
Kirkby Valley Golf Club

Amphibian Survey Report

Compiled by Ecology Services Ltd.

on behalf of

L32 Trading Ltd.

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1.0 Introduction

- 1.1 Ecology Services Ltd. was commissioned by L32 Trading Ltd. in May 2019, April 2020, January 2021 and April 2021, to undertake amphibian surveys of ponds at Kirkby Golf Club, L32 4SS, hereinafter referred to as 'the site'. The centre of the site is located by National Grid Reference (NGR) 339582, 397944. The site location and pond / waterbody locations are shown on Figure 1.
- 1.2 Initially 5 waterbodies were identified in 2019 within 250m of the undeveloped parcel of land on the western side of the River Alt (Waterbodies 1, 2, 3, 4 & 8). Waterbody 4 was scoped out as it lay on the opposing side of Aintree Lane to the west, which is kerbed and considered to act as a barrier to dispersing amphibians. Waterbody 8 was dry during the survey period. Information was received during the initial site meeting (M. Hanlon, **pers. comm.**, 8th May 2019) that waterbodies 1 & 3) had been recently created as part of the golf course landscaping works. eDNA surveys of these ponds were undertaken in May 2019 to establish presence or absence of great crested newt.
- 1.3 eDNA surveys of ponds within 250m of the development proposals east of the River Alt (Waterbodies 5 and 9) plus Waterbody 6 which was identified after the 2019 survey season had ended were undertaken in April 2020. Waterbody 8 was again found to be dry in April 2020.
- 1.4 Waterbodies 10, 11, 13, 15/16, 18, 19, 20, 21, 22 & 23 within the wider golf course were surveyed in April and May 2021.
- 1.5 The results of the eDNA surveys are discussed in the below sections.
- 1.6 Four parcels of land within the site, collectively referred to as the 'development area', are proposed for (re)development as shown on Figure 2 including:
 - Reconfiguration and other improvement work across the entire golf course including the reshaping of tees / greens / bunkers and erection of a footbridge over the River Alt to provide both an improved 18 hole course and a new 9 hole course.
 - New and improved irrigation / drainage works (including irrigation lake), extensive tree and shrub planting, and ecological mitigation measures across the course.
 - The erection of a new 2 storey Clubhouse;
 - Retention, repair, refurbishment, alteration and extension of the existing clubhouse and its change of use to form a golf-related / grounds maintenance training academy (existing access and car park retained / unchanged).
 - A new greenkeeper's compound with associated building and security fencing.
 - Provision of an adventure golf course and practice putting green; and provision of a golf driving range with an associated 2 storey building and external lighting and safety netting.
 - 6 synthetic outdoor 5-a-side football pitches, 5 synthetic outdoor tennis courts, 1 external bowling green with associated timber players' hut plus associated lighting and means of enclosure.
 - Car and cycle parking spaces to serve all of the above facilities with two new vehicular accesses created onto Valley Road.
 - Formation of a new gated access via Aintree Lane for the use of construction traffic and (upon completion of development works) for the sole use of course operatives for the purposes of course management / maintenance.
- 1.7 Two lakes are proposed to assist with course irrigation and ecological mitigation at the site
- 1.8 The aims were to:

- 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 the waterbodies.
- Undertake an eDNA survey of suitable waterbodies, following Natural England's recommended survey methodology.
- To identify the presence/absence of great crested newt (*Triturus cristatus*).

1.9 Natural England's Guidance (English Nature, 2001) advises that surveys of ponds up to around 500m from a 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.

1.10 In this instance a 500m survey area was not considered to be required because suitable foraging and hibernation habitat lies in close proximity to those ponds. Additionally, the golf lies course between the development areas and those ponds and does not provide ideal conditions for dispersal.

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. 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 Environment 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 Ecological data and records searches were undertaken by contacting the sources listed in Table 1.

Table 1: Ecological Desktop Study Sources

Source of information	Information supplied
North Merseyside Local Biodiversity Action Plan (NMBAP)	Identification of LBAP species and habitats known to occur in the region.
Natural Environment and Rural Communities (NERC) Act 2006	Review of Habitats/Species of Principal Importance known to occur in the region.
Multi Agency Geographical Information for the Countryside (MAGIC)	Statutory protected sites within 2km of the site. Records of great crested newt relating to Natural England EPS licences within the vicinity of the site.
Merseyside Biobank	Non-statutory protected sites designated for their great crested newt interest and any records of great crested newt within 1km of the site.

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.

Source of information	Information supplied
Natural England Open Data Geoportal	To review records of great crested newt survey licence returns within 2km of the site.
NBN Atlas	Review of Species of Principal Importance known to occur in the region.

