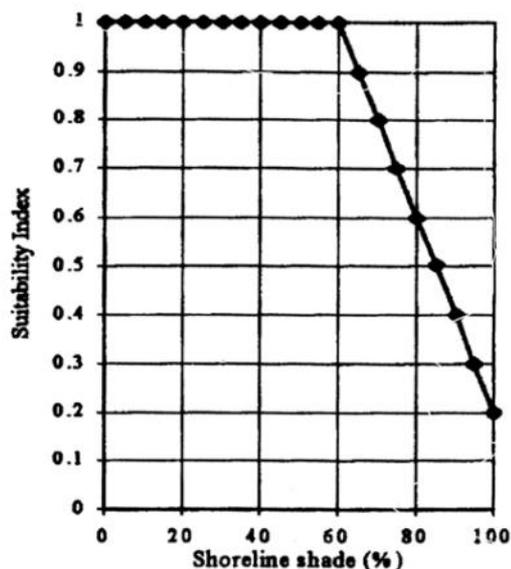
## Factor 4. Water quality

The assessment of water quality is subjective and should be based on invertebrate diversity, the presence of submerged water plants and knowledge of the water sources feeding the pond. Water quality should not be confused with water clarity. Sometimes clear water can be devoid of invertebrates, and turbid ponds can support a wealth of invertebrates. There is no quick and simple invertebrate index of water quality. However, some species are indicators of water quality.

Category	SI	Criteria
Good	1.0	Water supports an abundant and diverse invertebrate community. Netting reveals handfuls of diverse invertebrates, including groups such as mayfly larvae and water shrimps.
Moderate	0.67	Moderate invertebrate diversity
Poor	0.33	Low invertebrate diversity (e.g. species such as midge and mosquito larvae). Few submerged plants.
Bad	0.01	Clearly polluted, only pollution-tolerant invertebrates (such as rat-tailed maggots), no submerged plants.

Other cues may also provide information about water quality. For example, ponds subject to agricultural inputs are likely to have poor water quality.

## Factor 5. Shade



Estimate percentage pond perimeter shaded, to at least 1 m from the shore. Shading is usually from trees, but can include buildings. Shading should not include emergent pond vegetation. The estimate should be made during the period from May to the end of September.

## Factor 6. Waterfowl

This factor is concerned with the impact of waterfowl upon the pond and newts. At high densities, as created when waterfowl are encouraged to use a pond by provision of food, the birds can remove all aquatic vegetation, pollute water and persistently stir sediments. Some waterfowl may also actively hunt adult newts and their larvae. Score as one of three categories.

Category	SI	Criteria
Absent	1	No evidence of waterfowl impact (moorhens may be present).
Minor	0.67	Waterfowl present, but little indication of impact on pond vegetation. Pond still supports submerged plants and banks are not denuded of vegetation.
Major	0.01	Severe impact of waterfowl. Little or no evidence of submerged plants, water turbid, pond banks showing patches where vegetation removed, evidence of provisioning waterfowl.

'Waterfowl' includes most water birds, such as ducks, geese and swans. Moorhens should be excluded because almost every pond has at least one or two.

## Factor 7. Fish

Information on fish should be gleaned from local knowledge and the surveyor's own observations. Pond owners will usually be aware of stocking with fish for commercial or aesthetic reasons. However, stickleback (which can be significant predators of great crested newt larvae, when present in large numbers) are unlikely to be deliberately introduced to a pond, but may arrive through other means. Netting is useful in detecting smaller fish, such as sticklebacks, or the fry of larger species.

Category	SI	Criteria
Absent	1	No records of fish stocking and no fish revealed by netting or observed by torchlight.
Possible	0.67	No evidence of fish, but local conditions suggest that they may be present.
Minor	0.33	Small numbers of crucian carp, goldfish or stickleback known to be present.
Major	0.01	Dense populations of fish known to be present.

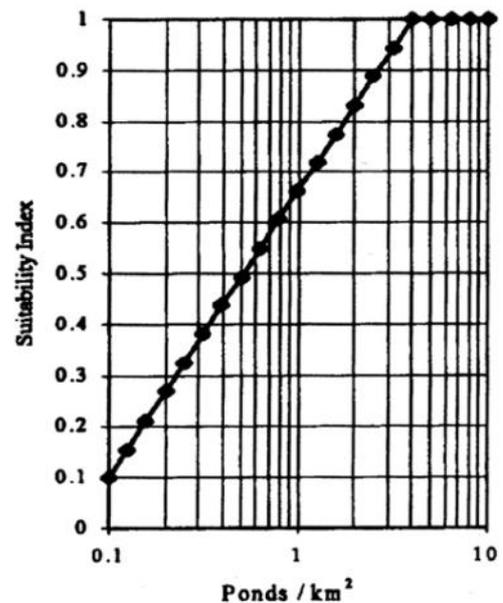
### Factor 8. Pond count

This is the number of ponds occurring within 1 km of survey pond. Do not count the survey pond itself. Ponds on the far side of major barriers, such as main roads, should not be counted. Use 1:25,000 scale O.S. data, such as Explorer maps, GIS or web-based mapping sources, such as:

Getamap [www.ordnancesurvey.co.uk/oswebsite/getamap/](http://www.ordnancesurvey.co.uk/oswebsite/getamap/)  
 Magic [www.magic.gov.uk/site\\_map.html](http://www.magic.gov.uk/site_map.html)  
 Digimap [edina.ac.uk/digimap/](http://edina.ac.uk/digimap/)

Pond counts can be carried out a by a survey coordinator and so do not necessarily have to be performed by surveyors.

