
Chapel Lane, Widnes

Environmental DNA (eDNA) Survey Report for Great Crested Newt

Compiled by Ecology Services Ltd.

on behalf of

Taylor Wimpey

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1.0 Introduction

- 1.1 Ecology Services Limited was commissioned by Taylor Wimpey in February 2022, to undertake Ecological Survey Works in the form of a great crested newt environmental DNA (eDNA) survey of ponds at land at Chapel Lane, Widnes, National Grid Reference (NGR): 350040, 387800. See Figure 1, Site location plan and pond locations.
- 1.2 This Environmental DNA (eDNA) Survey Report for Great Crested Newt has been prepared by Ecology Services Limited on behalf of Taylor Wimpey UK Ltd to assist the local planning authority's consideration of an outline planning application for a site at Chapel Lane, Widnes for:
- "Outline planning application (with all matters other than access reserved) (to be determined by Halton Borough Council only) for the development of up to 350 dwellings, public open space, landscaping and associated infrastructure works. Full details for site access arrangements via a new junction on Chapel Lane (to be determined by Knowsley Borough Council only) and emergency access via Sandy Lane (to be determined by Halton Borough Council)."*
- 1.3 The proposed development site is located to the north-west of the Widnes urban area. The site is situated within the boundary of two separate allocations for residential development within the adopted Halton Delivery and Allocations Local Plan [DALP] (March 2022) under Policy RD1 (Residential Development Allocations). The allocations comprise two separate land parcels separated by Sandy Lane, (Site Ref. W4 to the west of Sandy Lane and Site Ref. W5 to the east). The application red line boundary does not extend to the full extent of the allocation.
- 1.4 The site is located on the outskirts of the town of Widnes, within the Borough of Halton but on the boundary with Knowsley Council and contains a mix of habitats including: improved and poor semi-improved grassland, species-rich and species-poor hedgerows, wet and dry ditches, two ponds, scattered trees and buildings. The land to the north and west of the site is largely under arable cultivation crossed by hedgerows and ditches with further ponds and an area of broadleaved woodland. Broadleaved woodland adjoins the site to the east, beyond which lies Queensbury Way residential development with further residential development to the south.
- 1.5 The aims of the survey were to:
- Undertake a walkover survey of the proposed development site and surrounding land, to locate all waterbodies within 250m (where there is land access).
 - Undertake a desktop study for existing records of amphibians, information on local population status of amphibians and a review of existing maps and satellite imagery where there is no land access.
 - Undertake an amphibian survey of waterbodies, following Natural England's recommended survey methodology.
 - To identify the presence/absence of great crested newt (*Triturus cristatus*) and other amphibians on site.
 - Undertake a population size class assessment of great crested newt populations (if found to be present).
- 1.6 Natural England's Guidance (English Nature, 2001) advises that surveys of ponds up to around 500m from the proposed development might need to be surveyed. The decision on whether to survey depends primarily on how likely it is that the development would affect newts using those ponds. For developments resulting in permanent or temporary habitat loss

at distances over 250m from the nearest pond, careful consideration should be given as to whether a survey is appropriate. Surveys of land at this distance from ponds are normally appropriate when all of the following conditions are met (Natural England's Great Crested Newt Licence Method Statement Template, 2015):

- (a) maps, aerial photos, walk-over surveys or other data indicate that the pond(s) has potential to support a large great crested newt population,
- (b) the footprint contains particularly favourable habitat, especially if it constitutes the majority available locally,
- (c) the development would have a substantial negative effect on that habitat, and
- (d) there is an absence of dispersal barriers.

- 1.7 In this instance a 250m survey area was considered appropriate due to due to additional ponds located 250-500m from site being located within the residential area or located on the opposite side of a busy road (A5080, Cronton Road) and therefore offer very little connectivity to the site.
- 1.8 eDNA is nuclear or mitochondrial DNA that has become separated from an organism into the environment. Sources of eDNA include secreted faeces, mucous, gametes, shed skin, hair and carcasses. In aquatic environments, eDNA is diluted and distributed while suspended in the water column where it persists for 7–21 days, depending on the conditions.
- 1.9 Recent research has shown that the DNA of a range of aquatic organisms can be detected in water samples at very low concentrations using qPCR (quantitative Polymerase Chain Reaction) methods.
- 1.10 A test primer has been developed (a length of artificial DNA which specifically binds to and amplifies the DNA of the target organism) which is able to detect great crested newt eDNA successfully in water samples.

2.0 Planning Policy and Legislation

Planning Policy

- 2.1 The National Planning Policy Framework (NPPF, 2021) places a clear responsibility on Local Planning Authorities (LPA) to contribute to conserving and enhancing the natural and local environment. LPAs should promote the conservation, restoration and enhancement of priority habitats, ecological networks and the protection and recovery of priority species (e.g. Species of Principal importance, Local Biodiversity Action Plan species); and identify and pursue opportunities for securing measurable net gains for biodiversity. The Office of the Deputy Prime Minister (ODPM) Circular 06/2005 provides administrative guidance on the application of the law in relation to planning and nature conservation.
- 2.2 A Local Planning Authority (LPA) has a duty to ensure that protected species and habitats within the UK are a 'material consideration' in the determination of a planning application. Therefore, an LPA is unlikely to determine an application until all relevant information relating to protected species or habitats is submitted to fully inform the application. Relevant information includes adequate surveys and, where required, mitigation strategies, which will need to be submitted to inform a planning application.
- 2.3 When dealing with planning applications where a European Protected Species (EPS) (great crested newt) may be affected, a Local Authority is a 'competent authority' within the meaning

of Regulation 7 of the Conservation of Habitats & Species Regulations 2017 (as amended). The Local Authority must therefore exercise their functions under the provisions made within the 2017 Regulations (as amended), and planning decisions should only be made when European Protected Species and their habitats are fully taken into account.

Legislation

- 2.4 The great crested newt is protected under The Conservation of Habitats and Species Regulations 2017 (as amended)¹ and The Wildlife and Countryside Act 1981 (as amended). Taken together this legislation makes it an offence to:
- Deliberately (intentionally) kill, injure or take a great crested newt;
 - Possess or control any live or dead specimen or anything derived from a great crested newt;
 - Deliberately (intentionally) or recklessly damage, destroy or obstruct access to a breeding site or any structure or place used for shelter or protection by a great crested newt; or
 - Deliberately (intentionally) or recklessly disturb a great crested newt while it is occupying a structure or place which it uses for that purpose.
 - Sell, offer for sale, possess or transport for the purpose of sale (live or dead animal, part or derivative).
- 2.5 The legislation applies to all life stages of great crested newts. The extent of legal protection covers both the species and its habitat. It is therefore important to identify the presence of great crested newts and also to identify suitable habitat on site in order that legal obligations regarding this species can be observed.
- 2.6 Any development or other activity that would result in a contravention of the above legislation would require a licence to avoid committing an offence. See Appendix 1 for further information on licensing.
- 2.7 Waterbodies that support all five species of British amphibians in high numbers may be afforded statutory protection as a Site of Special Scientific Interest (SSSI) under the Wildlife and Countryside Act 1981 (as amended).
- 2.8 Section 40 of the Natural Environment and Rural Communities (NERC) Act 2006 (as amended) places a statutory duty on public authorities, in exercising their functions, to conserve and enhance biodiversity in England. Species of Principal Importance for the conservation or enhancement of biodiversity in England, identified by the Secretary of State in consultation with Natural England, are listed under Section 41 of the NERC Act. The Local Planning Authority and government bodies (e.g. Natural England) will expect the overall design of the development to have regard for the conservation and enhancement of populations of these species. Great crested newt and common toad (*Bufo bufo*) are listed as Species of Principal Importance under Section 41 of the NERC Act.

¹ The Conservation of Habitats and Species (Amendment) (EU Exit) Regulations 2019 continue the same provision for European protected species, licensing requirements and protected areas after Brexit.

3.0 Methodology

Desktop Study

- 3.1 Information regarding historic species records of amphibians were collated from the following sources listed in Table 1.

Table 1: Desktop Study Resources and Record Centres Consulted

Sources of Information	Information Supplied
Merseyside Environmental Advisory Service (MEAS) Cheshire records office - RECORD	To identify locally protected sites or species of interest within 2km of the site.
Natural Environmental and Rural Communities (NERC) Act 2006 (as amended)	Identification of Species of Principal Importance known to occur in the region.
Local Biodiversity Action Plan	Identification of local priority amphibian species known to occur in the region.
Multi-Agency Geographic Information for the Countryside (MAGIC) https://magic.defra.gov.uk/	Identification of European Protected Species records (granted licences, class licences and District Level Licensing pond surveys) within 2km of the site.

- 3.2 The aim of the desktop study was to check for any great crested newt records and information on great crested newt status in the local area. Where great crested newts are confirmed present and a licence required to permit the works, desktop study data is a requirement of licensing. The data search with MEAS was undertaken on 14th June 2022 and the RECORD data search was undertaken on the 27.6.2022.
- 3.3 A review of existing maps such as Ordnance Survey and digital based maps and aerial photographs such as the Multi-Agency Geographic Information for the Countryside (MAGIC) website (<https://magic.defra.gov.uk/>), MARIO Maps (mario.lancashire.gov.uk/) and Google Earth was undertaken to identify water bodies within 250m of the site.
- eDNA Methodology**
- 3.4 The method used to collect the eDNA followed the Analytical and Methodological Development for Improved Surveillance of the Great Crested Newt (WC1067), Appendix 5 Technical Advice Note for Field and Laboratory Sampling of Great Crested Newt (*Triturus cristatus*) environmental DNA, dated 30th September 2014 (see Appendix 2).
- 3.5 See Appendix 2 Sampling Protocol for equipment used and sample collection protocol. Upon collection samples were sent by courier to SureScreen Scientifics for analysis. The analysis involves using qPCR with twelve replicates per pond). See Appendix 3 SureScreen Scientifics Technical Report for the results of the sample analysis and the number of positive replicates.
- 3.6 The research carried out by the Freshwater Habitats Trust (FHT) show that the test can be more effective in confirming the presence or absence of great crested newt than a combination of conventional survey techniques. Nevertheless, the eDNA methodology is not error free.
- 3.7 A recent study that designed a statistical model on a set of data provided by Natural England identified error within the two stages of the eDNA methodology and calculated approximate figures for this data set, as follows. Stage 1 false positive error $p=0.15$ (15% error probability), stage 1 false negative error $p=0.27$ (27% error probability), stage 2 false positive error $p=0.05$ (5% error probability), and stage 2 false negative $p=0.19$ (19% error probability) (Griffin et al., 2019), where stage 1 is the ecologists sampling stage and stage 2 is the labs analysis stage. These error probabilities are based on an existing dataset of 200 ponds with 1 eDNA sample taken per pond and as such these error probabilities may vary and are not site or

pond specific. Further samples taken from the same pond significantly reduces the probability of error.

- 3.8 There are variables within the environment and waterbody characteristics (covariates) known that may impact the probability of an error occurrence at stage 1 which include:
- eDNA usually sheds as clumps and is therefore spread around the pond disproportionately and target DNA may be missed during stage 1.
 - Dense mats of vegetation or wide shallow drawdown zones around the pond which may prevent the mixing of eDNA into the water column resulting in the possibility of target DNA being missed.
 - Water flowing between ponds allows the transport of eDNA from one pond to another and may even remove eDNA from the survey pond.
 - Disruption of sediment may cause the collection of historic DNA causing a false positive error at stage 1 or reduce efficiency of DNA extraction at stage 2.
- 3.9 Covariates Griffin et al., 2019, found to significantly affect error probability at stage 2:
- The maximum pond depth and presence of macrophytes in the pond significantly increases true positive probability therefore reducing error probability.
 - The pond length and pond density within an area significantly decrease true positive probability therefore increasing error probability.
 - The presence of fish decreases the probability of false positive therefore reducing error probability.
- 3.10 A pond description has been provided below and any known factors of error were taken into account while undertaking the survey, with anything that could potentially cause error has been noted within Table 1.

Timing

- 3.11 Samples were taken on the 20th of April 2022, pond 3 was sampled on the 6th of June 2022 which is within the accepted sample period of mid-April to June.

Weather Conditions

- 3.12 Weather conditions on the sample days were reasonable, with no appreciable rain or wind affecting the survey.

Personnel

- 3.13 The eDNA samples were undertaken by suitably experienced ecologists under Natural England Class Survey Licence (CLS) by experienced ecologist Mr. C. Smith (Class Licence number 2016-20464-CLS-CLS) Paige Hickey BSc MSc and Lorna Smithers MA BA (Hons). All ecologists were subject to training in line with the Freshwater Habitats Trust (FHT) sampling methods.