- 3.2 The data search with MEAS was undertaken on 15th January 2021.
- 3.3 As part of the desktop study, historic aerial imagery from 1937² and 1961² has been viewed which shows that there were no ponds present on the site at these times. Given that the site has become isolated from the wider landscape by the construction of busy roads to the east and south in the 1970s, the ponds on site are unlikely to have maintained an isolated population of great crested newts or a significant population of common toad due to the development of barriers in the 1970's. Generally, the ponds currently within the site are recently created and several are of an ephemeral nature with many drying out during the amphibian breeding season. Therefore, it is considered unlikely that great crested newt or significant populations of common amphibians would have colonised the ponds within the site.
- eDNA Methodology**
- 3.4 The eDNA sampling was undertaken by suitably trained and licensed ecologists and during the approved sample period. Sample protocols developed by the Freshwater Habitats Trust were strictly adhered to.
- 3.5 eDNA is nuclear or mitochondrial DNA that is released 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 in the water where it persists for 7–21 days, depending on the conditions.
- 3.6 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.
- 3.7 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.
- 3.8 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. This is for the periods between mid-April and the end of June.
- 3.9 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.
- 3.10 See Appendix 2 Sampling Protocol for equipment used and sample collection protocol. Upon collection samples were sent by courier to SureScreen Scientifics.

² (<https://maps.nls.uk/view/126522125>) ²<https://maps.nls.uk/view/91576426>

HSI Methodology

- 3.11 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).
- 3.12 The HSI is a geometric mean of ten suitability indices: $HSI = (SI1 \text{ (Geographic Location)} \times SI2 \text{ (Pond area)} \times SI3 \text{ (Pond permanence)} \times SI4 \text{ (Water quality)} \times SI5 \text{ (Shading)} \times SI6 \text{ (Presence of water fowl)} \times SI7 \text{ (presence of fish)} \times SI8 \text{ (Pond density in area)} \times SI9 \text{ (Terrestrial Habitat Quality)} \times SI10 \text{ (Macrophyte cover in pond)})^{1/10}$.
- 3.13 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

- 3.14 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.

- 3.15 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).

Timing

- 3.16 eDNA samples were taken on the 15th May 2019, 20th April 2020, 20th April 2021 and 4th May 2021 which is within the accepted sample period of mid-April to June.
- 3.17 HSI assessments can be undertaken at any time of the year, although some measurements including macro-invertebrate assemblages and macrophyte cover are best taken in the summer months. Macro-invertebrate samples were not taken on the grounds of health and safety.

Weather Conditions

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

Personnel

- 3.19 All ecologists were subject to training in line with the Freshwater Habitats Trust (FHT) sampling methods.
- 3.20 The eDNA samples were undertaken by suitably experienced ecologists under Natural England Class Survey Licence (CLS) Miss C. Wood MSc (Hons) (2017-32734-CLS-CLS), Mr. B. Meadows BSc (Hons) (2019-39994-CLS-CLS) and Miss N. Burdin MSc.
- 3.21 The HSI assessment was undertaken by Ecologist Miss. C. Wood.

Limitations

- 3.22 Approximately 20% of Waterbody 2 and 70% of Waterbody 6 could not be accessed due to deep water, steep banks and dense scrub.
- 3.23 Macro-invertebrate sampling as part of the HSI survey were not undertaken on health and safety grounds due to lone working. This has been factored into the score by allowing a maximum score to be applied for this index.

4.0 Results and Evaluation

Desktop Study Results

- 4.1 A desktop study was undertaken as part of the Preliminary Ecological Appraisal in May 2019 and updated in January 2021. A data search for local records within 2km of the site was provided by Merseyside BioBank. The study identified one record of great crested newt within 2km to the north east of the site from 2011 on the far side of the M57 (approximately 900m away). There are no direct habitat linkages between the location of this record and the site. No records of great crested newt were identified within 2km of the site using any of the additional sources in Table 1.

- 4.2 MAGIC map, NBN Atlas and the Natural England Open Data Geoportal found no records of great crested newt European Protected Species (EPS) licences or survey licence return records within 2km of the proposed development site.

Waterbody Descriptions

- 4.3 Waterbody 4 has been scoped out of this assessment and is therefore excluded from the waterbody descriptions as it lies on the opposing side of Aintree Lane to the west, which is kerbed and considered to act as a barrier to dispersing amphibians. Waterbodies 7, 8 and 24 were temporary areas of standing water where golf course works had been undertaken and have therefore also been scoped out.

