***Land off New Brighton Road, New Brighton***

# Environmental DNA (eDNA) & Habitat Suitability Index (H.S.I.) Survey Report for Great Crested Newt

**Compiled by Ecology Services Ltd.**

*on behalf of*

Stuart Milne Homes NW Ltd.

**July 2019 (rev. May 2021)**


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# 1.0 Introduction

1.1 Ecology Services Limited was commissioned by Stewart Milne Homes NW England Ltd. in May 2018, June 2019 and April 2021, to undertake eDNA great crested newt (GCN) surveys of ponds on land near New Brighton Road, Mold, CH7 6QJ; National Grid Reference; (NGR) 325193, 365564. See Drawing 1 showing Site Location Plan & Pond Locations.

1.2 The proposals for the site are for housing and associated infrastructure on land to the south of New Brighton Road and to the east of Argoed View. See Drawing 2 which shows the Proposed Layout (V23 07.04.21).

1.3 Subsequent to an appeal in relation to a previous planning application at the site, the scheme has been revised with a reduction in the number of units from 92 to 84, a new footpath along the northern boundary of the site, an increase in the size of the Local Equipped Area for Play (LEAP) to the south west of the site and of the Public Open Space (POS) to the north west of the site.

1.4 The aims of the survey were to:

- Undertake eDNA samples of waterbodies within 250m, following best practice protocol for samples.
- Undertake a desk-based Habitat Suitability Index (H.S.I) assessment for ponds with no access.

# 2.0 Background

2.1 Desktop study records obtained as part of the Preliminary Ecological Appraisal 2018 found four relevant records pertaining to great crested newt. See Appendix 3 for the Data Search Plan.

- The desktop study returned results for Pond 1 with 5 individual newts recorded located at 249m north of the site and is referenced as 'small pond, off Lake Offa' dated 1996.
- The second record is for great crested newts present at Tyn-y-Coed Farm located 178m north of the site dated 1995.
- The third record is for one male and one female within a garden pond located 201m north of the site dated 2002.
- The fourth record is an incidental record of an adult great crested newt located 29m south of the site dated 2015. The newt was found outside the cellar at the Beaufort Hotel by a member of staff and not a suitably experienced ecologist. The staff member was shown photographs of newt species and confirmed the finding to be great crested newt. The Beaufort Hotel is located to the east of the site at approximately 96m, not as the record indicates.

2.2 Located within 250m of the site are two ponds, located approximately 249m north west of the site boundary (Pond 1) and approximately 70m north of the site boundary (Pond 2), both located to the north of New Brighton Road. Access was granted to take eDNA samples from Pond 1 in 2018 and 2019, but refused in 2021 with access being refused to Pond 2 in all three years.

2.3 A visit was made to the property at the location of the third record above on 28th June 2019 and the owner confirmed that there is no pond in the residential garden and had no recollection that there ever had been. The current owners had lived there for approximately 5 years and prior to that it had been owned by the parents of family friends. No pond was

found to be present in the woodland at the location of the second record above during the site visit undertaken on 28th June 2019. The ponds to which these two records relate are either no longer present or the co-ordinates provided are incorrect.

2.4 The previous planning application was refused partially on the grounds of 'the potential to cause disturbance to great crested newts and/or loss or damage to their resting places'.

2.5 During the appeal to this planning decision, the planning inspector summarised that their 'overall conclusions in respect of GCNs are that the proposed development would not conflict with development plan or national policy or with the requirements of the Habitats Regulations'.

2.6 This conclusion was based on a number of factors including the precautionary terms used by the Council and Natural Resources Wales including 'the potential to cause disturbance' and that 'it is possible that the species uses the site', which was based on 4 records of GCN within the vicinity of the site. These records were investigated and concluded that 3 'no longer had much relevance' and 1 has not been confirmed as a GCN; evidence provided by local residents 'do not materially change the paucity of evidence of GCNs using the appeal site'; and the survey effort of the pond that had access was considered 'reasonable and adequate'.

## 3.0 Legislation and Planning

3.1 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[1] (as amended).

3.2 In brief, this legislation makes it is an offence to: -

- Deliberately capture, injure or kill any wild animal;
- Deliberately disturb wild animals;
- Damage or destroy a breeding site or resting place of such an animal.