Limitations

- 3.14 It is often the case that sites cannot be surveyed using all available techniques to full effect, as there are frequent restrictions such as season, weather and access, which limit survey intensity. In this case, access was restricted due to the very low water level on Pond 2, so only a small section of the pond was able to be sampled. There were also access issues with some areas of Pond 4 (60% was sampled). Pond 5 has some access issues due to very steep verges around approximately 60% of the pond. Pond 6 had very little access due to fencing and vegetation (20% was sampled). Pond 8 had some access restrictions due to scrub around the perimeter. Pond 3 was sampled at a later date due to access restrictions with heavy vegetation once vegetation clearance had been undertaken. The water level of

Pond 3 was very low and the perimeter had receded in areas to where the water could not be reached due to deep sediment (35% was sampled).

4.0 Results and Evaluation

Desktop Study

- 4.1 The desktop study returned four records of great crested newt with records ranging from 2005 to 2010 located approximately 2km to north of the site. The nearest record, albeit a low resolution record, is c. 835m to the north-east of the site on the opposite side of the A5080, which is a busy kerbed road likely to provide a barrier to movement of GCN. There are no European Protected Species records (granted licences, class licences and District Level Licensing pond surveys) within 2km of the site.

Species of Principal Importance

- 4.2 Great crested newt is listed as a Species of Principal Importance under Section 41 of the Natural Environmental and Rural Communities (NERC) Act 2006 (as amended).
- 4.3 The great crested newt is still quite widespread in Britain. The species is locally numerous in parts of lowland England and Wales but is absent or rare in Cornwall and Devon. It is widespread but local in Scotland and absent from Northern Ireland.
- 4.4 The species has suffered a decline in recent years with studies in the 1980s indicating a national rate of colony loss of approximately 2% over five years. It is estimated that there are a total of 18,000 great crested newt ponds within Britain, although only 3,000 of these have been identified. The British population is amongst the largest in Europe, where it is threatened in several countries.

Local Biodiversity Action Plan (LBAP)

- 4.5 The Halton Biodiversity Action Plan lists great crested newts as a species of local concern. The North West of England is a stronghold for this species in Britain as a whole.
- 4.6 Great crested newt surveys have been carried out on Ainsdale Sand Dunes NNR in 2001, 2002, 2004 and 2006. Evidence of great crested newt occupation was found in 31 ponds and temporary slacks. In 2004/05 the North Merseyside Amphibian & Reptile Group carried out a survey of the Sefton coast. Many of the ponds between Freshfield and Birkdale contained both adults and tadpoles.
- 4.7 Between 1995 and 1998, the Pond Life Project undertook a survey of 1000 ponds in the Northwest. Of these 500 were north of the Mersey although no sites surveyed were within the boroughs of Sefton, Knowsley, St Helens or Liverpool. The Pond Life survey showed that 25% of ponds in the northern part of the survey area contained great crested newts. Furthermore, in some urban areas (e.g. Wigan) the percentage of occupied ponds was higher.
- 4.8 There are good populations of great crested newt in the Greater Manchester area, the Sefton Coast, the outskirts of Liverpool and the coastal plain of Lancashire, including the Fylde, although there has been a notable loss of sites adjacent to urban areas.

Pond Description

- 4.9 A total of 9 ponds were located within 250 metres of the site during the Preliminary Ecological Appraisal and desktop study. The ponds and their locations are shown on Figure 1. Detailed pond descriptions of Ponds 1-8 are provided below (A further pond was found to be filled in).
- 4.10 Pond 1 is a circular pond with a perimeter of approximately 30m, located 35m to the northeast of the site. The pond has a mixture of banks including steep, shallow and engineered and

contained two culverts, with a leaf litter base and a depth of <0.5m and with clear water at the banks turning more turbid towards the middle of the pond. The pond is shaded by both trees and scrub and bulrush (*Typha latifolia*) was present. There were signs of waterfowl at the time of the survey.

- 4.11 Pond 2 is an oval pond with a perimeter of around 100m and located around 20m from the development boundary. The pond has very shallow banks and at the time of survey much of the pond was dry, with a small section close to a culvert that was <0.5m depth which was sampled. The water was very turbid and had a surface layer of scum with a leaf litter base. The pond was located within a wooded area and had some shading from surrounding trees. At the time of survey signs of waterfowl were also found.
- 4.12 Pond 3 is an oval pond with a perimeter of around 70m and is located within the site. It was very overgrown and silted up with much leaf litter from the surrounding scrub and trees and collapsed goat/grey willows within the pond. The pond has both steep and shallow banks, with a water level of less than 0.5m. The pond is shaded by around 70% trees and there had been some recent vegetation clearance around the margins to allow for access. Reed canary-grass was locally frequent in the pond. No signs of fish or waterfowl were found at the time of survey, however a mallard with ducklings and two nests were noted in the pond during the extended Phase 1 habitat survey undertaken on 24th June 2022.
- 4.13 Pond 4 is a circular field pond approximately 60m in perimeter and located within the west of the development site. The pond has steep banks with an approximately 1m water depth and very turbid water and a sediment base. There was no aquatic vegetation and occasional soft-rush around the margin. No water fowl signs were found at the time of survey although waterfowl have previously been recorded and the farmer previously advised that the pond is stocked with carp. The pond was found to have shading by both trees and scrub (a small horse chestnut tree, hawthorn bushes and a large crack willow were present).
- 4.14 Pond 5 is a circular pond with a perimeter of around 35m that is located on the southern boundary, partly inside the site with most of the pond found just outside the site boundary. It is mostly located within a residential garden. The pond has engineered stone banks for 75% of the perimeter with steep banks that are fenced at one side. The water depth was estimated to be at least 1.5m with a concrete base overlaid with sediment. The water was turbid. Watercress (*Rorippa nasturtium-aquaticum*) covered approximately 15% and approximately 30% of the pond was shaded by sycamore, silver birch (*Betula pendula*) and a conifer. Some bramble was present on one of the banks. The invasive species American skunk cabbage (*Lysichiton americanus*) was also present. There were signs of waterfowl at the time of survey.
- 4.15 Pond 6 is an oval pond that is approximately 60m in perimeter and is located east of the development site around 100m from the site boundary. The pond has steep banks and turbid water and is covered by around 40% bullrush. The water depth is under 0.5m with a sediment base. At the time of survey no signs of water fowl were found.
- 4.16 Pond 7 is an undulating shaped pond which is around 205m in perimeter and located around 167m to the west of the site. The pond has clear water with 40% vegetation cover which include marsh marigold, yellow flag iris and burr reed with shallow banks and a leaf litter base. The water depth at the time of survey was >0.5m and signs of water fowl were present.
- 4.17 Pond 8 is an oval pond approximately 76m in perimeter and around 200m north of the site boundary. The pond has turbid water, a depth of approximately 0.5m with a sediment and leaf litter base with shallow banks, The is substantial shading by trees and scrub and at the time of survey and evidence of water fowl was present.

eDNA Sample Results

- 4.18 Following analysis of the samples by SureScreen, the eDNA results are presented in Table 8 below. The SureScreen Scientifics Technical Report can be found in Appendix 3.

Table 2: eDNA Results

Pond No.	Sample date	% of pond accessed	Relevant notes	Positive/negative & Replicates
1	20.04.2022	90%	Some soft ground	Negative
2	20.04.2022	80%	Most of the pond was dry	Negative
3	06.06.2022	35%	Access limitations due to receded water level so soft sediment and overhanging willow	Negative
4	20.04.2022	60%	Some access issues due to scrub	Negative
5	20.04.2022	40%	Around half of the pond was inaccessible.	Negative
6	20.04.2022	20%	Most of the pond was in accessible	Negative
7	20.04.2022	90%		Negative
8	20.04.2022	75%	Some access issues due to scrub	Negative

Note: Amber cells denote risk factor, as sample area was either under 80% or there is a sediment warning in relation to positive results only.

- 4.19 The eDNA sampling method found all surveyed ponds to be negative and therefore ponds were not found to contain eDNA evidence of great crested newts. However, five ponds surveyed were limited by access and below the desired sample area of 80%, which reduces the confidence in the negative results.

5.0 Impacts & Recommendations

- 5.1 No evidence of great crested newt was discovered within any of the ponds at this site using eDNA sampling as described above. While there were constraints to sampling, given that:

- all of the samples have come back negative,
- some of the ponds (Ponds 1, 3 & 4) have been sampled twice (in 2020 (see Appendix 4) and 2022) and both times have been negative and
- there are no records of GCN within 500m, with only 4 records within 2km (the nearest record, albeit a low resolution record, is c. 835m to the north-east of the site on the opposite side of the A5080 which is a busy kerbed road likely to provide a barrier to movement of GCN,

it is considered unlikely that great crested newts are present on site. Therefore, there are no apparent implications with regards to great crested newts and development at this site.

- 5.2 As single round of traditional amphibian surveys of Ponds 1, 2 and 4 was undertaken in 2020 (Ecology Services, 2021, see Appendix 4) which identified smooth newt and common frog. Pond 1 was considered suitable for breeding common toad, which is a Species of Principal Importance in England (Section 41 of the Natural Environmental and Rural Communities (NERC) Act 2006). As a precautionary measure, all construction contractors should be made aware of the potential presence of common amphibians, including common toad, common frog and smooth newt on site and the potential risks to them. Should any common amphibians be encountered, they should be moved to suitable cover away from development works. If large numbers of common toad are found, then the acting consultant should be contacted for further advice on measures to minimise impacts.

- 5.3 Habitats on site are considered to be of low value to foraging amphibians and it is considered that far more suitable habitat for amphibians exists in the wider survey area to the east of the site where woodland and rank grassland exists.
- 5.4 Ponds (fulfilling certain criteria) are a Habitat of Principal Importance. The retention of ponds, ditches and associated habitats is therefore important. It is recommended that the ponds within the site are retained and protected during construction. When working close to water bodies appropriate pollution prevention measures must be implemented. Although withdrawn, The Environment Agency's guidance still provides useful advice. Current guidance on pollution prevention is provided on the Gov.uk website².
- 5.5 If a pond is to be impacted, an appropriate mitigation strategy will be required detailing appropriate working methods and mitigation procedures. Works, if necessary, should be carried under the supervision of a suitably qualified ecologist. The development also offers the opportunity to enhance the existing ponds by clearing accumulated silt and leaf litter and clearing fallen and overshadowing trees and/or create new ponds as part of the sustainable drainage system for the development.
- 5.6 Any work on ponds must take full account of protected species such as water vole, amphibians and aquatic invertebrates.

Enhancement Measures

- 5.7 In line with current planning policy to provide net gains for biodiversity, habitat enhancement measures have been designed and include:
- Enhancement of existing ponds and hedgerows to increase species diversity.
 - The use of Sustainable Drainage Systems (SuDS).
 - Enhancement of the brook and creation of a species-rich habitat corridor along the northern boundary of the site.

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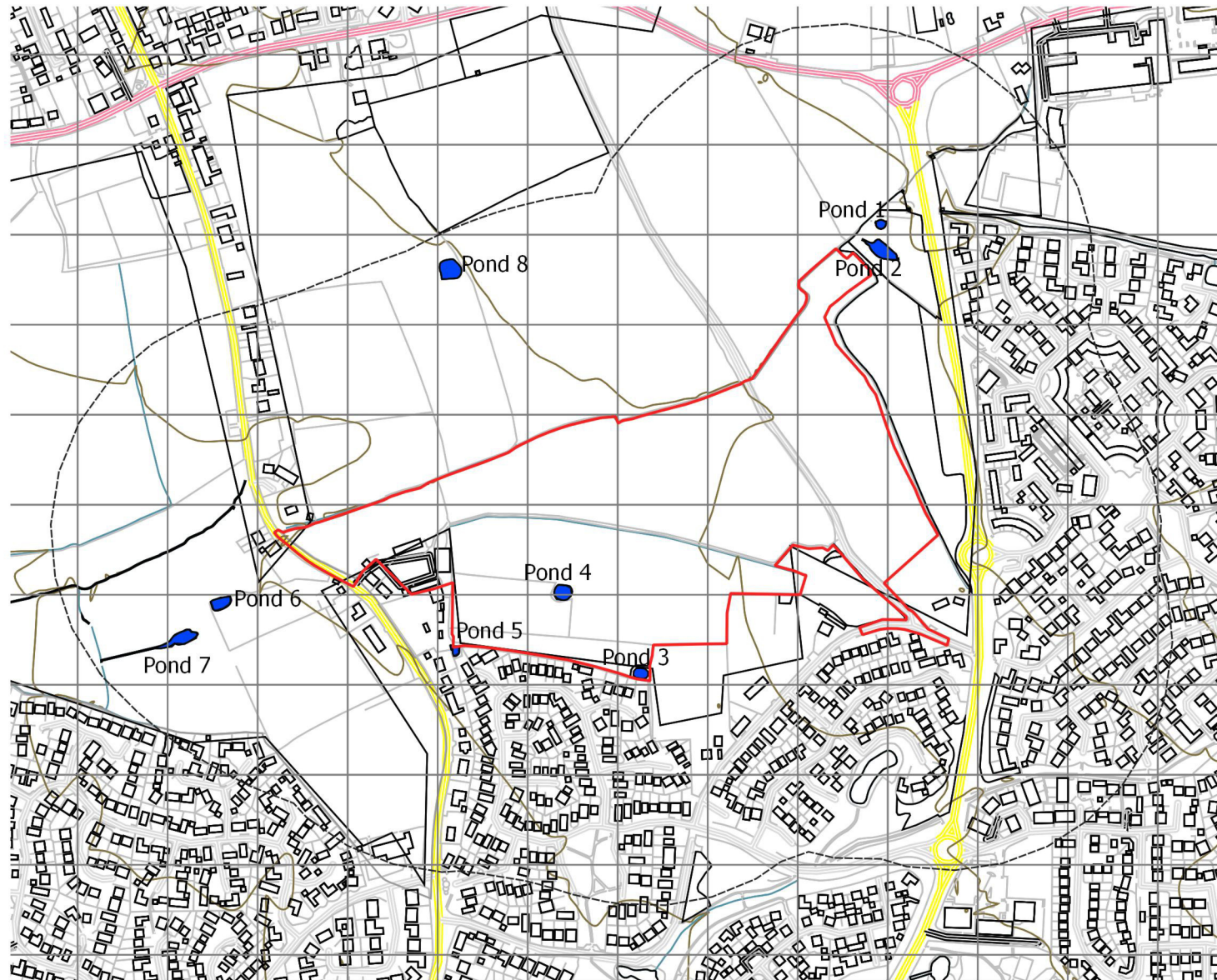
Wildlife & Countryside Act 1981 (as amended).