Table 2: Waterbody Descriptions

Waterbody No.	Description
1	A rectangular pond with a perimeter of approximately 90m, located in the south of the golf course approximately 13m from the development area. It has shallow banks with a water depth of <1m and a sediment base. It is a relatively new pond with a limited amount of floating sweet grass (<i>Glyceria fluitans</i>) present and it is not shaded.
2	A ditch system located in the west of the golf course approximately 10m from the development area. It contained standing water and there was no evidence of any flow. It has steep banks with dense vegetation on both banks. The base is sand/sediment. Aquatic and marginal vegetation includes floating sweet grass, bulrush (<i>Typha latifolia</i>) and soft rush (<i>Juncus effusus</i>).
3	A circular pond with a perimeter of approximately 40m, located in the north west of the golf course approximately 235m from the development area. It has shallow banks with a water depth of <1m and a sediment base. It is a relatively new pond with no aquatic vegetation present and it is not shaded.
5	A small pond where three drainage ditches intersect, located approximately 210m to the north of the eastern area of the development area. It is approximately 1m wide, with shallow water and dominated by bulrush.
6	A small shallow pond with no aquatic vegetation located on the northern boundary of the western portion of the development area. It is shaded by surrounding scrub and tall ruderal vegetation. This pond was discovered during a badger survey in July, outside of the amphibian survey season.
9	A ditch located on the north eastern boundary of the eastern portion of the development area. It is approximately 1m wide and deep and c.185m long, it has been seen both dry, with the base supporting abundant Yorkshire fog and occasional great willowherb and also filled with standing water over multiple site visits across the year indicating that it is of an ephemeral nature.
10	An oval pond with a perimeter of 60m, turbid water and 5% vegetation cover (bulrush). The pond has shallow, vertical banks, a sediment base and a water depth of approximately 1m. The pond is surrounded by rough grassland with scattered trees (25% shaded). The pond had recently been reworked with mounds of earth splitting the original pond and creating a new pond (Pond 17).
11	A kidney shaped pond (perimeter c. 150m) with vertical banks, c. 1m deep, turbid water and a sediment base. There was some newly establishing bulrush (25% marginal vegetation cover) and the pond was 25% shaded.
12	A very small, shallow pond (<0.5m deep) in late succession on the edge of the golf course. It was approximately 8 square metres and triangular in shape with a central mat of bulrush on which terrestrial plants including creeping buttercup were growing (90% vegetation cover). Pendulous sedge was also present. The water was clear and the pond had a sediment base with leaf litter and 40% shading by dense scrub. It was dry in late April 2021.
13	A recently excavated, largely unshaded, rectangular waterbody, approximately 6m x 7m identified during the August 2020 Phase 1 habitat survey update. It had shallow, vertical banks and a sediment base. There was very little aquatic/marginal vegetation with some bulrush colonising. By January 2021 it had been extended to 7m wide and 21m long.

Waterbody No.	Description
14	A shallow pond (<0.5m water depth) approximately 12 x 12 metres located on the edge of the golf course in an area of rough grassland. The banks were steep, the water turbid with a sediment base and had 25% shading by trees. The pond contained bullrush and small areas of establishing floating sweet-grass (c. 50% vegetation cover). The pond was dry in late April 2021.
15	A large, ephemeral, shallow (<0.5m), circular waterbody with clear water located on the golf course edge that is thought to frequently fluctuate in area. The pond is considered to be dry for most months of the year with associated plants being soft rush and spike rush (50% cover).
16	A recently excavated, steep-sided ditch, approximately 100m long and 2m wide with no vegetation and occasional standing water leading to a pond with dense bulrush vegetation (60% cover). The water was turbid, <0.5m deep and there was 50% shading by dense scrub.
17	A circular pond, c. 5m diameter, with turbid water (<0.5m deep), steep banks and a sediment base. There was no aquatic vegetation and the pond was 25% shaded. A newly created pond due to reworking of Pond 10 with mounds of earth splitting the original pond. Was dry in late April 2021. The pond is surrounded by rough grassland with scattered trees.
18	An established pond located on the northern boundary of the golf course. It comprises two oval waterbodies that are linked via a narrow flooded central section. The banks were shallow and vertical with a sediment base to the pond. The water was turbid water with a reddish colour and c. 1m deep. The pond margins and central section are dominated by bulrush (30% vegetation cover). A kingfisher was noted feeding from this pond on numerous occasions.
19	A small pool of water resulting from a digger excavation by the northern boundary fence within an area of dense bramble and reed canary grass. It was c. 3m x 1.5m, with steep, earth sides, turbid water and 100% covered with duckweed (<i>Lemna</i> sp.). It was not possible to establish the water depth. There was no marginal/emergent vegetation. There were two tall poplars to the north and one to the east. There was no evidence of waterfowl, but could potentially be small fish. A shallow ditch leads off to the south towards the golf course, possibly feeding the pond with run-off.
20	A small pool at the junction of two ditches with vertical banks and a sediment base, approximately 4m x 2m with no aquatic/emergent vegetation and turbid water. Water was running into the pool from the ditch to the west and was approximately 1.5m in January 2021 although considered to have fluctuating water level. It was surrounded by poplars except on the north eastern side. There was no evidence of waterfowl.
21	One of two fairly recently excavated shallow ponds located on the edge of cleared vegetation and coniferous trees within the golf course. An irregular shaped waterbody, c. 30m long and 8m wide with shallow (<0.5m) clear water, steep banks and some adjacent dense scrub. Some fallen trees had been dumped within one end of the pond moved from elsewhere on the site. Some bulrush was present in the pond.
22	One of two fairly recently excavated shallow ponds located on the edge of cleared vegetation and coniferous trees within the golf course. The pond was kidney shaped and c. 18m long and 5m wide with turbid water. It has shallow, vertical banks, with c. 1m deep water and a sediment base. There was c. 5% vegetation cover and 5% shading from trees.
23	A 40m long by 6m wide undulating pond located within the woodland on the eastern side of the golf course with turbid water, vertical banks and a sediment base with leaf litter. It is likely to be dry during the summer months but was fully wet during the survey (<0.5m water depth). There were no associated aquatic plant species. It was dry in early May 2021.

eDNA Sample Results

- 4.4 Following analysis of the samples by SureScreen, the eDNA results are presented in Table 2 below. The SureScreen Scientifics Technical Report can be found in Appendix 3.