3.3 Disturbance is defined as that which is likely:

1. to impair their ability –
   - to survive, to breed or reproduce, or to rear or nurture their young, or
   - in the case of animals of a hibernating or migratory species, to hibernate or migrate; or
2. to affect significantly the local distribution or abundance of the species to which they belong.

3.3 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 Resources Wales and can only be applied for, once planning permission is granted, if planning permission is required.

3.4 Natural Resources Wales (NRW) has the powers to grant an EPS licence for the following purposes;

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

- 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.

3.5 In addition, NRW 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 favorable conservation status in their natural range.

3.5 When dealing with cases where a European Protected Species (EPS) 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 and planning decisions should only be made when European Protected Species and their habitats are fully taken into account.

3.6 The Environment (Wales) Act 2016, sets out the requirement for the 'sustainable management of natural resources' together with new ways of working to achieve this. Part 1 of the Environment Act sets out Wales' approach to planning and managing natural resources at a national and local level with a general purpose linked to statutory 'principles of sustainable management of natural resources' defined within the Act.

<u>Section 6 – Biodiversity and resilience of ecosystems duty</u>

3.7 Section 6 under Part 1 of the Environment (Wales) Act 2016 introduced an enhanced biodiversity and resilience of ecosystems duty (the S6 duty) for public authorities in the exercise of functions in relation to Wales. The S6 duty requires that public authorities must seek to maintain and enhance biodiversity so far as consistent with the proper exercise of their functions and in so doing promote the resilience of ecosystems.

<u>Section 7 - Biodiversity lists and duty to take steps to maintain and enhance biodiversity</u>

3.8 This section replaces the duty in Section 42 of the Natural Environment and Rural Communities (NERC) Act 2006. The Welsh Ministers will publish, review and revise lists of living organisms and types of habitat in Wales, which they consider are of key significance to sustain and improve biodiversity in relation to Wales. Great crested newt and common toad are listed.

3.9 The Welsh Ministers must also take all reasonable steps to maintain and enhance the living organisms and types of habitat included in any list published under this section, and encourage others to take such steps. Part 1 of the Act, including Sections 6 and 7, came in to force on May 21, 2016.

3.10 Planning Policy Wales (PPW) Edition 11 (February 2021) sets out the land use planning policies of the Welsh Government and is supplemented by a number of Technical Advice Notes (TANs) which combined sets out the national planning policy for Wales. In particular, TAN 5: Nature Conservation and Planning (2009) details how the land use planning

systems should contribute to protecting and enhancing biodiversity and geological conservation. All local planning authorities have a statutory duty to make arrangements to secure continuous improvement in the exercise of their functions and should aim to enhance the sustainable quality of life and environment for local citizens and communities.

3.11 Development plan strategies, policies and development proposals must consider the need to: support the conservation of biodiversity, in particular the conservation of wildlife and habitats; ensure action in Wales contributes to meeting international responsibilities and obligations for biodiversity and habitats; safeguard protected and priority species and existing biodiversity assets from impacts which directly affect their nature conservation interests and compromise the resilience of ecological networks and the components which underpin them; and secure enhancement of and improvements to ecosystem resilience by improving diversity, condition, extent and connectivity of ecological networks.

3.12 Flintshire Local Development Plan (2015) Biodiversity and Nature Conservation (Topic Paper No 1) notes that biodiversity conservation and enhancement is an essential contributor to sustainability. One of the key objectives is therefore to conserve and enhance species and their habitats that are of international, national and local importance and which may be threatened by new development.

# 4.0 eDNA Summary

4.1 Environmental DNA (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.

4.2 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.

4.3 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.

4.4 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 June.

# 5.0 Methodology

***eDNA***

5.1 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.

5.2 See Appendix 1 Sampling Protocol for equipment used and sample collection protocol. Upon collection samples were sent by courier to SureScreen Scientifics.

5.3 SureScreen Scientifics developed the methodology to detect aquatic and semi-aquatic species from a water sample, by analysing the DNA released by organisms into the environment (environmental DNA (eDNA)), which was accepted by Natural England.

***Habitat Suitability Index (HSI)***

5.4 As access was denied for the survey of Pond 2 (Appendix 2), a desk-based Habitat Suitability Index (HSI) has been undertaken based upon information sourced via Google maps. For the purpose of this review, where any indices cannot be accurately taken a worst-case precautionary approach is adopted and the maximum score applied.

