

recommended by Natural England (see Appendix A for eDNA testing methodology). The samples were received by ADAS on the 7th of May 2021 and tested on the 12th of May 2021.

A Habitat Suitability Index (HSI) value was calculated for the waterbodies. See Table 1 below for HSI value and waterbody description. Waterbody locations and HSI data are shown in Appendix B and C respectively.

Table 1: HSI Value and Description

Waterbody Reference	Distance From Site Boundary (m)	Size (m ²)	Description	Surrounding Habitat	HSI Value
1	Within the site boundary	570	A single pond, steep banks, consisting of aquatic vegetation to the north/east and south. Waterfowl present with young. 'Good' water quality.	Vegetated banks with the surrounding habitat amenity grassland.	0.714 (Good)

No evidence of GCN eDNA was found within any of the samples and no signs of degradation; therefore, it is considered unlikely that GCN are currently using the pond within the site boundary.

Recommendations

Although GCN are not considered to be currently using the waterbodies and are considered to be absent from the site, it is recommended that the site could be enhanced post-development for amphibians by improving terrestrial and aquatic habitats. This could include pond creation within open spaces, ditch improvement for commuting newts, the creation of habitat corridors seeded with wildflower and grassland mixes and the incorporation of hibernacula is suitable areas.

Yours sincerely,

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References

Biggs J et al. (2014). Analytical and methodological development for improved surveillance of the Great Crested Newt. Appendix 5. Technical advice note for field and laboratory sampling of great crested newt (*Triturus cristatus*) environmental DNA. Freshwater Habitats Trust, Oxford.

English Nature (2001) Great Crested Newt Mitigation Guidelines. English Nature, Peterborough.

National Planning Policy Framework (2019) ISBN: 9781409834137.

Appendix A - eDNA Testing Methodology.

The following survey techniques are recommended by Natural England:

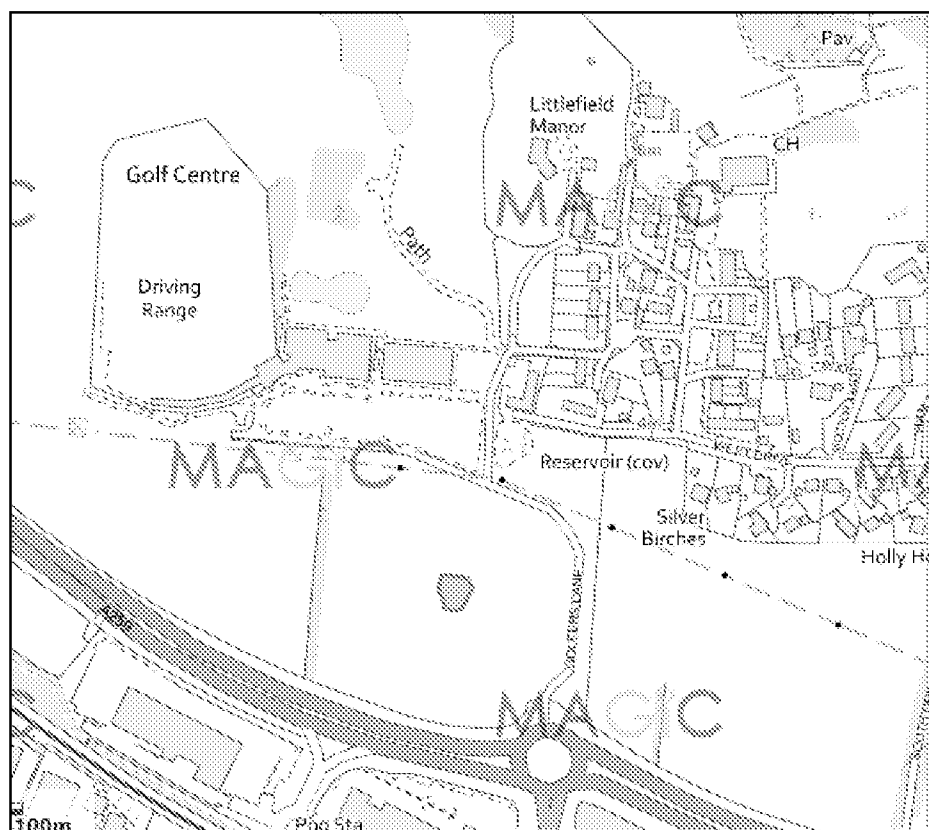
- Wearing gloves, twenty 30ml water samples were collected at even intervals (no closer than 2m apart) around the perimeter of each pond and combined in a container. Care was taken not to enter the water at any point to avoid cross-contamination of DNA between ponds.
- Six 15ml samples were then preserved in alcohol and sent to ADAS for analysis, following Natural England guidelines.

The following analysis techniques outlined by Biggs *et al.* (2014) were followed by ADAS and are approved by Natural England:

- The method detects pond occupancy from GCN using traces of DNA shed into the pond environment (eDNA). The detection of GCN eDNA is carried out using real time Polymerase chain reaction (PCR) to amplify part of the cytochrome 1 gene found in mitochondrial DNA. The method followed is detailed in Biggs J., *et al.*, (2014).

The results are defined as follows:

- **Positive:** DNA from the species was detected.
- **eDNA Score:** Number of positive replicates from a series of twelve.
- **Negative:** DNA from the species was not detected; in the case of negative samples the DNA extract is further tested for PCR inhibitors and degradation of the sample.
- **Inconclusive:** Controls indicate degradation or inhibition of the sample, therefore the lack of detection of GCN DNA is not conclusive evidence for determining the absence of the species in the sample.

Appendix B – Location of On-site Pond**Appendix C – HSI Data**

Factor	Waterbody 1	
	Field Score	SI Value
Location	Zone A	1
Area (m ²)	500-700	1
Permanence	Never	0.9
Water quality	Poor	0.67
Shade %	0-60	1
Fowl	Absent	1
Fish	Absent	1
Pond density	>12	1
Terrestrial Habitat	Poor	0.67
Macrophyte cover %	1-5	0.35
HSI value	0.714	
Pond Suitability	Good	