Table 2: eDNA Results

Waterbody No.	Sample date	Sample time	% of waterbody accessed	Relevant notes	Positive/negative & Replicates
1	15.05.2019	10.30	100%	None	Negative
2	15.05.2019	11.20	80%	None	Negative
3	15.05.2019	12.10	100%	None	Negative
5	20.04.2020	10.15	90%	None	Negative
6	20.04.2020	13.00	70%	Access difficulties due to dense scrub and steep banks.	Negative
9	20.04.2020	11:00	100%	None	Negative
10	20.04.2021	14:30	100%	Waterfowl presence	Negative
11	20.04.2021	13:10	100%	None	Negative
12	DRY				
13	20.04.2021	11:15	100%	None	Negative
14	DRY				
15 & 16	20.04.2021	12:15	80%	High water levels had resulted in these waterbodies being connected and therefore sampled as one waterbody. Access difficulties due to steep banks. Waterfowl presence.	Negative
17	DRY				
18	20.04.2021	13:45	100%	Waterfowl presence.	Negative
19	04.05.2021	11:00	100%	None	Negative
20	04.05.2021	11:15	100%	None	Negative
21	04.05.2021	11:30	100%	None	Negative
22	04.05.2021	11:45	95%	None	Negative
23	DRY				

- 4.5 The eDNA sampling method did not find any positive evidence of great crested newt in 16 out of 20 waterbodies (80%) sampled within 250m the site (excluding Waterbody 4 which was scoped out of this assessment. Four of the waterbodies (12, 14, 17 and 23) were found to be dry at the time of the sampling visit. Waterbodies 7, 8 and 24 were the result of temporary golf works and were therefore scoped out.

Habitat Suitability Index Results

- 4.6 The habitat suitability index results for Waterbodies 1-3, 5-6 and 9-23 are presented in Table 3 below. The raw data tables can be found in Appendix 4.

Table 3: Summary Results of Habitat Suitability Index³

Pond No.	HSI Score	Pond Suitability	Summary of Contributing Factors
1	0.821	Good	Never dries, moderate terrestrial habitat, minimal shade, waterfowl and fish are absent, poor macrophyte cover.
2	0.961	Excellent	Rarely dries, optimal size, minimal shade, good terrestrial habitat, waterfowl and fish are absent.
3	0.734	Good	Never dries, minimal shade, poor terrestrial habitat, waterfowl and fish are absent, poor macrophyte cover.
5	0.722	Good	Sometimes dries, no shade, good terrestrial habitat, waterfowl and fish are absent.
6	0.597	Average	Sometimes dries, good terrestrial habitat, waterfowl and fish are absent, high shade, poor macrophyte cover.

³ The maximum score has been given for macro invertebrate assemblages because the waterbodies were not netted.

Pond No.	HSI Score	Pond Suitability	Summary of Contributing Factors
9	0.622	Average	Dries annually, some shade, good terrestrial habitat, waterfowl and fish are absent, no macrophyte cover.
10	0.781	Good	Never dries, no shading, waterfowl absent, fish possible, good terrestrial habitat and low macrophyte cover.
11	0.817	Excellent	Never dries, no shade, good terrestrial habitat, waterfowl absent but fish are possible. Macrophyte cover low.
12	0.495	Poor	Dries annually, no shade, waterfowl and fish absent, good terrestrial habitat and high macrophyte cover.
13	0.733	Good	Sometimes dries, no shading, waterfowl and fish absent, good terrestrial habitat and no macrophyte cover.
14	0.809	Excellent	Sometimes dries, no shading, waterfowl and fish absent, good terrestrial habitat and good macrophyte cover.
15	0.833	Excellent	Sometimes dries, no shading, waterfowl and fish absent, good terrestrial habitat and macrophyte cover.
16	0.717	Good	Dries annually, no shading, waterfowl and fish absent, good terrestrial habitat and macrophyte cover.
17	0.657	Average (dry)	Sometimes dries, low shading, waterfowl and fish absent, good terrestrial habitat and no macrophyte cover.
18	0.858	Excellent	Never dries, no shading, waterfowl absent, fish possible, good terrestrial habitat and low macrophyte cover.
19	0.559	Below average	Sometimes dries, 100% shaded, waterfowl and fish absent, good terrestrial habitat and no macrophyte cover.
20	0.490	Poor	Dries annually, 50% shaded, waterfowl and fish absent, good terrestrial habitat and no macrophyte cover.
21	0.745	Good	Sometimes dries, no shading, waterfowl and fish absent, good terrestrial habitat and no macrophyte cover.
22	0.743	Good	Sometimes dries, low shading, waterfowl absent but fish possible, good terrestrial habitat and low macrophyte cover.
23	0.557	Below average	Dries annually, 100% shaded, waterfowl and fish absent, good terrestrial habitat and no macrophyte cover.