5.5 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.

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

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

***Timing***

5.8 The eDNA samples were taken on the 27th of June 2018 which within the accepted sample period of mid-April to June. A second set of samples were taken on 28th June 2019, also within the accepted sample period.

5.9 The HSI assessment of the Pond 2 was desk based and undertaken on the 1st of November 2018.

***Weather Conditions***

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

***Personnel***

5.11 All Ecologists were subject to training in line with the Freshwater Habitats Trust (FHT) eDNA sampling methods.

5.12 The eDNA samples were undertaken under Natural England Class Survey Licence (CLS) by experienced Ecologist Miss. C. Wood (Class Licence number 2017- 32734-CLS-CLS).

***Limitations of Survey***

5.13 There were various limitations when taking the samples, which were mainly due to access restrictions where the desired 80% of pond sample areas around the ponds were not achievable. See Table 1 for relevant notes. Where the % of ponds sample is under the desired 80%, caution should be taken, especially where a negative result has been found.

5.14 Where positive results have been found in conjunction with disturbance to sediments, it can be the case that historic eDNA in sediments are being recorded.

***Access constraints***

5.15 Access was not permitted to the large pond (Pond 2) to the north of the site.

5.16 Access to Pond 1 was refused in 2021, however, Pond 1 has been sampled twice in recent years and is on the outer limit of the 250m buffer zone around the site, therefore this is not considered a significant constraint to the assessment.

# 6.0 Results

***eDNA Sample Results***

6.1 Following analysis of the samples by SureScreen, the eDNA results are presented in Table 1 below. The SureScreen Scientifics Technical Report can be found in Appendix 3.

**Table 1: eDNA Results**

| Pond No. | Sample date | Sample time | % of pond accessed | Relevant notes | Positive/ negative & Replicates |
|---|---|---|---|---|---|
| 1 | 27.06.18 | 10:15 | 60% | Shallow water depth, cattle poached margins, evidence of waterfowl and disturbed sediment | Negative |
| 1 | 28.06.19 | 14:20 | 75% | Steep banks and dense scrub, some areas of dense rush restricting access. Water very turbid with large fish present. | 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.*

***Habitat Suitability Index Results***

6.2 Owing to access not being permitted to Pond 2, the pond was subjected to a desk-based Habitat Suitability Index (HSI) assessment to determine the ponds suitability to support great crested newt and provide supportive evidence. A summary of the results is shown in Table 2 below.

6.3 Reference sources for the desk based HSI include: Google Earth.

**Table 2**: Summary Results of Habitat Suitability Index Assessment

| Pond No. | HSI Score | Pond Suitability | Summary of Contributing Factors |
|---|---|---|---|
| 1 | 0.828 | Good | Geographic location; pond area (>2,000m$^2$); pond density; waterfowl are largely absent and fish are possible; good terrestrial habitat quality. |

| 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 |

# 7.0 Implications & Recommendations

7.1 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.

7.2 The two rounds of eDNA sampling found no evidence of great crested newt in Pond 1, although it must be noted as in Table 1, the survey triggered the risk factor for ponds with less than 80% of the pond margin being surveyed and therefore, did not meet the required standard. However, following a detailed review of this result and survey conditions, the surveyor felt that the first set of samples were taken from a wide range of niche habitats within the waterbody which appeared favourable to great crested newt. During the second sampling, a greater percentage of the pond margin was able to be sampled, although still slightly below the desired 80% or more. The risk factor is also employed for ponds with high turbidity as this can sway the results in favour of great crested newt being falsely present. With this in mind, the low detectability warning for percentage cover could potentially be ignored as there was a high turbidity at the time of the survey and yet, the result found negative evidence for great crested newt. There are therefore, no apparent implications arising from great crested newt and Pond 1.