Figure 1:
Pond Location Map

Figure 1:
Pond Location Map

Map Ref (NGR): 350040,387800

-  250m Buffer
-  Site Boundary
-  Ponds



0 100 200 m



Appendix 1: Statutory & Planning Context (England)

Disclaimer: Appendix 1 is a guide to legislation and procedure relating to biodiversity in England. It is general guidance and it does not give specific advice in relation to any site, species or project. It represents Ecology Services Ltd interpretation of legislation and procedure as at June 2022. Readers should note that legislation and procedure changes continually and is interpreted on a case-specific basis. Nothing in Appendix 1 should be construed as an offer of advice or legal opinion.

Great Crested Newts

Great crested newts and their habitat (aquatic and terrestrial) are afforded strict protection under the Wildlife and Countryside Act 1981 (as amended) and the Conservation of Habitats and Species Regulations 2017 (as amended).

Taken together, this legislation makes it an offence to:

- Deliberately (intentionally) kill, injure or take a great crested newt;
- Possess or control any live or dead specimen or anything derived from a great crested newt;
- Deliberately (intentionally) or recklessly damage, destroy or obstruct access to a breeding site or any structure or place used for shelter or protection by a great crested newt; or
- Deliberately (intentionally) or recklessly disturb a great crested newt while it is occupying a structure or place which it uses for that purpose.
- Sell, offer for sale, possess or transport for the purpose of sale (live or dead animal, part or derivative).

The above legislation applies to all life stages of a great crested newt, including eggs, juveniles and adults. The extent of legal protection covers both the species and habitat.

The Conservation of Habitats and Species (Amendment) (EU Exit) Regulations 2019 continue the same provision for European protected species, licensing requirements and protected areas after Brexit.

The Office of the Deputy Prime Minister (ODPM) Circular 06/2005, states that the presence of a protected species is a material consideration when a planning authority is considering a development proposal that, if carried out, would be likely to result in harm to the species or its habitat.

Great crested newts are also protected by the Protection of Animals Act 1911, which prohibits cruelty and mistreatment. Releasing a great crested newt in such a way as to cause undue suffering may be an offence under the Abandonment of Animals Act 1960. In addition to the above, there are various statutory provisions relating to the transport of animals, designed to ensure their welfare.

Great crested newt is listed as a Species of Principal Importance under Section 41 of The Natural Environmental and Rural Communities (NERC) Act 2006 (as amended). Under Section 40 of the NERC Act 2006, Local Planning Authorities must, in exercising their functions, have regard, so far as is consistent with the proper exercise of those functions, to the purpose of conserving and enhancing biodiversity.

Local Planning Authorities are required to consider the impact of any development upon Species of Principal Importance listed under the NERC Act 2006 and European Protected Species (EPS) and their habitat.

Therefore, it is important to identify the presence of great crested newt individuals and also to identify suitable habitat on site in order that legal obligations regarding this species can be observed.

A Local Planning Authority (LPA) has a duty to ensure that protected species and habitats within the UK are a “material consideration” in the determination of a planning application. Therefore, a LPA is

unlikely to determine an application until all relevant information relating to protected species or habitats is submitted to fully inform the application. Relevant information includes adequate surveys and a mitigation strategy (the latter only if required) for their approval which will need to be submitted to inform any planning application.

If the survey identifies the presence of great crested newt, a Natural England Rapid Risk Assessment should be undertaken to determine if a European Protected Species (EPS) licence is required. If the Rapid Risk Assessment finds the risks to be Green: offence highly unlikely or Amber: offence likely it may be possible to undertake works using non-licensed avoidance measures.

If the risks are Red: offence highly likely, an EPS licence is likely to be required and a mitigation scheme would need to be developed, which minimises direct threats to newts and compensates for loss of habitat.

Where great crested newts are affected by development then a licence to derogate from the Conservation of Habitats and Species Regulations 2017 (as amended) would be required. European Protected Species (EPS) licence applications are processed and issued by Natural England and the EPS licence can only be applied for, once planning permission is granted, if planning permission is required.

Natural England has powers to grant a EPS licence for the following purposes;

- Regulation 55(2)(e) - 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; or
- Regulation 55(2)(f) - preventing the spread of disease; or
- Regulation 55(2)(g) - preventing serious damage to livestock, foodstuffs for livestock, crops, vegetables, fruit, growing timber or any other form of property or to fisheries. In addition, Natural England can only issue a licence if it is satisfied that the activity meets one of the above purposes and is also satisfied of the following;
- Regulation 55(9)(a) - that there is no satisfactory alternative, and
- Regulation 55(9)(b) - that the action authorised will not be detrimental to the maintenance of the population of the species concerned at a favourable conservation status in their natural range.

An alternative option may be to pay to join a District Level Licensing Scheme³ which avoids the need to undertake surveys or plan and carry out mitigation works.

In December 2016, Natural England introduced four policies for European Protected Species (EPS) licensing. The policies were revised in January 2022. The policies seek to achieve better outcomes for EPS and reduce unnecessary costs, delays and uncertainty that were inherent in the current system. The four policies are as follows:

Policy 1 - Reduce mitigation measures for impacts on EPS, allows compensation for EPS impacts to be delivered without the need to relocate or exclude populations where: exclusion or relocation measures are not necessary to maintain the conservation status of the local population; the avoid-mitigate-compensate hierarchy is followed; and compensation provides greater benefits to the local population than would exclusion and/or relocation. This policy can be used to reduce mitigation measures, such as exclusion or relocation of EPS, by increasing compensation. This policy allows killing of EPS and destruction of their habitat without needing to exclude or relocate individual animals.

³ <https://www.gov.uk/government/publications/great-crested-newts-district-level-licensing-schemes>

Policy 2 - Location of compensation habitats, allows for the provision of off-site compensation measures where the licensing tests are met, the avoid-mitigate-compensate hierarchy is followed, there are good reasons for maximising development on the site of EPS impacts and where an off-site solution provides greater benefit to the local population than an on-site solution.

Policy 3 - Let EPS use temporary habitats, relates to developments, such as mineral extraction, which temporarily create habitat which is likely to attract EPS and enables works to proceed without the exclusion of EPS where the conservation status of the local population would not be detrimentally affected. On completion of development, such sites must contribute to the conservation status of the local population as much as or more than the land use which preceded development. The measures to achieve this should be set out in a management plan and secured by a legal agreement.

Under **Policy 4** - Alternative sources of evidence to reduce standard survey requirements, Natural England may accept a lower than standard survey effort where: the costs or delays associated with carrying out standard survey requirements would be disproportionate to the additional certainty that it would bring; the ecological impacts of development can be predicted with sufficient certainty; and mitigation or compensation will ensure that the licensed activity does not detrimentally affect the conservation status of the local population of any EPS.

Other Amphibians

Where such an amphibian survey rules out the presence of great crested newts, there may be the need to develop a scheme for the conservation of the less protected amphibians as a planning condition, should the development pose a risk to a locally significant population of amphibians.

Under the Wildlife and Countryside Act 1981 (as amended) the five widespread amphibian species, smooth newt (*Lissotriton vulgaris*), palmate newt (*Lissotriton helveticus*), common toad (*Bufo bufo*) and common frog (*Rana temporaria*) receive limited protection through Section 9(5) only which makes selling, offering for sale, possessing or transporting for the purpose of sale (live or dead animal, part or derivative) an offence.

Common toad is a Species of Principal Importance, under Section 41 of the Natural Environmental and Rural Communities (NERC) Act 2006 (as amended). The Office of the Deputy Prime Minister (ODPM) Circular 06/2005, states that habitats and species listed as priorities, with reference to the former UK BAP, are capable of being a material consideration in the making of planning decisions. Amphibians may also be listed as priorities under Local Biodiversity Action Plans.

Due to general declines in most British amphibian species in recent years, many local authorities require amphibian surveys as a planning condition, or as part of environmental information submitted as part of a planning application, even where the presence of great crested newts is ruled out.

Waterbodies that support all five species of British amphibians in high numbers may be afforded statutory protection as a Site of Special Scientific Interest (SSSI) or in local plans as Sites of Importance for Nature Conservation (SINC) or a similar equivalent, for sites of local importance.

This legislation and planning overview exclude species such as natterjack toad (*Epidalea calamita*) and pool frog (*Pelophylax lessonae*) as these are not within the range of the survey.

Appendix 2 Sampling Protocol

The following text is a direct extract from:

**Analytical and methodological development for improved surveillance of the
Great Crested Newt (WC 1067)
Appendix 5 Technical advice note for field and laboratory sampling of great crested newt
(*Triturus cristatus*) environmental DNA 30th September 2014**

3. Field protocol

Field sampling should be undertaken by a suitably trained and experienced great crested newt surveyor (trained volunteer or professional). At present it is believed that eDNA water sampling does not disturb newts enough to justify the procedure being licensed by the national regulatory authority. A single visit to the target pond should be made between mid-April and June, during the newt breeding season. eDNA samples can be collected at any time of day and in any reasonable weather conditions, including light rain. It may be best to avoid heavy rain as this makes sampling more difficult and might increase the risk of cross contamination (e.g. splashing of mud which could contain great crested newt DNA from wet ground). There is evidence that unpreserved amphibian eDNA decays slightly more quickly in full sun than shaded conditions, becoming undetectable after 8 and 11 days respectively (Pilliod et al., 2014), but as long as samples are preserved the impact on detection should be slight.

3.1 Sampling equipment

The field sampling equipment used by Biggs et al. (2014) has five components (Figure 2):

- A sterile 30 mL ladle
- A sterile self-supporting Whirl-Pak plastic bag with 1 L capacity
- A sterile 10 mL pipette to resample the pond water
- Six sterile 50 mL centrifuge tubes containing preservative (Absolute Ethanol (200 Proof), Molecular Biology Grade, Fisher BioReagents (Product Code: 10644795), sodium acetate and other markers)
- Two pairs of sterile gloves

Kits can be stored at room temperature before use in an appropriate solvent store, consistent with Home Office regulations, and should be used within about two weeks of receipt. The time between kit receipt and use should be noted (see Section 5.1). Use one kit per pond up to an area of 1 ha. Beyond this, use an additional kit per hectare. However, note that as yet there is no practical experience of the effectiveness of kits used on ponds greater than 1 ha in area. Note that sampling techniques are still developing rapidly in this field and alternative preservatives to ethanol are currently being sought.

3.2 Field water sample collection protocol

The field sampling protocol should follow the steps outlined below. Gloves should be worn at all times during the sampling process, replacing the gloves between sample collection from the pond and pipetting into the sterile sub-sample tubes. Samples should be collected without entering the water, i.e. the surveyor stands only on the pond bank or muddy pond edges. This prevents disturbance of the substrate and may limit cross-contamination.

Stages of field sampling protocol

Step 1 Identify where 20 samples will be taken from the pond. The location of sub-samples should be spaced as evenly as possible around the pond margin, and if possible targeted to areas where there is vegetation which may be being used as egg laying substrate and open water areas which newts may be using for displaying.

Step 2 Open the sterile Whirl-Pak bag by tearing off the clear plastic strip c 1cm from the top (along the perforated line), then pulling the tabs. The bag will stand-up by itself.

Step 3 Collect 20 samples of 30 mL of pond water from around the pond (see 1 above) using the ladle (fill the ladle), and empty each sample into the Whirl-Pak bag. At the end the Whirl-Pak bag should be just under half full (600 mL).

NOTE: Before each ladle sample is taken, the pond water column should be mixed by gently using the ladle to stir the water from the surface to close to the pond bottom without disturbing the sediment on the bed of the pond. It is advisable not to sample very shallow water (less than 5-10 cm deep).