Divide the number of ponds by  $\pi$  (3.14) to calculate the density of ponds per km<sup>2</sup> and read off the SI value from graph.



### Factor 9. Terrestrial habitat

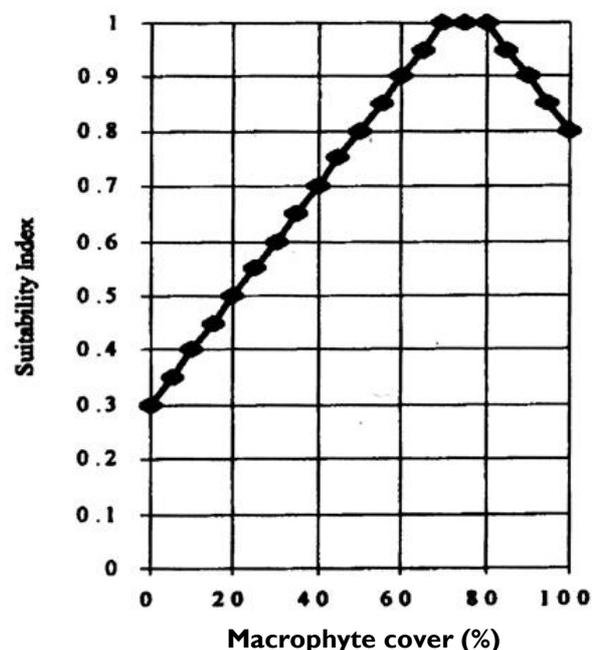
Scoring terrestrial habitat depends on the surveyor’s understanding of newt habitat quality. Good terrestrial habitat offers cover and foraging opportunities and includes meadow, rough grassland with tall sward height, scrub, woodland or mature gardens. Terrestrial habitat should be considered within approximately 250 m from the pond, but only on the near side of any major barriers to dispersal (e.g. main roads or large expanses of bare habitat).

Category	SI	Criteria
Good	1	Habitat that offers good opportunities for foraging and shelter (e.g. most semi-natural environments, such as rough grassland, scrub or woodland, also brownfield sites and low intensity farmland) covers more than 75% of available area.
Moderate	0.67	Habitat offers opportunities for foraging and shelter but may not be extensive (25-75%) of available area.
Poor	0.33	Habitat with poor structure (e.g. amenity grassland, improved pasture and arable) that offers limited opportunities (less than 25% of available area) for foraging and shelter.
None	0.01	No suitable habitat around pond (e.g. centre of arable field or large expanse of bare habitat).

Great crested newts do not have specific terrestrial habitat requirements. However, good quality terrestrial habitat has structure. The presence of hedges, ditches, stone walls, old farm buildings, piles of loose stone or rock, rabbit burrows and small mammal holes all contribute towards ‘good’ terrestrial habitat. Note that it is rare to encounter a pond falling within the terrestrial habitat category of ‘none’.