- 4.7 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. The results above indicate that the ponds within the ownership boundary are of a lower suitability to support amphibians.

5.0 Impacts and Recommendations

- 5.1 The eDNA sampling resulted in a negative result for all 16 waterbodies sampled i.e. great crested newts were absent. The survey data for Waterbodies 1, 2 and 3 is now more than two survey seasons old and therefore out of date. Given the number of other waterbodies since sampled (16) which have all tested negative for eDNA it is considered highly unlikely that Waterbodies 1, 2 and 3 would have become colonised by great crested newts in the intervening period.

- 5.2 Waterbodies 12, 14, 17 and 23 were found to have dried by late April/early May 2021. With the exception of Waterbody 14 which had an HSI of 0.809 (Excellent), Waterbodies 12, 17 and 23 had HSI scores which were average, below average or poor. It is also noted that these scores are based on a maximum score for macro-invertebrates, because sampling was not undertaken for this index on the grounds of health and safety. However, Natural England guidance states: a HSI is not a substitute for undertaking newt surveys; it indicates but cannot confirm presence or absence.
- 5.3 For Waterbodies 12, 14, 17 and 23, it is noted that winter 2020/21 had well above the average rainfall (122%), these Waterbodies having been identified in mid-January 2021. Rainfall in March was below average (89%) with April being the driest on record since 1980 (28% average rainfall). Waterbodies 14 and 17 are in close proximity to other waterbodies which tested negative for great crested newt and being of recent origin and it is considered unlikely that these two waterbodies support great crested newts. Waterbodies 12 and 23 lie 115m and 265m respectively to the north of Waterbody 9 which tested negative for great crested newt eDNA. While some of the waterbodies were dry at the time of the eDNA sampling, in total 16 out of 20 waterbodies (80%) were sampled and found to be negative for great crested newt eDNA. If great crested newts had been using the waterbodies which had dried by late April/early May, those newts are likely to have been displaced to other nearby waterbodies still holding water, none of which tested positive for great crested newt eDNA.
- 5.4 Additionally, there are no records of great crested newts, which are fundamentally linked to the site. The only record for great crested newt is located approximately 900m to the east and is isolated from the site by the M57 motorway which acts as a barrier to dispersal.
- 5.5 Given the above findings, it is considered that it likely that great crested newts are absent and all works can proceed with the below recommendation.
- 5.6 As a precautionary measure all construction contractors should be made aware of the potential presence of amphibians 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. In the unlikely event that a great crested newt is discovered on site, at any time during the development works, all works must cease immediately and either Natural England or the acting ecologist should be contacted for advice.
- 5.7 No waterbodies will be lost to the part of the site to be developed, however, comprehensive engineering, reconfiguration, new and improved irrigation / drainage works and other improvement works across the wider golf course) is likely to result in impacts to waterbodies.
- 5.8 To provide continued aquatic habitat for amphibians and other species, it is recommended that the same number of waterbodies is maintained across the golf in the long term. Ideally these should be designed and managed to encourage wildlife to provide an overall biodiversity enhancement. Ideal ponds for amphibians are:
- Surface area between 100 and 300m²
 - Varying depth; both deep (up to around 4m) and shallow ponds may be used; Optimum maximum depth of between 1m and 3.5m
 - The pond should hold water throughout at least one summer in every 3 years
 - Substantial cover of submerged and marginal vegetation
 - Open areas to facilitate courtship behaviour
 - Ponds in clusters, rather than in isolation
 - Absence of shading on the south side
 - Absence of fish
 - Absence or low density of waterfowl

- 5.9 Habitats within the development area, including rough, species-poor semi-improved grassland, swamp, tall ruderal, scrub and plantation woodland habitats, provide suitable terrestrial habitat for amphibians as well as refuge opportunities. Further refuge and hibernation opportunities for amphibians are provided by banks of earth and rubble created during pond and ditch excavation works. Habitats within the wider golf course are dominated by mown amenity grassland with small areas of rough grassland, tall ruderal, scattered trees, semi-natural broadleaved woodland and dense scrub.
- 5.10 There will be loss of rough, species-poor semi-improved grassland, swamp, tall ruderal, scrub and plantation woodland habitats to the development and temporary loss of habitats during comprehensive engineering, reconfiguration, new and improved irrigation / drainage works and other improvement works across the wider golf course. The developed part of the site will include approximately 10.8ha semi-rough, rough and long rough grassland with areas seeded with wildflower seed mix plus 6,310m² new woodland planting which will provide continued terrestrial habitat for amphibians. A further 152,400m² within the wider golf course will be enhanced to provide compensation for loss of habitat within the south western part of the site providing rough grassland and scrub/understorey habitats.
- 5.11 To provide further enhancement for amphibians and other fauna, a number of artificial hibernacula (see Appendix 5) and log/brush piles should be created in suitable areas throughout the golf course. The existing waterbodies could also be enhanced by planting with suitable native marginal plants.