Step 4 Once 20 samples have been taken, close the bag securely using the top tabs and shake the Whirl-Pak bag for 10 seconds. This mixes any DNA across the whole water sample.

Step 5 Put on a new pair of gloves to keep the next stage as uncontaminated as possible.

Step 6 Using the clear plastic pipette provided take c15 mL of water from the Whirl-Pak bag and pipette into a sterile tube containing 35 mL of ethanol to preserve the eDNA sample (i.e. fill tube to the 50 mL mark). Close the tube ensuring the cap is tight.

Step 7 Shake the tube vigorously for 10 seconds to mix the sample and preservative. This is essential to prevent DNA degradation. Repeat for each of the 6 conical tubes in the kit. Before taking each sample, stir the water in the bag to homogenize the sample - this is because the DNA will constantly sink to the bottom.

Step 8 Empty the remaining water from the Whirl-Pak bag back into the pond.

Step 9 The box of preserved sub-samples is then returned at ambient temperature immediately for analysis. If batches of samples are collected and stored prior to analysis they should be refrigerated at 2-4° C. Kits can be stored for up to one month in a refrigerator before analysis. It is not necessary to freeze samples. Freezing may damage storage bottles, which can lead to leaking during transit, and also unnecessarily increases costs by requiring refrigerated transport. The length of time eDNA samples are stored in a refrigerator prior to analysis should be recorded and passed on to the analysing laboratory. Use an appropriate labelling system to ensure that the kits are supplied with a unique reference number.

See eDNA protocol form below and overleaf

Sample Kit ID:			
Kit received (Date):		Signed into store by:	
Kit signed out (Date)		Signed out by:	
Project name:			
Project no.:			
Have previous pond surveys been undertaken this year:	Yes/No		
	Date:		
If Yes, has evidence of anti-contamination been provided	Yes/No		
Pond number:			
Date of survey:		Survey time:	
Lead surveyor name:			
Surveyor Gcn Licence: (Must be licensed)		Surveyor eDNA trained: (Survey must be trained)	Yes/No
Number of samples taken at pond: (20 samples required)		% of pond sampled: (Min 80/90% desired)	
Is the pond <1ha?	Yes/No		
Notes of any constraints to sampling: (Circle as required)	Access difficulties, surveyor entered water, potential contamination of sampling equipment, loss of ethanol, sediment disturbance, shallow water, cattle disturbance of sediment, noticeable distance of sediment by waterfowl, other...		
Return to fridge: (Date and time)		Signed into fridge by:	
Fridge temperature check: (Date & time)		Fridge temperature checked:	
Collected by Courier: (date)		Collected by Courier: (Staff responsible)	
eDNA sampling result	Positive/Negative		
Other amphibian survey result:	Visit 00 of 4/6/Date/Method/Results		

Appendix 3

SureScreen Scientifics Technical report



Folio No: E12969
 Report No: 1
 Purchase Order: 22012
 Client: ECOLOGY SERVICES LTD
 Contact: Paige Hickey

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: 26/04/2022
Date Reported: 05/05/2022
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
1174	Pond 6 Chapel Lane	SJ 49420 87665	Pass	Pass	Pass	Negative	0
1177	Pond 4	49830 87711	Pass	Pass	Pass	Negative	0
1178	Pond 1	SO 1918 8811	Pass	Pass	Pass	Negative	0
1180	6a	SJ	Pass	Pass	Pass	Negative	0
1182	Pond 2	SJ 80191 8811	Pass	Pass	Pass	Negative	0

If you have any questions regarding results, please contact us: ForensicEcology@surescreen.com

Reported by: Chris Troth

Approved by: Esther Strafford

Pond 6a = Pond 7



Folio No: E12951
 Report No: 1
 Purchase Order: 22012
 Client: ECOLOGY SERVICES LTD
 Contact: Paige Hickey

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: 26/04/2022
Date Reported: 28/04/2022
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
1179	Pond 8 Chapel Lane	49705 88067	Pass	Pass	Pass	Negative	0
1181	Pond 5 Chapel Lane	SJ 493 882	Pass	Pass	Pass	Negative	0

If you have any questions regarding results, please contact us: ForensicEcology@surescreen.com

Reported by: Chris Troth

Approved by: Esther Strafford



Folio No: E13976
 Report No: 1
 Purchase Order: 22012
 Client: ECOLOGY SERVICES LTD
 Contact: Lorna Smithers

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: 07/06/2022
Date Reported: 14/06/2022
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
1176	Pond 3 Chapel Lane Crost Farm	SJ 49919 87618	Pass	Pass	Pass	Negative	0

If you have any questions regarding results, please contact us: ForensicEcology@surescreen.com

Reported by: Esther Trafford

Approved by: Chelsea Warner



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 4
Chapel Lane, Widnes Amphibian Survey Report 2021, Ecology Services (2021)

Chapel Lane, Widnes

Amphibian Survey Report

Compiled by Ecology Services Ltd.

on behalf of

Taylor Wimpey

January 2021



Ref. number: 20024
Written by: BM
Checked: LR
Approved: LR

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Executive Summary

Ecology Services Limited was commissioned by Taylor Wimpey in March 2020 to undertake an amphibian survey and great crested newt environmental DNA (eDNA) survey of ponds within land at Chapel Lane, Widnes; National Grid Reference (NGR) 35004, 38780. The site lies within an area of countryside characterised by improved grassland and semi-improved pasture, crossed by hedgerows and ditches and with scattered ponds.

Ponds accessible within the site and its 250m buffer include:

- Pond 1 (Below-average HSI)
- Pond 2 (Poor HSI)
- Pond 3 (Below-average HSI)
- Pond 4 (Below-average HSI)

A review of Ordnance Survey maps and aerial photographs indicate there are an additional four ponds within 250m of the site. Access to third party land was not sought due to the COVID-19 pandemic. Surveys of these ponds will be required to fully inform a planning application.

An amphibian survey of accessible waterbodies located within the site and its 250m buffer was undertaken in spring 2020. The surveys were carried out in accordance with survey methodologies stated within English Nature's Great Crested Newt Mitigation Guidelines (2001). eDNA surveys were undertaken in conjunction with the amphibian survey and amphibian surveys stopped on receipt of a negative eDNA result.

Due to the ongoing COVID-19 pandemic access was restricted to several of the ponds for both amphibian and eDNA surveys along with presence of barbed wire fences.

The amphibian surveys found great crested newts to be absent at the site; all ponds surveyed for eDNA were found to be negative. Other amphibians recorded included: smooth newt and common frog. All three ponds surveyed were limited by access and below the desired sample area of 80%, which reduces the confidence in the negative results. Due to the access constraints during the eDNA surveys, it is recommended that repeat eDNA surveys are undertaken of Ponds 1-4 to try and attain a higher percentage of sample area and to further back up the negative result.

It is recommended that the ponds within the site are retained and protected during construction. If a pond is to be impacted, an appropriate mitigation strategy will be required detailing appropriate working methods and mitigation procedures. Works, if necessary, should be carried under the supervision of a suitably qualified ecologist.

As a precautionary measure, all construction contractors should be made aware of the potential presence of common amphibians, including common toad, common frog, smooth newt and palmate newt, on site and the potential risks to them. Should any common amphibians be encountered, they should be moved to suitable cover away from development works. If large numbers of common toad are found, then the acting consultant should be contacted for further advice on measures to minimise impacts.

The development also offers the opportunity to enhance the existing ponds by clearing accumulated silt and leaf litter and clearing fallen and overshadowing trees and/or create new ponds as part of the sustainable drainage system for the development. Any work on ponds must take full account of protected species such as water vole, amphibians and aquatic invertebrates. The brook along the northern boundary of the site will also be enhanced including the creation of a species-rich habitat corridor.

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Appendix 1: Statutory & Policy Context
Appendix 2: Results of Habitat Suitability Index Assessment
Appendix 3: eDNA Sampling Protocol
Appendix 4: SureScreen Scientifics Technical Report

1.0 Introduction

- 1.1 Ecology Services Limited was commissioned by Taylor Wimpey in March 2020 to undertake an amphibian survey and great crested newt environmental DNA (eDNA) survey of ponds within land at Chapel Lane, Widnes; National Grid Reference (NGR) 35004, 38780. See Figure 1, Site location plan and pond locations.

Site Description

- 1.2 The site is located on the outskirts of the town of Widnes, within the Borough of Halton but on the boundary with Knowsley Council and contains a mix of habitats including: improved and poor semi-improved grassland, species-rich and species-poor hedgerows, wet and dry ditches, two ponds, scattered trees and buildings. The land to the north and west of the site is largely under arable cultivation crossed by hedgerows and ditches with further ponds and an area of broadleaved woodland. Broadleaved woodland adjoins the site to the east, beyond which lies Queensbury Way residential development with further residential development to the south.
- 1.3 The site is currently included in the draft Halton Borough Council Delivery and Allocations Local Plan 2014-2037 (2018) as a residential development site. At the time of compiling this report, no development scheme has been prepared and only basic biodiversity enhancements are provided. This report shall be updated to ensure that mitigation and enhancement measures are incorporated once the scheme is finalised.
- 1.4 The aims of the survey were to:
- Undertake a walkover survey of the proposed development site and surrounding land, to locate all waterbodies within 250m (where there is land access).
 - Undertake a desktop study for existing records of amphibians, information on local population status of amphibians and a review of existing maps and satellite imagery where there is no land access.
 - Undertake a Habitat Suitability Index (HSI) assessment of all water-bodies with land access.
 - Undertake an amphibian survey and eDNA sampling of waterbodies, following Natural England's recommended survey methodology.
 - To identify the presence/absence of great crested newt (*Triturus cristatus*) and other amphibians on site.
 - Undertake a population size class assessment of great crested newt populations (if found to be present).
- 1.5 Natural England's Guidance (English Nature, 2001) advises that surveys of ponds up to around 500m from the proposed development might need to be surveyed. The decision on whether to survey depends primarily on how likely it is that the development would affect newts using those ponds. For developments resulting in permanent or temporary habitat loss at distances over 250m from the nearest pond, careful consideration should be given as to whether a survey is appropriate. Surveys of land at this distance from ponds are normally appropriate when all of the following conditions are met (Natural England's Great Crested Newt Licence Method Statement Template, 2015):
- (a) maps, aerial photos, walk-over surveys or other data indicate that the pond(s) has potential to support a large great crested newt population,
 - (b) the footprint contains particularly favourable habitat, especially if it constitutes the majority available locally,

(c) the development would have a substantial negative effect on that habitat, and

(d) there is an absence of dispersal barriers.

- 1.6 In this instance a 250m survey area was considered appropriate due to additional ponds located up to 500m from site, are located within the residential area or located the other side of a busy road (Cronton Road) and therefore offer very little connectivity to the site.
- 1.7 eDNA surveys were undertaken in conjunction with the amphibian surveys. This was due to a population size class assessment only being required if great crested newts were found to be present, therefore if eDNA surveys found great crested newts to be absent, amphibian surveys would no longer be required.
- 1.8 eDNA is nuclear or mitochondrial DNA that has become separated from an organism into the environment. Sources of eDNA include secreted faeces, mucous, gametes, shed skin, hair and carcasses. In aquatic environments, eDNA is diluted and distributed while suspended in the water column where it persists for 7–21 days, depending on the conditions.
- 1.9 Recent research has shown that the DNA of a range of aquatic organisms can be detected in water samples at very low concentrations using qPCR (quantitative Polymerase Chain Reaction) methods.
- 1.10 A test primer has been developed (a length of artificial DNA which specifically binds to and amplifies the DNA of the target organism) which is able to detect great crested newt eDNA successfully in water samples.

2.0 Planning Policy and Legislation

Planning Policy

- 2.1 The National Planning Policy Framework (NPPF, 2019) places a clear responsibility on Local Planning Authorities (LPA) to contribute to conserving and enhancing the natural and local environment. LPAs should promote the conservation, restoration and enhancement of priority habitats, ecological networks and the protection and recovery of priority species (e.g. Species of Principal importance, Local Biodiversity Action Plan species); and identify and pursue opportunities for securing measurable net gains for biodiversity. The Office of the Deputy Prime Minister (ODPM) Circular 06/2005 provides administrative guidance on the application of the law in relation to planning and nature conservation.
- 2.2 A Local Planning Authority (LPA) has a duty to ensure that protected species and habitats within the UK are a 'material consideration' in the determination of a planning application. Therefore, an LPA is unlikely to determine an application until all relevant information relating to protected species or habitats is submitted to fully inform the application. Relevant information includes adequate surveys and, where required, mitigation strategies, which will need to be submitted to inform a planning application.
- 2.3 When dealing with planning applications where a European Protected Species (EPS) (great crested newt) may be affected, a Local Authority is a 'competent authority' within the meaning of Regulation 7 of the Conservation of Habitats & Species Regulations 2017. The Local Authority must therefore exercise their functions under the provisions made within the 2017 Regulations, and planning decisions should only be made when European Protected Species and their habitats are fully taken into account.