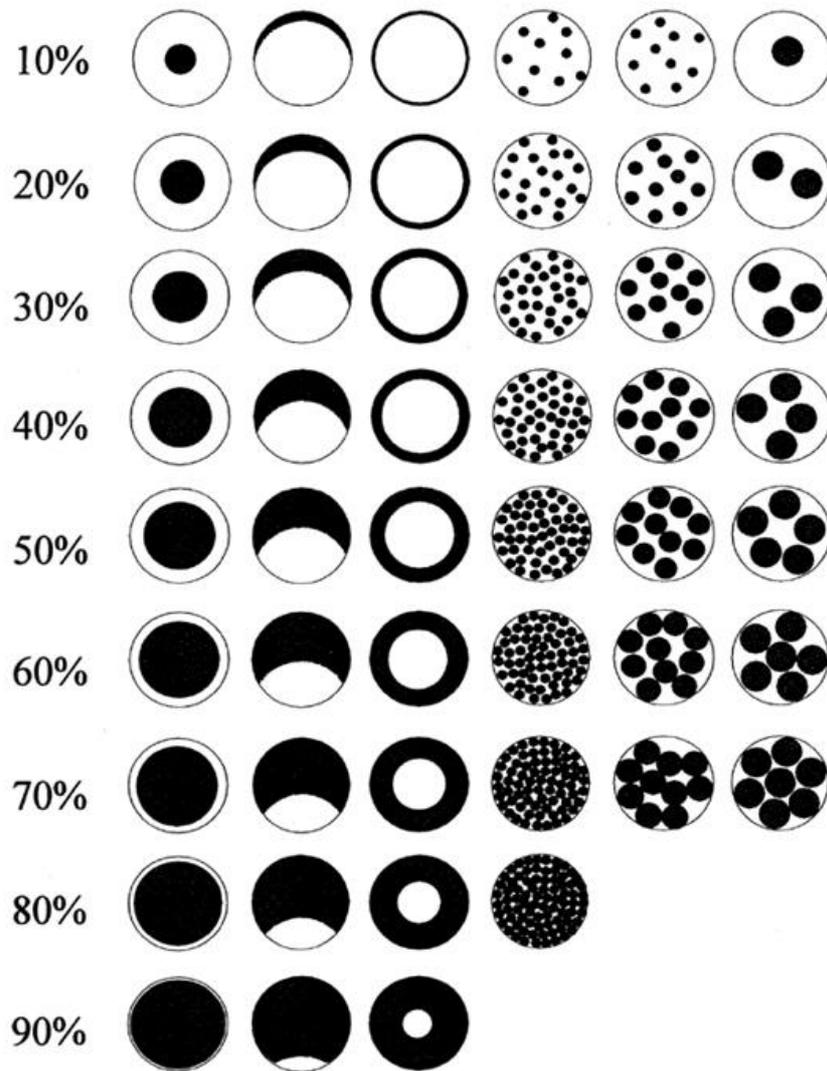
### Factor 10. Macrophytes

Estimate the percentage of the pond surface area occupied by macrophyte cover. This includes emergents, floating plants (excluding duckweed) and submerged plants reaching the surface. Make an estimate between March and the end of September. Read off the SI value from graph.



## Guide for assessment of macrophyte cover in a pond

The areas of dark shading simulate a variety of vegetation dispersion patterns.



### Reference

Oldham R.S., Keeble J., Swan M.J.S. & Jeffcote M. (2000). Evaluating the suitability of habitat for the Great Crested Newt (*Triturus cristatus*). *Herpetological Journal* 10(4), 143-155.

This Advice Note is an output from a workshop held at the Herpetofauna Workers' Meeting in January 2007. ARG UK is grateful to Lee Brady, Rob Oldham, David Sewell and John Baker for leading the workshop and/or contributing to this note, and workshop participants for providing useful suggestions. ARG UK is also grateful to the British Herpetological Society for permission to use graphics from the original paper on HSI, published in the *Herpetological Journal*.

This Advice Note can be downloaded from the ARG UK website [www.arguk.org](http://www.arguk.org) and should be cited as: ARG UK (2010). ARG UK Advice Note 5: Great Crested Newt Habitat Suitability Index. Amphibian and Reptile Groups of the United Kingdom.

Publication date: May 2010.

ARG UK is the network of volunteer conservation groups concerned with the native amphibians and reptiles of the UK.



amphibian and reptile  
conservation



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## Summary of scoring system

### SI<sub>1</sub> Location

Field score	SI
A (optimal)	1
B (marginal)	0.5
C (unsuitable)	0.01

### SI<sub>2</sub> Pond area

Field score	SI
Measure pond surface area (m <sup>2</sup> ) and round to nearest 50 m <sup>2</sup>	Read off graph.

### SI<sub>3</sub> Pond drying

Field score	SI	Criteria
Never	0.9	Never dries
Rarely	1.0	Dries no more than two years in ten or only in drought.
Sometimes	0.5	Dries between three years in ten to most years
Annually	0.1	Dries annually

### SI<sub>4</sub> Water quality

Field score	SI	Criteria
Good	1.0	Abundant and diverse invertebrate community.
Moderate	0.67	Moderate invertebrate diversity
Poor	0.33	Low invertebrate diversity, few submerged plants
Bad	0.01	Clearly polluted, only pollution-tolerant invertebrates, no submerged plants.

### SI<sub>5</sub> Shade

Field score	SI
Estimate percentage perimeter shaded to a least 1 m from shore.	Read off graph.

### SI<sub>6</sub> Fowl

Field score	SI	Criteria
Absent	1	No evidence of water fowl (although moorhen may be present)
Minor	0.67	Waterfowl present, but little sign of impacts
Major	0.01	Severe impact of waterfowl

### SI<sub>7</sub> Fish

Category	SI	Criteria
Absent	1	No records of fish stocking and no fish revealed during survey.
Possible	0.67	No evidence of fish, but local conditions suggest that they may be present.
Minor	0.33	Small numbers of crucian carp, goldfish or stickleback known to be present.
Major	0.01	Dense populations of fish known to be present.

### SI<sub>8</sub> Pond count

Field score	SI
Count the number of ponds within 1 km of the survey pond (not separated by major barriers) and divide by 3.14. This can be done from maps rather than in the field.	Read off graph.

### SI<sub>9</sub> Terrestrial habitat

Category	SI
Good	1
Moderate	0.67
Poor	0.33
None	0.01

### SI<sub>10</sub> Macrophytes

Field score	SI
Estimate the percentage of the pond surface area occupied by macrophyte cover (between May and the end of September)	Read off graph.

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