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Wildlife & Countryside Act 1981 (as amended)

Figure 1:
Pond/ Waterbody Location Plan

Figure 1:
Pond / Waterbody plan

Map Centre Ref: (NGR) 339582, 397944
Map Scale: 1:450 @ A3

-  Site boundary
-  Pond / waterbody
P = Pond / waterbody number

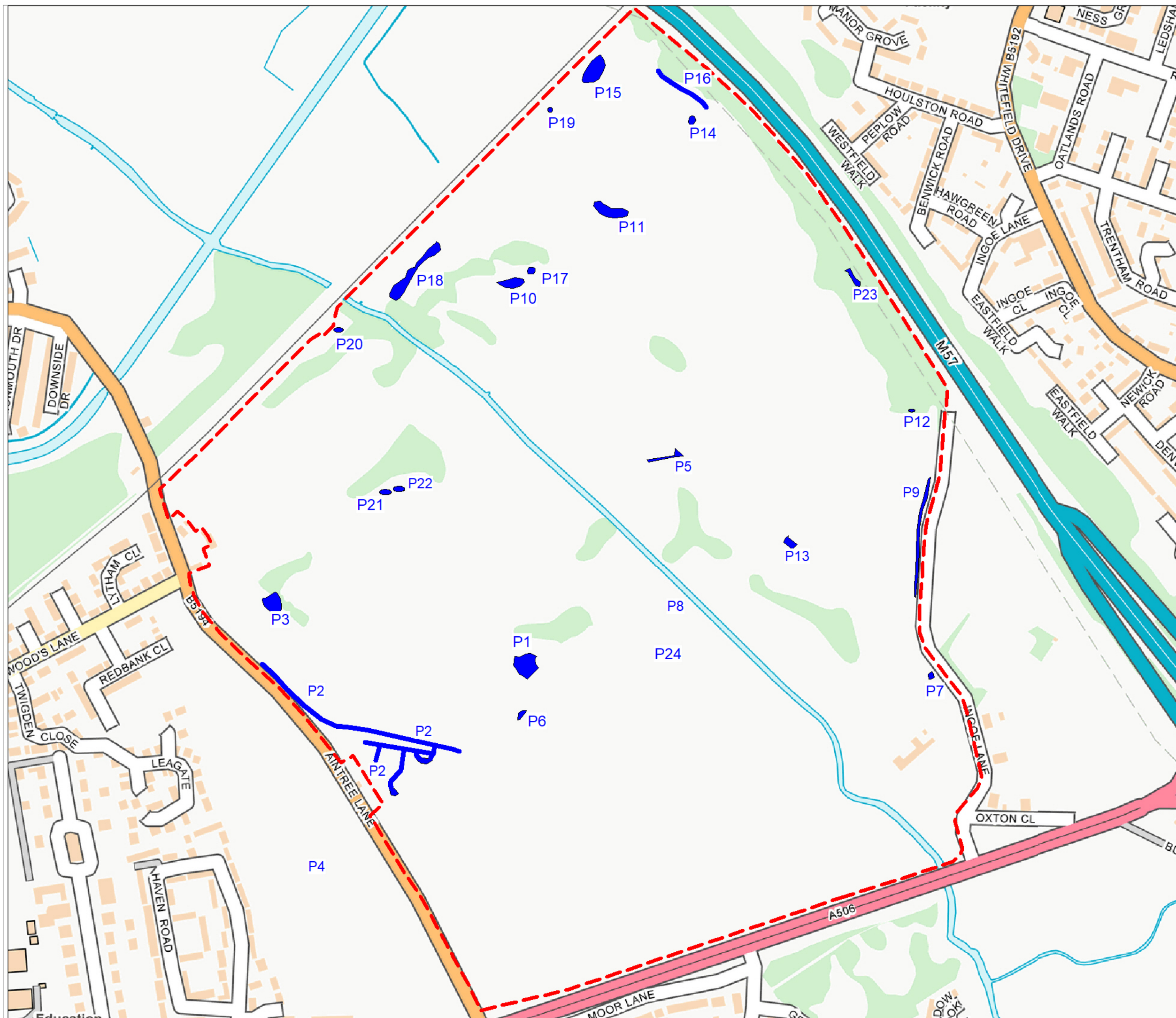
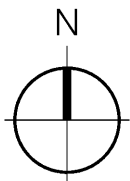