Legislation

- 2.4 The great crested newt is protected under The Conservation of Habitats and Species

Regulations 2017¹. Regulation 43 (1) makes it an offence to:

- Deliberately capture or kill a great crested newt
- Deliberately disturb a great crested newt
- Deliberately take or destroy the eggs of a great crested newt
- Damage or destroy a breeding site or resting place of a great crested newt

2.5 The species is also protected under The Wildlife and Countryside Act 1981 (as amended), which makes it an offence to:

- Intentionally kill, injure or take a great crested newt
- Possess or control any live or dead specimen or anything derived from a great crested newt
- Intentionally or recklessly damage, destroy or obstruct access to any structure or place used for shelter or protection by a great crested newt
- Intentionally or recklessly disturb a great crested newt while it is occupying a structure or place which it uses for that purpose

2.6 The legislation applies to all life stages of great crested newts.

2.7 Section 40 of the Natural Environmental and Rural Communities (NERC) Act 2006 places a statutory duty on public authorities, in exercising their functions, to conserve biodiversity in England. Species of Principal Importance for the conservation of biodiversity in England, identified by the Secretary of State in consultation with Natural England, are listed Section 41 of the NERC Act. The Local Planning Authority and government bodies (e.g. Natural England) will expect the overall design of the development to have regard for the conservation of these species. Section 41 of the NERC Act lists great crested newt and common toad (*Bufo bufo*) as Species of Principal Importance.

3.0 Methodology

Desktop Study

3.1 Information regarding historic species records of amphibians were collated from the following sources; listed in Table 1.

Table 1: Desktop Study Resources and Record Centres Consulted

Sources of Information	Information Supplied
Biodiversity Information Centre for Cheshire, Halton, Warrington, and Wirral - RECORD Merseyside Biobank	To identify locally protected sites or species of interest within 2km of the site.
Natural Environmental and Rural Communities (NERC) Act 2006	Identification of Species of Principal Importance known to occur in the region.
Halton Biodiversity Action Plan (the North Merseyside Biodiversity Action Plan (LBAP) has also been reviewed as the site lies on the boundary between the two LBAPs).	Identification of local priority amphibian species known to occur in the region.
Multi-Agency Geographic Information for the Countryside (MAGIC) https://magic.defra.gov.uk/	Identification of European Protected Species mitigation licences, Class Survey Licence Returns and District Level Licensing Pond Surveys for great crested newt.

¹ https://naturalengland-defra.opendata.arcgis.com/datasets/2bd21192cf0a413fbce2176c0752c5a2_0

3.2 The aim of the desktop study was to check for any great crested newt records and information on great crested newt status in the local area. Where great crested newts are confirmed

¹ The Conservation of Habitats and Species (Amendment) (EU Exit) Regulations 2019 continue the same provision for European protected species, licensing requirements and protected areas after Brexit.

present and a licence required to permit the works, desktop study data is a requirement of licensing.

3.3 The data searches were undertaken as part of the Preliminary Ecological Appraisal with RECORD and Merseyside Biobank were undertaken on 10th and 11th April 2019 respectively. However, the data obtained is now over twelve months old and should be updated prior to submission of a planning application.

3.4 A review of existing maps such as Ordnance Survey and digital based maps and aerial photographs, such as the Multi-Agency Geographic Information for the Countryside (MAGIC) website (<https://magic.defra.gov.uk/>), was undertaken to identify water bodies within 250m of the site.

Habitat Suitability Index (HSI) Assessment

3.5 The Habitat Suitability Index (HSI) for the great crested newt was developed by Oldham et al. (2000). It is a scoring system of evaluating habitat quality and quantity using a numerical index, between 0 and 1. 0 indicates unsuitable habitat, and 1 represents optimal habitat. The HSI for the great crested newt incorporates ten suitability indices, all of which are factors thought to affect great crested newts (NARRS, undated).

3.6 The HSI is a geometric mean of ten suitability indices: $HSI = (SI1 \text{ (Geographic Location)} \times SI2 \text{ (Pond area)} \times SI3 \text{ (Pond permanence)} \times SI4 \text{ (Water quality)} \times SI5 \text{ (Shading)} \times SI6 \text{ (Presence of water fowl)} \times SI7 \text{ (presence of fish)} \times SI8 \text{ (Pond density in area)} \times SI9 \text{ (Terrestrial Habitat Quality)} \times SI10 \text{ (Macrophyte cover in pond)})^{1/10}$.

3.7 Lee Brady has developed a system for using HSI scores to define pond suitability for great crested newts on a categorical scale (NARRS, undated):

HSI Scores	Pond Suitability
<0.5	Poor
0.5 – 0.59	Below average
0.6 – 0.69	Average
0.7 – 0.79	Good
>0.8	Excellent

3.8 The great crested newt HSI is potentially a useful tool in survey and mitigation. One benefit is that it can be undertaken in a single field visit (with supporting desk work), and at any time of the year (though some variables are more easily measured in spring and summer). Its main uses are:

1) in surveys, to assess habitat quality in a repeatable, objective manner. In particular, the HSI allows individual factors that influence newt presence to be easily identified. These factors could help explain a very high or very low count. A high HSI can justify employing additional survey effort or methods if no newts are found initially.

2) in impact assessments, to allow a measure of how damaging a development could be. HSI might also be used as a screening tool to select no impact or minimal impact options in conjunction with (3) below.

3) in risk assessments, helping to decide whether an offence might be committed, and therefore whether a licence should be applied for. If a pond has a very low HSI score (say <0.5) then there would typically be a minimal chance of great crested newt presence. Hence, with due care and in limited circumstances (see also caveats below), the HSI might be used in the absence of newt survey to help conclude that an offence is highly unlikely and therefore work could proceed in that area without a licence. This application of the HSI should only be used where the predicted impacts

- were newts to be present - would be low (e.g. development at least 100m from pond, permanent habitat loss <0.5ha or temporary habitat loss <5ha). The developer and consultant should realise that there would still be a risk of committing an offence, but it would typically be so low as to be negligible. Obviously, note that if HSI >0.5, this is not confirmation of newt presence; a newt survey would be required to confirm this.

4) in habitat enhancement, HSI could be used to identify the low-scoring factors in an existing pond that need addressing to improve its quality for newts.

5) in post-development monitoring, to allow an assessment of habitat condition.

3.9 The HSI is not a substitute for undertaking newt surveys; it indicates but cannot confirm presence or absence. 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 allow the conclusion that any particular pond with a high score will support newts, or that any pond with a low score will not do so. Very low HSI scores may be used along with scheme details to infer a minimal chance of committing an offence in low impact situations, as explained above. Whilst current data indicate a generally good relationship, HSI scores should not be used to predict population size. Care should be taken when interpreting low HSI scores; for example, a low scoring pond close to an occupied newt pond may still support newts (Natural England Great Crested Newt Licence Method Statement Template, 2015).

3.10 The following survey methodologies adhere to Natural England's survey guidelines. A combination of survey techniques was utilised at this site, including bottle trapping, torch light surveys, egg searches and netting, to give a full picture of amphibian activity and to compensate for limitations of any one technique.

Torch Light Survey Methodology

3.11 Waterbodies were surveyed by walking the perimeter of the waterbodies after dusk and searching the water with a powerful torch (Nightlite, one million candle power). It is often not possible to achieve 100 % coverage of shorelines of all waterbodies using the torch light surveys because of access difficulties, for example dense marginal vegetation. Estimates of the percentage of shoreline of each waterbody surveyed and other factors affecting torch light counts are provided.

Bottle Trap Survey Methodology

3.12 The bottle traps used were constructed from a two litre plastic bottle, with an inverted neck, secured to the pond bottom by a cane passing through the trap at an angle and operated with an air bubble.

3.13 Bottle traps were set at an average spacing of 2m along accessible shorelines (to allow estimation of population size). Where significant lengths of shoreline are inaccessible, or if the pond is very large, bottle trapping will be used in the accessible areas, using 2m spacing as a means of standardising survey effort.

3.14 Traps were set in the evening and operated overnight, emptying the traps early the following morning. Newts are particularly at risk in traps on warm, sunny days in shallow water. Traps were operated well within the maximum time limits set by Natural England (2001):

- Up to 17 hours for traps with an air bubble throughout the survey period,
- 12 hours in March through to April for traps without an air bubble,
- 10 hours in May for traps without an air bubble,
- 8 hours June for traps without an air bubble.

- 3.15 Estimates of the percentage of shoreline of each waterbody surveyed and other factors affecting bottle counts are provided. Where it was not possible to logistically trap an entire shoreline, sample sections were trapped (with bottles at 2m spacing) in locations deemed most likely to yield newts e.g. amongst egg-laying vegetation for females and in open water for displaying males.

Egg Search Methodology

- 3.16 Great crested newts lay their eggs singly on the leaves of submerged vegetation. The vegetation is folded over the egg to form a protective 'purse'. The eggs of great crested newts can be distinguished from those of the two smaller newt species, smooth and palmate newts because they are slightly larger (3 – 4 mm) in size, with a pale lemon coloured yolk. The smooth and palmate newt eggs are 2 – 3 mm in size, with a white or grey tinged yolk. A female great crested newt can lay 300 + eggs, so detection rates for eggs are higher than for adults. However, eggs can prove difficult to find in heavily vegetated ponds with small newt populations, or those with no accessible vegetation. The presence of eggs confirms the waterbody as a breeding site. However, it is impossible to obtain any reliable population estimate on the basis of a newt egg count. Also, it is undesirable for the conservation of the species to survey for eggs intensively, as the unfolding of vegetation to confirm type of egg will tend to render the egg more vulnerable to predation or to being dislodged. Therefore, no attempt will be made to quantify the number of eggs in a pond; searching will be stopped once an egg has been found.
- 3.17 Searching for newt eggs is useful between March and July (peak egg laying period April - June). Not all eggs are viable, so although most eggs will have hatched by June, non-viable eggs will remain on vegetation longer, before decaying or becoming predated. Aquatic vegetation was searched for newt eggs around the shoreline, looking for the characteristic shape of folded leaves on favoured plants for ovipositing.

Netting Methodology

- 3.18 Waterbodies were hand netted during the day to search for great crested larvae and other amphibians. The standard procedure for hand netting was used; a D-net swept vigorously through the water in 2m sweeps with a survey effort of 15 minutes per 50m of shoreline (English Nature, 2001, Froglife Advice Sheet 11).

Evaluation Methodology

- 3.19 Using JNCC/Natural England's population size class assessment (Gent and Gibson, 2003/English Nature, 2001), the populations of amphibians present can be classified as follows:

Table 2: Amphibian Population Size Class Assessment

Species	Method	Low/ small population	Good/ medium population	Exceptional/ large population
Great crested newt	Seen or netted in a day	<5	5-50	>50
	Counted at night	<10	10-100	>100
Smooth / palmate newt	Netted in day/ counted at night	<10	10-100	>100
Common toad	Estimated	<500	500-5,000	>5,000
	Counted	<100	100-1,000	>1,000
Common frog	Spawn clumps counted	<50	50-500	>500

Timing

- 3.20 Torch light surveys, bottle trapping, netting and egg searches were employed during the peak period of breeding activity (April). Surveys were carried out on the following dates, using the following methods, as shown in Table 3:

Table 3: Summary of Field Survey Techniques, 2020

Survey dates	Torch light counts	Bottle trapping	Egg/spawn searches	Netting
15 th /16 th April 2020	✓	✓	✓	✓

Weather Conditions

- 3.21 Weather conditions on the nights of survey were reasonable, with no appreciable rain or wind affecting survey. Temperatures ranged from 7°C to 16°C at midnight and at the end of surveys.

eDNA Methodology

- 3.22 The method used to collect the eDNA followed the Analytical and Methodological Development for Improved Surveillance of the Great Crested Newt (WC1067), Appendix 3 Technical Advice Note for Field and Laboratory Sampling of Great Crested Newt (*Triturus cristatus*) environmental DNA, dated 30th September 2014.
- 3.23 See Appendix 3 Sampling Protocol for equipment used and sample collection protocol. Upon collection samples were sent by courier to SureScreen Scientifics for analysis. The analysis involves using qPCR with twelve replicates per pond). See Appendix 4 SureScreen Scientifics Technical Report for the results of the sample analysis and the number of positive replicates.
- 3.24 The research carried out by the Freshwater Habitats Trust (FHT) shows that the test can be more effective in confirming the presence or absence of great crested newt than a combination of conventional survey techniques. Nevertheless, the eDNA methodology is not error free.
- 3.25 A recent study that designed a statistical model on a set of data provided by Natural England identified error within the two stages of the eDNA methodology and calculated approximate figures for this data set, as follows. Stage 1 false positive error $p=0.15$ (15% error probability), stage 1 false negative error $p=0.27$ (27% error probability), stage 2 false positive error $p=0.05$ (5% error probability) and stage 2 false negative $p=0.19$ (19% error probability) (Griffin et al., 2019), where stage 1 is the ecologists sampling stage and stage 2 is the labs analysis stage. These error probabilities are based on an existing dataset of 200 ponds with 1 eDNA sample taken per pond and as such these error probabilities may vary and are not site or pond specific. Further samples taken from the same pond significantly reduces the probability of error.
- 3.26 There are variables within the environment and waterbody characteristics (covariates) known that may impact the probability of an error occurrence at stage 1 which include:
- eDNA usually sheds as clumps and is therefore spread around the pond disproportionately and target DNA may be missed during stage 1.
 - Dense mats of vegetation or wide shallow drawdown zones around the pond which may prevent the mixing of eDNA into the water column resulting in the possibility of target DNA being missed.
 - Water flowing between ponds allows the transport of eDNA from one pond to another and may even remove eDNA from the survey pond.
 - Disruption of sediment may cause the collection of historic DNA causing a false positive error at stage 1 or reduce efficiency of DNA extraction at stage 2.

- 3.27 Griffin et al., 2019, found covariates to significantly affect error probability at stage 2:
- The maximum pond depth and presence of macrophytes in the pond significantly increases true positive probability therefore reducing error probability.
 - The pond length and pond density within an area significantly decrease true positive probability therefore increasing error probability.
 - The presence of fish decreases the probability of false positive therefore reducing error probability.
- 3.28 A pond description has been provided below and any known factors of error were taken into account while undertaking the survey, with anything that could potentially cause error noted within Table 8.
- 3.29 Samples were taken on the 15th April for Ponds 1, 2, and 4, and the 19th May for Pond 3. All of which are within the accepted sample period of mid-April to June.

Weather Conditions

- 3.30 Weather conditions on the sample days were reasonable, with no appreciable rain or wind affecting the survey.

Personnel

- 3.31 Torch light surveying of known great crested newt ponds, catching great crested newts in bottle traps, or hand nets, disturbing refugia and egg searches are all licensed activities. Torch light, bottle trap, netting and egg searching were conducted under Natural England Class Survey Licence (CLS) by Miss C. Wood MSc BSc (Hons) (2019-39994-CLS-CLS) and Mr. B. Meadows BSc (Hons), ACIEEM (2017-32734-CLS-CLS). For health and safety reasons assistants were employed.

Limitations

- 3.32 It is often the case that sites cannot be surveyed using all available techniques to full effect, as there are frequent restrictions such as season, weather and access, which limit survey intensity. In this case, limitations were present with all ponds surveyed.
- Pond 1 was partially inaccessible (approximately 50% of the pond's perimeter) due to steep banks, scrub and two outfalls being located on the perimeter of the pond blocking access. The torchlight survey was also limited due to the pond being covered in duckweed. Netting was used as an additional methodology to further increase the survey effort.
eDNA samples were limited by access restrictions, as mentioned above (approximately 70% of the pond sampled).
 - Pond 2 was mostly dry at the time of the survey and could not be bottle trapped. The only area of water present was highly polluted and contained no vegetation, therefore egg searches could also not be performed. A torch light survey and netting were still performed even though it is thought that Pond 2 is very unlikely to contain great crested newts.
eDNA samples could not be taken of Pond 2 due to there being very little water present.
 - Pond 3 was not accessible during the amphibian survey due to it only being accessible through the landowner's farmyard and, because of the COVID-19 national lockdown, access through the farmyard was not permitted. Access was later permitted to take eDNA samples.
eDNA samples were limited by access restrictions from steep banks, dense scrub, and barbed wire fence (approximately 20% of the pond sampled).
 - Pond 4 was partially inaccessible (approximately 50% of the pond's perimeter) due to steep banks, scrub and a barbed wire fence. Netting was used as an additional methodology to further increase the survey effort.

eDNA samples were limited by access restrictions, as mentioned above (approximately 50% of the pond sampled).

- In view of the COVID-19 pandemic, Taylor Wimpey did not pursue access to Ponds 5, 6, 7 and 8 and were therefore not surveyed by either HSI assessment, amphibian surveys or eDNA surveys.

3.33 There were no constraints identified when undertaking the HSI assessments of Ponds 1-4.

3.34 Where the percentage of eDNA pond sample areas is under the desired 80%, caution should be taken, especially where a negative result has been found.

4.0 Results and Evaluation

Desktop Study

4.1 The desktop study found records of great crested newt and common toad within 2 km of the development site. The records for great crested newt are all located to the north of the A5080 (Cronton Lane), the nearest record being approximately 430m north east of the site. There were no records identified on MAGIC within 2km of the site.

Species of Principal Importance

4.2 Section 41 of the Natural Environmental and Rural Communities (NERC) Act 2006 lists great crested newt and common toad as a Species of Principal Importance.

4.3 The great crested newt is still quite widespread in Britain. The species is locally numerous in parts of lowland England and Wales but is absent or rare in Cornwall and Devon. It is widespread but local in Scotland and absent from Northern Ireland.

4.4 The species has suffered a decline in recent years with studies in the 1980s indicating a national rate of colony loss of approximately 2% over five years. It is estimated that there are a total of 18,000 great crested newt ponds within Britain, although only 3,000 of these have been identified. The British population is amongst the largest in Europe, where it is threatened in several countries.

4.5 The common toad is a widespread amphibian found throughout Britain although is absent from Ireland, however the species is considered to be in notable decline.

Local Biodiversity Action Plan (LBAP)

4.6 The North Merseyside Biodiversity Action Plan and the Cheshire Region Biodiversity Action Plan both list great crested newt as a species of local concern. The North West of England is a stronghold for this species in Britain as a whole.

Pond Description

4.7 A total of 8 ponds were located within 250 metres of the site during the walkover surveys and investigations, two of which (Ponds 3 and 4) are within the site. At the time of the amphibian survey only 3 ponds were accessible with an additional pond which was subsequently sampled for eDNA. The ponds and their locations are shown on Figure 1. Detailed pond descriptions for the 4 ponds accessed are provided below.

4.8 Pond 1 was approximately 7-8 m wide, surrounded by grey/goat willow (95% pond margin) with inlet and outlet pipes. Bulrush (*Typha latifolia*) covered approximately 35% of the pond together with some common duckweed (*Lemna minor*). No other aquatic or emergent vegetation was present. The water was slightly turbid.

4.9 Pond 2 consisted of a small area of swamp vegetation dominated by common reed, most of which had no standing water. There was a small area of shallow standing water at the southern end near an inlet pipe with no aquatic or emergent vegetation. There is an outfall

from this swamp area to the ditch to north. This swamp area may be designed to take run-off from Queensbury Way to the east. The standing water was very turbid and appeared to be polluted at the time of the survey.

- 4.10 Pond 3 was very overgrown, approximately 15m diameter, with collapsed goat/grey willows within the pond. The pond probably contains a lot of leaf litter from the surrounding trees, however the water quality appeared to be reasonable. No aquatic/emergent vegetation was present and rosebay willowherb and some bracken (*Pteridium aquilinum*) was present on the banks.
- 4.11 Pond 4 was approximately 25 m in diameter with no aquatic or emergent vegetation. Bramble scrub was present on the banks with some common nettle, honeysuckle (*Lonicera periclymenum*) and bittercress (*Cardamine* sp.) together with a small horse chestnut tree, a hawthorn bush and a large crack willow. The water quality appeared average and a little turbid. Four mallards (*Anas platyrhynchos*), two males and two females, were present during the Preliminary Ecological Appraisal survey and the farmer advised that the pond is stocked with carp.

Habitat Suitability Index Assessment

- 4.12 Ponds 1-4 were subjected to a Habitat Suitability Index (HSI) assessment to determine the pond's suitability to support great crested newt. A summary of the results is shown in Table 4 and the raw data is within Appendix 2.

Table 4: Summary Results of Habitat Suitability Index Assessment

Pond No.	HSI Score	Pond Suitability	Summary of Contributing Factors
1	0.542	Below Average	Pond sometimes dries; waterfowl and fish are absent; moderate terrestrial habitat quality.
2	0.348	Poor	Pond dries annually; waterfowl and fish are absent; moderate terrestrial habitat quality.
3	0.555	Below Average	Pond never dries; waterfowl are minor and fish are possible; moderate terrestrial habitat quality.
4	0.555	Below Average	Pond never dries; waterfowl are minor and fish are minor; poor terrestrial habitat quality.

Amphibian Survey Results

- 4.13 No evidence of great crested newts was discovered at this site, using all the methods described above. Smooth newts and common frog were recorded by bottle trapping, torch light survey, visual observations and netting. Only one survey of Ponds 1, 2 and 4 were undertaken due to negative eDNA results being returned for all three ponds. The results of the surveys are detailed in Tables 5 & 6 below.

Table 5: Great Crested Newt 2020 Survey Results

Pond number/reference:		Pond 1		Survey Method:	Torch Light Survey			Bottle-trapping			Netting			Egg search	Larvae
No. of survey visits to this pond			1		Torch power: >=1,000,000 cp			No. of traps used:		8				GCN eggs found?	Larvae found? (via any method)
Great Crested Newt:			Sex/life stage:		Male	Female	Imm.	Male	Female	Imm.	Male	Female	Imm.		
Date	Air temp. (°C)	Veg. cover	Turbidity	Adult totals:	0	0	0	0	0	0	0	0	0	No	No
15.04.2020	16	5	2 <th colspan="2">0</th> <th colspan="2">0</th> <th colspan="2">0</th>		0			0			0				
GCN Peak adult count for this pond in any one visit (by torch, trap or net):					0										

Comments: Duckweed present limiting torch light survey effectiveness, netting was used as an additional measure

Vegetation cover score (0-5); 0 = no vegetation obscuring survey; 5 = vegetation completely obscuring survey; Turbidity score (0-5); 0 = completely clear; 5 = very turbid. X = Method not used.

Pond number/reference:		Pond 2		Survey Method:	Torch Light Survey			Bottle-trapping		Netting			Egg search	Larvae	
No. of survey visits to this pond			1		Torch power: >=1,000,000 cp			No. of traps used:	0				GCN eggs found?	Larvae found? (via any method)	
Great Crested Newt:			Sex/life stage:		Male	Female	Imm.	Male	Female	Imm.	Male	Female			Imm.
Date	Air temp. (°C)	Veg. cover	Turbidity	Adult totals:	0	0	0	X	X	X	0	0	0	X	No
15.04.2020	16	0	5		0			X			0				
GCN Peak adult count for this pond in any one visit (by torch, trap or net):					0										

Comments: Very little water present (too little to bottle trap), water clearly very polluted and turbid, netting was used as an additional measure.

Vegetation cover score (0-5); 0 = no vegetation obscuring survey; 5 = vegetation completely obscuring survey; Turbidity score (0-5); 0 = completely clear; 5 = very turbid. X = Method not used.

Pond number/reference:		Pond 4		Survey Method:	Torch Light Survey			Bottle-trapping			Netting			Egg search	Larvae
No. of survey visits to this pond		1	Torch power: >=1,000,000 cp			No. of traps used:		4	GCN eggs found?	Larvae found? (via any method)					
Great Crested Newt:		Sex/life stage:			Male	Female	Imm.	Male	Female	Imm.	Male	Female	Imm.		
Date	Air temp. (°C)	Veg. cover	Turbidity	Adult totals:	0	0	0	0	0	0	0	0	0	No	No
15.04.2020	16	0	2		0			0			0				
GCN Peak adult count for this pond in any one visit (by torch, trap or net):					0										

Comments: Access was restricted to one side of the pond due to steep banks, scrub, and a barbed wire fence.

Vegetation cover score (0-5); 0 = no vegetation obscuring survey; 5 = vegetation completely obscuring survey; Turbidity score (0-5); 0 = completely clear; 5 = very turbid. X = Method not used.

All visits had weather suitable for GCN survey (air temp above 5°C, little/no wind, no or only intermittent light showers). Generally, veg cover and relatively clear water allowed torch survey effort (albeit constrained by restricted access). Pond 1 had high vegetation cover in the form of duckweed sp. and netting was used as an additional measure. Pond 2 had high turbidity and netting was used as an additional measure. Availability of suitable egg laying substrate was limited in all the ponds; therefore, GCN egg/leaf fold search was undertaken where possible to be in accordance with the EN GCN MG. Restricted access limited the deployment of bottle traps. Some hand netting was carried out albeit limited to avoid undue disturbance.

Table 6: Other Amphibian Survey Results

Date of Survey	Torch				Bottle Trap				Net			
15.04.2020	Lv	Lh	Bb	Rt	Lv	Lh	Bb	Rt	Lv	Lh	Bb	Rt
Pond 1	0	0	0	1, 8Tp	3M,2F	0	0	0	0	0	0	0
Pond 2	0	0	0	0	X	X	X	X	0	0	0	0
Pond 4	0	0	0	0	0	0	0	0	0	0	0	0

Lv = smooth newt; Lh = palmate newt; Rt = common frog; Bb = common toad; M = male; F = female; J = juvenile; L = newt larva; S = spawn; Tp = Rt/Bb tadpole; X = survey method not used.