Figure 2:
Proposed Development Plan



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SITE SPECIFIC HAZARDS

IN ACCORDANCE WITH THE REQUIREMENTS OF THE CON REGULATIONS 2015 THE FOLLOWING SIGNIFICANT RESIDUAL HAZARDS HAVE NOT BEEN DESIGNED OUT OF THIS PROJECT AND MUST BE TAKEN INTO CONSIDERATION BY CONTRACTORS PLANNING TO UNDERTAKE THE WORKS SHOWN ON THIS DRAWING

ORDNANCE SURVEY LICENCE

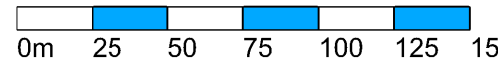
REPRODUCED FROM ORDNANCE SURVEY MASTERMAP DIGITAL DATA BY PERMISSION OF ORDNANCE SURVEY ON BEHALF OF HER MAJESTY'S STATIONARY OFFICE © CROWN COPYRIGHT 2015. ALL RIGHTS RESERVED. REFERENCE NUMBER 100009006.

NOTES

— SITE BOUNDARY 80.3055 HECTARES

— OTHER LAND IN THE OWNERSHIP OF THE CLIENT

SCALE 1:2500



- ROOT PROTECTION AREA
- CATEGORY B TREE
- CATEGORY C TREE
- CATEGORY U TREE
- CROWN SPREAD
- TREE/SHRUB REMOVED

REV C 26.04.21 HIGHWAY UPDATE
REV B 16.03.21 GENERAL UPDATE
REV A 15.12.20 UPDATED
REV DATE NOTES

SPL
SPL
RSL
INT

CLIENT / PROJECT
MAGHULL DEVELOPMENTS LTD
KIRKBY GOLF CLUB - VALLEY ROAD
KIRKBY
DRAWING TITLE
PROPOSED ESTATE PLAN

STATUS
PLANNING

DATE 17.11.20 DRAWN RSL SCALE @ A1 1:2500

PROJECT NUMBER	UNIT / BLOCK	C / S/B CODE	TYPE & NUMBER	REVISION LETTER
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9627			L22	C
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Site Location Plans	L	GA Plans	P	Elevations	E
Sections	S	Details	D	Profile Colour	C

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Appendix 1: Statutory & Planning Context

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 April 2021. 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 newt 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 will 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 (as amended) lists great crested newt as a UK 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, 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 a method statement (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 an 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 license 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 UK Species of Principal Importance, under Section 41 of the Natural Environmental 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: 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: E5136
 Report No: 1
 Order No: 19046
 Client: ECOLOGY SERVICES LTD
 Contact: Charlotte Wood
 Contact Details: charlotte.wood@ecologyservices.co.uk
 Date: 21/05/2019

TECHNICAL REPORT

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

Date sample received at Laboratory: 16/05/2019
Date Reported: 21/05/2019
Matters Affecting Results: None

RESULTS

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
1040	Kirkby Golf Club	SJ 390 979	Pass	Pass	Pass	Negative	0
2597	Kirkby Golf Club	SJ 393 978	Pass	Pass	Pass	Negative	0
2598	Kirkby Golf Club	SJ 390 978	Pass	Pass	Pass	Negative	0

SUMMARY

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

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

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 SureScreen Scientifics Division Ltd, Morley Retreat, Church Lane, Morley, Derbyshire, DE7 6DE
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 Company Registration No. 08950940

Page 1 of 3



RESULTS INTERPRETATION

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

Site Name- Information on the pond.

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

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

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

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

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

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

METHODOLOGY

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

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

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

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

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

Reported by: Chris Troth

Approved by: Sarah Evans

End Of Report

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Page 3 of 3



Folio No: E6818
 Report No: 1
 Purchase Order: 20044
 Client: ECOLOGY SERVICES LTD
 Contact: Charlotte Wood

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: 21/04/2020
 Date Reported: 24/04/2020
 Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
0028	Pond 6, Kirkby Golf Club	SJ 393978	Pass	Pass	Pass	Negative	0
0030	Pond 5, Kirkby Golf Club	SJ 395981	Pass	Pass	Pass	Negative	0
0032	Pond 9, Kirkby Golf Club	SJ 397984	Pass	Pass	Pass	Negative	0

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

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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.



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Appendix 4: HSI Raw Data

		Pond 1		Pond 2		Pond 3		Pond 5		Pond 6		Pond 9	
SI Ref	Description of Index	Measure / Comment	SI score	Measure / Comment	SI score	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	A	1	A	1
SI2	Pond Area m2	300	0.6	500	1	200	0.4	50	0.1	50	0.1	150	0.3
SI3	Pond permanence	Never dries	0.9	Rarely dries	1	Never dries	0.9	Sometimes dries	0.5	Sometimes dries	0.5	Dries annually	0.1
SI4	Water quality	Good	1	Good	1	Good	1	Good	1	Good	1	Good	1
SI5	Shading %	30	1	40	1	15	1	0	1	90	0.4	50	1
SI6	Presence of waterfowl	Absent	1	Absent	1	Absent	1	Absent	1	Absent	1	Absent	1
SI7	Presence of fish	Absent	1	Absent	1	Absent	1	Absent	1	Absent	1	Absent	1
SI8	Pond Density in area	11	0.96	11	0.96	11	0.96	11	0.96	11	0.96	11	0.96
SI9	Terrestrial Habitat Quality	Moderate	0.67	Good	1	Poor	0.33	Good	1	Good	1	Good	1
SI10	Macrophyte cover in pond	10	0.4	20	0.7	10	0.4	100	0.8	0	0.3	0	0.3
HSI	Overall HSI for Pond:		0.821		0.961		0.734		0.722		0.597		0.622
	Comments:	Good		Excellent		Good		Good		Average		Average	