- 4.14 No great crested newt, smooth newt or palmate newt eggs were found in any of the ponds during the surveys.

Amphibian Survey Summary and Evaluation

- 4.15 Ponds surveyed within the site and its 250m buffer include:
- Pond 1 (Below Average HSI)
 - Pond 2 (Poor HSI)
 - Pond 3 (Below Average HSI)
 - Pond 4 (Below Average HSI)
- 4.16 An HSI can only make an assessment of a pond's suitability to support amphibians and it cannot determine the presence/absence of an amphibian species.
- 4.17 No evidence of great crested newt was discovered within the ponds surveyed, using any of the methods described above. The amphibian survey methods strictly followed the methods stated within this report, which adhere to Natural England's recommended survey techniques.
- 4.18 The amphibian survey found smooth newt, palmate newt and common frog to be present at this site with the following peak counts recorded.

Table 7: Peak Counts of Amphibian Species Recorded

Pond ref.	Lv	Rt
1	5	1
2	0	0
4	0	0

Lv = smooth newt; Rt = common frog.

- 4.19 Using JNCC/Natural England's population size class assessment, smooth newt populations at this site can be classed as being small in size, albeit based on a single survey.
- 4.20 The 2020 survey was specifically designed to identify presence or absence of great crested newt and was undertaken during the peak newt breeding season. Pond 1 is considered suitable for breeding common toad. Common toad and common frog's breeding seasons are slightly earlier in the year than the newt breeding season and therefore a population assessment cannot be accurately derived based upon the presence of tadpoles only if they were to have been found.
- 4.21 A small number of common frog tadpoles were identified during the survey, indicating a small sized population breeding within Pond 1.

eDNA Sample Results

- 4.22 Following analysis of the samples by SureScreen, the eDNA results are presented in Table 8 below. The SureScreen Scientifics Technical Report can be found in Appendix 4.

Table 8: eDNA Results

Pond No.	Sample date	Sample time	% of pond accessed	Relevant notes	Positive/negative & Replicates
1	16.04.20	10:15	70%	Shallow Water with 100% duckweed cover; silty sediment which was easily disturbed; Areas of pond not accessible due to steep banks and scrub.	Negative

Pond No.	Sample date	Sample time	% of pond accessed	Relevant notes	Positive/negative & Replicates
2	16.04.20	N/A	N/A	Pond was dry	N/A
3	19.05.20	13:30	20%	Large part of pond inaccessible due to steep banks, barbed wire/electric fence, and dense scrub.	Negative
4	16.04.20	9:30	50%	Pond located in a field of sheep which use the pond; Areas of pond not accessible due to scrub, steep banks and barbed wire.	Negative

Note: Amber cells denote risk factor, as sample area was either under 80% or there is a sediment warning in relation to positive results only.

- 4.23 The eDNA sampling method found all surveyed ponds to be negative and therefore ponds were not found to contain eDNA evidence of great crested newts. However, all three ponds surveyed were limited by access and below the desired sample area of 80%, which reduces the confidence in the negative results.

5.0 Impacts & Recommendations

- 5.1 No evidence of great crested newt was discovered within Ponds 1-4 at this site using any of the survey methods during the amphibian survey or eDNA sampling as described above. The amphibian survey identified that smooth newt and common frog are present within 250m of the development site.
- 5.2 Habitats on site are considered to be of low value to foraging amphibians and it is considered that far more suitable habitat for amphibians exists in the wider survey area to the east of the site where woodland and rank grassland exists.
- 5.3 Due to the national lockdown as a result of COVID-19 pandemic, there were several constraints regarding access to ponds (during both the amphibian surveys and eDNA surveys), which mainly stemmed from refusal of access from the land owner. Where access was restricted while surveying accessible ponds, additional survey methods were used during the amphibian survey and all ponds which had eDNA surveys were found to be 'below average' or 'poor' on the HSI. Due to the access constraints during the eDNA surveys, it is recommended that repeat eDNA surveys are undertaken of Ponds 1-4 to try and attain a higher percentage of sample area and to further back up the negative result.
- 5.4 Surveys of ponds 5-8 within 250m of the site, will be required to fully inform a planning application for the site. The Local Planning Authority will require the updated survey report and a mitigation strategy to be submitted to inform any planning application, to adhere to current Planning Policy. In line with current planning policy to provide net gains for biodiversity, the strategy should include appropriate enhancement measures.
- 5.5 Ponds (fulfilling certain criteria) are a Habitat of Principal Importance. The retention of ponds, ditches and associated habitats is therefore important. It is recommended that the ponds within the site are retained and protected during construction. When working close to water bodies appropriate pollution prevention measures must be implemented. Although withdrawn, The Environment Agency's guidance still provides useful advice.
- 5.6 If a pond is to be impacted, an appropriate mitigation strategy will be required detailing appropriate working methods and mitigation procedures. Works, if necessary, should be carried under the supervision of a suitably qualified ecologist. The development also offers the opportunity to enhance the existing ponds by clearing accumulated silt and leaf litter and clearing fallen and overshadowing trees and/or create new ponds as part of the sustainable drainage system for the development.

- 5.7 Any work on ponds must take full account of protected species such as water vole, amphibians and aquatic invertebrates.
- 5.8 Although common toad was not recorded during the surveys, only limited amphibian surveys were undertaken and Pond 1 is considered suitable for breeding common toad. Common toad, is a Species of Principal Importance in England (Section 41 of the Natural Environmental and Rural Communities (NERC) Act 2006). Therefore, as a precautionary measure, all construction contractors should be made aware of the potential presence of common amphibians, including common toad, common frog, smooth newt and palmate newt, on site and the potential risks to them. Should any common amphibians be encountered, they should be moved to suitable cover away from development works. If large numbers of common toad are found, then the acting consultant should be contacted for further advice on measures to minimise impacts.
- 5.9 Please note that the 2020 surveys were designed upon the information provided, if the development area is altered or working areas are located to the outer edge of the survey area, additional survey works may be required to extend the survey area as appropriate.

Enhancement Measures

- 5.10 In line with current planning policy to provide net gains for biodiversity, habitat enhancement measures have been designed and include:
- Enhancement of existing ponds and hedgerows to increase species diversity.
 - The use of Sustainable Drainage Systems (SuDS).
 - Enhancement of the brook and creation of a species-rich habitat corridor along the northern boundary of the site.

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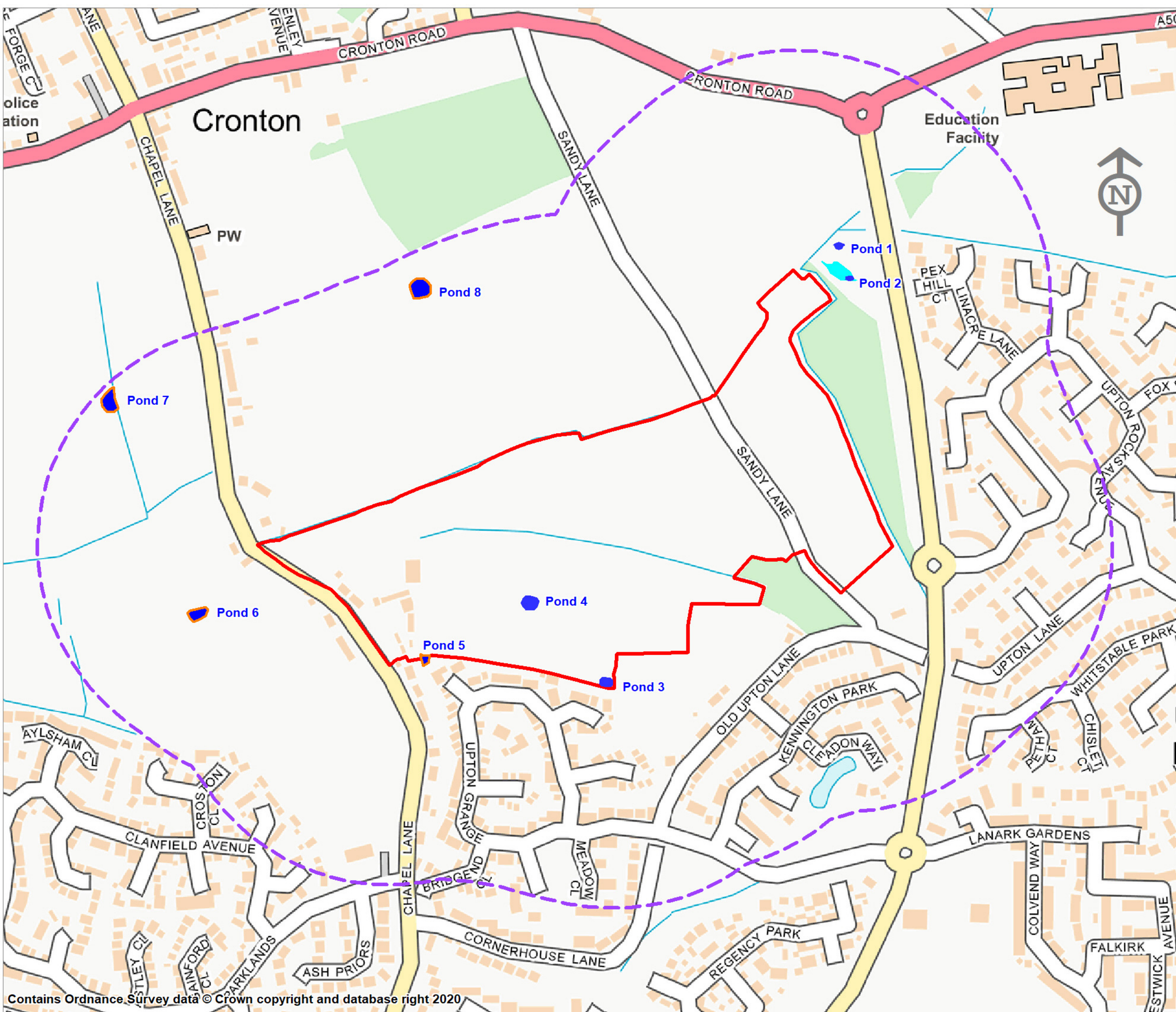
Figure 1:
Pond Location Plan

Figure 1:
Pond Location Plan.

Map Ref: (NGR) 35004, 38780
Map Scale: 1:4,000 @ A3

KEY

- Site boundary
- 250m site buffer
- Pond (accessible)
- Pond (no access)
- Swamp



Appendix 1: Statutory & Planning Context (England)

Disclaimer: Appendix 1 is a guide to legislation and procedure relating to biodiversity in England. It is general guidance and it does not give specific advice in relation to any site, species or project. It represents Ecology Services Ltd interpretation of legislation and procedure as at November 2020. Readers should note that legislation and procedure changes continually and is interpreted on a case-specific basis. Nothing in Appendix 1 should be construed as an offer of advice or legal opinion.

Great Crested Newts

Great crested newts and their habitat (aquatic and terrestrial) are afforded strict protection under the Wildlife and Countryside Act 1981 (as amended) and the Conservation of Habitats and Species Regulations 2017.

Taken together, this legislation makes it an offence to:

- Deliberately (intentionally) kill, injure or take a great crested newt;
- Possess or control any live or dead specimen or anything derived from a great crested newt;
- Deliberately (intentionally) or recklessly damage, destroy or obstruct access to a breeding site or any structure or place used for shelter or protection by a great crested newt; or
- Deliberately (intentionally) or recklessly disturb a great crested newt while it is occupying a structure or place which it uses for that purpose.
- Sell, offer for sale, possess or transport for the purpose of sale (live or dead animal, part or derivative).

The above legislation applies to all life stages of a great crested newt, including eggs, juveniles and adults. The extent of legal protection covers both the species and habitat.

The Conservation of Habitats and Species (Amendment) (EU Exit) Regulations 2019 continue the same provision for European protected species, licensing requirements and protected areas after Brexit.

The Office of the Deputy Prime Minister (ODPM) Circular 06/2005, states that the presence of a protected species is a material consideration when a planning authority is considering a development proposal that, if carried out, would be likely to result in harm to the species or its habitat.

Great crested newts are also protected by the Protection of Animals Act 1911, which prohibits cruelty and mistreatment. Releasing a great crested newt in such a way as to cause undue suffering may be an offence under the Abandonment of Animals Act 1960. In addition to the above, there are various statutory provisions relating to the transport of animals, designed to ensure their welfare.

The Natural Environmental and Rural Communities (NERC) Act 2006 lists great crested newt as a Species of Principal Importance, under Section 41 of the Act. Under Section 40 of the NERC Act 2006, Local Planning Authorities must, in exercising their functions, have regard, so far as is consistent with the proper exercise of those functions, to the purpose of conserving biodiversity.

Local Planning Authorities are required to consider the impact of any development upon Species of Principal Importance listed under the NERC Act 2006 and European Protected Species (EPS) and their habitat.

Therefore it is important to identify the presence of great crested newt individuals and also to identify suitable habitat on site in order that legal obligations regarding this species can be observed.

A Local Planning Authority (LPA) has a duty to ensure that protected species and habitats within the UK are a “material consideration” in the determination of a planning application. Therefore, a LPA is unlikely to determine an application until all relevant information relating to protected species or

habitats is submitted to fully inform the application. Relevant information includes adequate surveys and a mitigation strategy (the latter only if required) for their approval which will need to be submitted to inform any planning application.

If the survey identifies the presence of great crested newt, a Natural England Rapid Risk Assessment should be undertaken to determine if a European Protected Species (EPS) licence is required. If the Rapid Risk Assessment finds the risks to be Green: offence highly unlikely or Amber: offence likely it may be possible to undertake works using non-licensed avoidance measures.

If the risks are Red: offence highly likely, an EPS licence is likely to be required and a mitigation scheme would need to be developed, which minimises direct threats to newts and compensates for loss of habitat.

Where great crested newts are affected by development then a licence to derogate from the Conservation of Habitats and Species Regulations 2017 would be required. European Protected Species (EPS) licence applications are processed and issued by Natural England and the EPS licence can only be applied for, once planning permission is granted, if planning permission is required.

Natural England has powers to grant a EPS licence for the following purposes;

- Regulation 55(2)(e) - 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; or
- Regulation 55(2)(f) - preventing the spread of disease; or
- Regulation 55(2)(g) - preventing serious damage to livestock, foodstuffs for livestock, crops, vegetables, fruit, growing timber or any other form of property or to fisheries. In addition, Natural England can only issue a licence if it is satisfied that the activity meets one of the above purposes and is also satisfied of the following;
- Regulation 55(9)(a) - that there is no satisfactory alternative, and
- Regulation 55(9)(b) - that the action authorised will not be detrimental to the maintenance of the population of the species concerned at a favourable conservation status in their natural range.

In December 2016, Natural England introduced four policies for European Protected Species licensing. The policies seek to achieve better outcomes for EPS and reduce unnecessary costs, delays and uncertainty that were inherent in the current system. In brief, the four policies are:

- **Policy 1** - Greater flexibility when excluding and relocating European Protected Species (EPS) from development sites;
- **Policy 2** - Greater flexibility in the location of newly created habitats that compensate for habitats that will be lost;
- **Policy 3** - Allowing EPS to have access to temporary habitats that will be developed at a later date; and
- **Policy 4** - Appropriate and relevant surveys where the impacts of development can be confidently predicted.

Policy 1 allows compensation for EPS impacts to be delivered without the need to relocate or exclude populations where: exclusion or relocation measures are not necessary to maintain the conservation status of the local population; the avoid-mitigate-compensate hierarchy is followed; and compensation provides greater benefits to the local population than would exclusion and/or relocation.

Policy 2 allows for the provision of off-site compensation measures where the licensing tests are met, the avoid-mitigate-compensate hierarchy is followed, there are good reasons for maximising

development on the site of EPS impacts and where an off-site solution provides greater benefit to the local population than an on-site solution.

Policy 3 relates to developments, such as mineral extraction, which temporarily create habitat which is likely to attract EPS and enables works to proceed without the exclusion of EPS where the conservation status of the local population would not be detrimentally affected. On completion of development such sites must contribute to the conservation status of the local population as much as or more than the land use which preceded development. The measures to achieve this should be set out in a management plan and secured by a legal agreement.

Under **Policy 4** Natural England may accept a lower than standard survey effort where: the costs or delays associated with carrying out standard survey requirements would be disproportionate to the additional certainty that it would bring; the ecological impacts of development can be predicted with sufficient certainty; and mitigation or compensation will ensure that the licensed activity does not detrimentally affect the conservation status of the local population of any EPS.

Other Amphibians

Where such an amphibian survey rules out the presence of great crested newts, there may be the need to develop a scheme for the conservation of the less protected amphibians as a planning condition, should the development pose a risk to a locally significant population of amphibians.

Common toad is a Species of Principal Importance, under Section 41 of the Natural Environment and Rural Communities (NERC) Act 2006. The Office of the Deputy Prime Minister (ODPM) Circular 06/2005, states that habitats and species listed as priorities, with reference to the former UK BAP, are capable of being a material consideration in the making of planning decisions. Amphibians may also be listed as priorities under Local Biodiversity Action Plans.

Due to general declines in most British amphibian species in recent years, many local authorities require amphibian surveys as a planning condition, or as part of environmental information submitted as part of a planning application, even where the presence of great crested newts is ruled out.

Waterbodies that support all five species of British amphibians in high numbers may be afforded statutory protection as a Site of Special Scientific Interest (SSSI) or in local plans as Sites of Importance for Nature Conservation (SINC) or a similar equivalent, for sites of local importance.

This legislation and planning overview exclude species such as natterjack toad (*Epidalea calamita*) and pool frog (*Pelophylax lessonae*) as these are not within the range of the survey.

Appendix 2:

Results of Habitat Suitability Index Assessment

		Pond 1		Pond 2		Pond 3		Pond 4	
SI Ref	Description of Index	Measure / Comment	SI score	Measure / Comment	SI score	Measure / Comment	SI score	Measure / Comment	SI score
SI1	Geographic Location	A	1	A	1	A	1	A	1
SI2	Pond Area m2	100	0.2	400	0.8	300	0.6	250	0.5
SI3	Pond permanence	Sometimes dries	0.5	Dries annually	0.1	Never dries	0.9	Never dries	0.9
SI4	Water quality	Moderate	0.67	Bad	0.01	Poor	0.33	Poor	0.33
SI5	Shading %	100	0.2	100	0.2	100	0.2	50	1
SI6	Presence of waterfowl	Absent	1	Absent	1	Minor	0.67	Minor	0.67
SI7	Presence of fish	Absent	1	Absent	1	Possible	0.67	Minor	0.33
SI8	Pond Density in area	6	0.815	6	0.815	7	0.855	7	0.855
SI9	Terrestrial Habitat Quality	Moderate	0.67	Moderate	0.67	Moderate	0.67	Poor	0.33
SI10	Macrophyte cover in pond	0	0.3	0	0.3	0	0.3	0	0.3
HSI	Overall HSI for Pond:		0.542		0.348		0.555		0.555
	Comments:	Below Average		Poor		Below Average		Below Average	

Appendix 3: eDNA Sampling Protocol

The following text is a direct extract from:

**Analytical and methodological development for improved surveillance of the
Great Crested Newt (WC 1067)
Appendix 5 Technical advice note for field and laboratory sampling of great crested newt
(*Triturus cristatus*) environmental DNA 30th September 2014**

3. Field protocol

Field sampling should be undertaken by a suitably trained and experienced great crested newt surveyor (trained volunteer or professional). At present it is believed that eDNA water sampling does not disturb newts enough to justify the procedure being licensed by the national regulatory authority. A single visit to the target pond should be made between mid-April and June, during the newt breeding season. eDNA samples can be collected at any time of day and in any reasonable weather conditions, including light rain. It may be best to avoid heavy rain as this makes sampling more difficult and might increase the risk of cross contamination (e.g. splashing of mud which could contain great crested newt DNA from wet ground). There is evidence that unpreserved amphibian eDNA decays slightly more quickly in full sun than shaded conditions, becoming undetectable after 8 and 11 days respectively (Pilliod et al., 2014), but as long as samples are preserved the impact on detection should be slight.

3.1 Sampling equipment

The field sampling equipment used by Biggs et al. (2014) has five components (Figure 2):

- A sterile 30 mL ladle
- A sterile self-supporting Whirl-Pak plastic bag with 1 L capacity
- A sterile 10 mL pipette to resample the pond water
- Six sterile 50 mL centrifuge tubes containing preservative (Absolute Ethanol (200 Proof), Molecular Biology Grade, Fisher BioReagents (Product Code: 10644795), sodium acetate and other markers)
- Two pairs of sterile gloves

Kits can be stored at room temperature before use in an appropriate solvent store, consistent with Home Office regulations, and should be used within about two weeks of receipt. The time between kit receipt and use should be noted (see Section 5.1). Use one kit per pond up to an area of 1 ha. Beyond this, use an additional kit per hectare. However, note that as yet there is no practical experience of the effectiveness of kits used on ponds greater than 1 ha in area. Note that sampling techniques are still developing rapidly in this field and alternative preservatives to ethanol are currently being sought.

3.2 Field water sample collection protocol

The field sampling protocol should follow the steps outlined below. Gloves should be worn at all times during the sampling process, replacing the gloves between sample collection from the pond and pipetting into the sterile sub-sample tubes. Samples should be collected without entering the water, i.e. the surveyor stands only on the pond bank or muddy pond edges. This prevents disturbance of the substrate and may limit cross-contamination.

Stages of field sampling protocol

Step 1 Identify where 20 samples will be taken from the pond. The location of sub-samples should be spaced as evenly as possible around the pond margin, and if possible targeted to areas where there is vegetation which may be being used as egg laying substrate and open water areas which newts may be using for displaying.

Step 2 Open the sterile Whirl-Pak bag by tearing off the clear plastic strip c 1cm from the top (along the perforated line), then pulling the tabs. The bag will stand-up by itself.

Step 3 Collect 20 samples of 30 mL of pond water from around the pond (see 1 above) using the ladle (fill the ladle), and empty each sample into the Whirl-Pak bag. At the end the Whirl-Pak bag should be just under half full (600 mL).

NOTE: Before each ladle sample is taken, the pond water column should be mixed by gently using the ladle to stir the water from the surface to close to the pond bottom without disturbing the sediment on the bed of the pond. It is advisable not to sample very shallow water (less than 5-10 cm deep).

Step 4 Once 20 samples have been taken, close the bag securely using the top tabs and shake the Whirl-Pak bag for 10 seconds. This mixes any DNA across the whole water sample.

Step 5 Put on a new pair of gloves to keep the next stage as uncontaminated as possible.

Step 6 Using the clear plastic pipette provided take c15 mL of water from the Whirl-Pak bag and pipette into a sterile tube containing 35 mL of ethanol to preserve the eDNA sample (i.e. fill tube to the 50 mL mark). Close the tube ensuring the cap is tight.

Step 7 Shake the tube vigorously for 10 seconds to mix the sample and preservative. This is essential to prevent DNA degradation. Repeat for each of the 6 conical tubes in the kit. Before taking each sample, stir the water in the bag to homogenize the sample - this is because the DNA will constantly sink to the bottom.

Step 8 Empty the remaining water from the Whirl-Pak bag back into the pond.

Step 9 The box of preserved sub-samples is then returned at ambient temperature immediately for analysis. If batches of samples are collected and stored prior to analysis they should be refrigerated at 2-4° C. Kits can be stored for up to one month in a refrigerator before analysis. It is not necessary to freeze samples. Freezing may damage storage bottles, which can lead to leaking during transit, and also unnecessarily increases costs by requiring refrigerated transport. The length of time eDNA samples are stored in a refrigerator prior to analysis should be recorded and passed on to the analysing laboratory. Use an appropriate labelling system to ensure that the kits are supplied with a unique reference number.

See eDNA protocol form below and overleaf

Sample Kit ID:			
Kit received (Date):		Signed into store by:	
Kit signed out (Date)		Signed out by:	
Project name:			
Project no.:			
Have previous pond surveys been undertaken this year:	Yes/No	Date:	
If Yes, has evidence of anti-contamination been provided	Yes/No		
Pond number:			
Date of survey:		Survey time:	
Lead surveyor name:			
Surveyor Gcn Licence: (Must be licensed)		Surveyor eDNA trained: (Survey must be trained)	Yes/No
Number of samples taken at pond: (20 samples required)		% of pond sampled: (Min 80/90% desired)	
Is the pond <1ha?	Yes/No		
Notes of any constraints to sampling: (Circle as required)	Access difficulties, surveyor entered water, potential contamination of sampling equipment, loss of ethanol, sediment disturbance, shallow water, cattle disturbance of sediment, noticeable distance of sediment by waterfowl, other...		
Return to fridge: (Date and time)		Signed into fridge by:	
Fridge temperature check: (Date & time)		Fridge temperature checked:	
Collected by Courier: (date)		Collected by Courier: (Staff responsible)	
eDNA sampling result	Positive/Negative		
Other amphibian survey result:	Visit 00 of 4/6/Date/Method/Results		

Appendix 4: SureScreen Scientifics Technical Report

TECHNICAL REPORT

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

Date sample received at Laboratory: 17/04/2020
 Date Reported: 19/04/2020
 Matters Affecting Results: None

RESULTS

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
0025	Pond 4, Chapel Lane, Widnes	SJ 498877	Pass	Pass	Pass	Negative	0
0026	Pond 1, Chapel Lane, Widnes	SJ 501881	Pass	Pass	Pass	Negative	0

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: 20/05/2020
 Date Reported: 26/05/2020
 Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
0027	Pond 3, Chadel Lane	SJ 499 876	Pass	Pass	Pass	Negative	0



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.