		Pond 10		Pond 11		Pond 12		Pond 13		Pond 14		Pond 15	
SI Ref	Description of Index	Measure / Comment	SI score	Measure / Comment	SI score	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	A	1	A	1
SI2	Pond Area m2	240	0.4	200	0.4	15	0.03	150	0.3	150	0.3	200	0.4
SI3	Pond permanence	Never dries	0.9	Never dries	0.9	Dries annually	0.1	Sometimes dries	0.5	Sometimes dries	0.5	Sometimes dries	0.5
SI4	Water quality	Good	1	Good	1	Poor	0.33	Good	1	Good	1	Good	1
SI5	Shading %	0	1	0	1	0	1	0	1	0	1	0	1
SI6	Presence of waterfowl	Absent	1	Absent	1	Absent	1	Absent	1	Absent	1	Absent	1
SI7	Presence of fish	Possible	0.67	Possible	0.67	Absent	1	Absent	1	Absent	1	Absent	1
SI8	Pond Density in area	13	1	13	1	13	1	13	1	13	1	13	1
SI9	Terrestrial Habitat Quality	Good	1	Good	1	Good	1	Good	1	Good	1	Good	1
SI10	Macrophyte cover in pond	5	0.35	25	0.55	90	0.9	0	0.3	50	0.8	50	0.8
HSI	Overall HSI for Pond:		0.781		0.817		0.495		0.733		0.809		0.833
	Comments:	Good		Excellent		Poor		Good		Excellent		Excellent	

		Pond 16		Pond 17		Pond 18		Pond 19		Pond 20		Pond 21	
SI Ref	Description of Index	Measure / Comment	SI score	Measure / Comment	SI score	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	A	1	A	1
SI2	Pond Area m2	200	0.4	50	0.1	300	0.6	50	0.1	20	0.03	200	0.4
SI3	Pond permanence	Dries annually	0.1	Sometimes dries	0.5	Never dries	0.9	Sometimes dries	0.5	Dries annually	0.1	Sometimes dries	0.5
SI4	Water quality	Good	1	Good	1	Good	1	Good	1	Good	1	Good	1
SI5	Shading %	0	1	0	1	0	1	100	0.2	50	1	0	1
SI6	Presence of waterfowl	Absent	1	Absent	1	Absent	1	Absent	1	Absent	1	Absent	1
SI7	Presence of fish	Absent	1	Absent	1	Possible	0.67	Absent	1	Absent	1	Absent	1
SI8	Pond Density in area	13	1	13	1	13	1	13	1	8	0.88	8	0.88
SI9	Terrestrial Habitat Quality	Good	1	Good	1	Good	1	Good	1	Good	1	Good	1
SI10	Macrophyte cover in pond	60	0.9	0	0.3	30	0.6	0	0.3	0	0.3	0	0.3
HSI	Overall HSI for Pond:		0.717		0.657		0.858		0.559		0.490		0.745
	Comments:	Good		Average		Excellent		Below average		Poor		Good	

		Pond 22		Pond 23	
SI Ref	Description of Index	Measure / Comment	SI score	Measure / Comment	SI score
SI1	Geographic Location	A	1	A	1
SI2	Pond Area m2	200	0.4	250	0.5
SI3	Pond permanence	Sometimes dries	0.5	Dries annually	0.1
SI4	Water quality	Good	1	Good	1
SI5	Shading %	15	1	100	0.2
SI6	Presence of waterfowl	Absent	1	Absent	1
SI7	Presence of fish	Possible	0.67	Absent	1
SI8	Pond Density in area	11	0.96	11	0.96
SI9	Terrestrial Habitat Quality	Good	1	Good	1
SI10	Macrophyte cover in pond	10	0.4	0	0.3
HSI	Overall HSI for Pond:		0.743		0.557
	Comments:	Good		Below average (DRY)	

Appendix 5: Hibernaculum Design (English Nature, 2001)

Figure 1: Suggested hibernaculum design

This design mimics artificial and natural conditions in which great crested newts have frequently been found over-wintering. Dimensions should not be below 2m length x 1m width x 1m height. The illustrated design would be suitable for locating on an impermeable substrate. On free-draining substrates, the design is largely similar but the bulk of the fill is sited in an excavated depression in the ground. Hibernacula should ideally be positioned across a site, both close to and distant from breeding ponds, always in suitable terrestrial habitat and above the flood-line.