7.3 Pond 2 was not surveyed due to access not being permitted (Appendix 2) and so the pond's suitability was assessed using the HSI methodology to provide additional information. The HSI is based upon the precautionary approach scoring maximum values for all indices that couldn't be physically scored due to the lack of access, for example: water quality, macrophyte cover, absence of fish and water fowl. This resulted in a score of 0.828 (Good) for great crested newt. However, based upon the appearance of the pond using aerial imagery and the opinion of a professional ecologist experienced in amphibian surveys, it is likely that a lower result would have been achieved with a site-based survey. Taking into account that waterfowl and fish would likely be present in such a large pond and that water quality and macrophyte cover score would be lower because of this, a more realistic score of 0.257 (Poor) is possible when the HSI scores are run again. While an HSI assessment is not a substitute for undertaking surveys, it is considered unlikely that GCN would be present in Pond 2 given the above. The pond is however considered to be ideal for common toad.

7.4 Great crested newts have been found to move over considerable distances (up to 1.3km from breeding sites). However, the vast majority of newts will inhabit an area much closer to the pond, and the exact distribution and migration patterns of newts on land depends on a variety of factors. The quality of terrestrial habitat near to breeding ponds is important, as are the lack of barriers to dispersal (such as fast-flowing rivers or very busy roads). The distribution of ponds and hibernation opportunities may also influence movements (English Nature, 2001). Although a maximum routine migratory range for GCN has been estimated as approximately 250m from a breeding pond (Franklin, 1993; Oldham and Nicholson, 1986; Jehle, 2000), Jehle (2000) determined a terrestrial zone of 63m, within which 95% of summer refuges were located. In addition, following the breeding season, Jehle and Arntzen (2000) recorded 64% of newts within 20m of the pond edge.

7.5 An assessment of the efficiency of capture techniques and the value of different habitats for the great crested newt *Triturus cristatus* (1990-2001) in England (Cresswell and Whitworth, 2004) identified a clear inverse relationship between distance from the breeding pond and captures along drift fences, for those projects which used drift fencing away from ponds as part of an exclusion and relocation project. Captures were greatest within 50m of a pond and few captures were recorded greater than 100m from a pond. The report recommended

that the most comprehensive mitigation, in relation to avoiding disturbance, killing or injury is appropriate within approximately 50m of a breeding pond. It will also almost always be necessary actively to capture newts 50-100m away. However, at distances greater than 100m, there should be careful consideration as to whether attempts to capture newts are necessary or the most effective option to avoid incidental mortality. At distances greater than 200-250m, capture operations will hardly ever be appropriate.

With regards Pond 2, in the unlikely event that GCN are present, there is a significant amount of high quality terrestrial habitat (woodland, approximately 2.48ha) within approximately 50m of the pond with a further approximately 1.1ha within 250m to the west (Drawing 3). This represents a significant high quality terrestrial habitat resource for GCN, if present, as opposed to the more intensively managed agricultural land, including the proposed development site.

7.6 Research evidence would suggest that the majority of the GCN population is likely to remain within 100m of a breeding pond. Given the significant amount of good quality terrestrial habitat within 50m of Pond 2, there is no reason to believe GCN (if present) would disperse over a wider area. The majority of the proposed development site (89.4%) lies beyond 100m of Pond 2 and it is therefore considered unlikely that GCN would be using the proposed development site.

7.7 In addition to the above survey and desk-based assessment, the site was subject to reptile surveys during the 2018 season when amphibians are also active moving to and from ponds. The survey did not detect any presence for great crested newt, although eleven common toad were found beneath refugia. The application site provides foraging habitat only as it comprises mostly semi-improved grassland, with the exception of boundary hedgerows (which are mostly being retained) and some areas of taller vegetation around the boundaries and associated with some scattered trees centrally within the site. There is nothing so dense to be considered ideal for refuge or hibernation.

7.8 Taking into consideration the following it is considered unlikely that great crested newts are using the site:

- the date and reliability of the desktop study records for great crested newt;
- the negative result for great crested newt in Pond 1;
- the likely low HSI score of Pond 2;
- availability of good quality terrestrial habitat in close proximity to Pond 2;
- fairly busy road between the ponds and the site; and
- only common toad was detected during reptile refugia searches at the site,

7.9 As requested by NRW in their pre-application consultation response dated 5th June 2019 in relation to the previous planning application at the site, a separate precautionary compensatory scheme has been prepared which details the precautionary measures to be implemented at the site.

7.10 If at any time a great crested newt is suspected or found on site, works should cease in that area and the acting consultant or Natural Resources for Wales contacted for advice.

# 8.0 References

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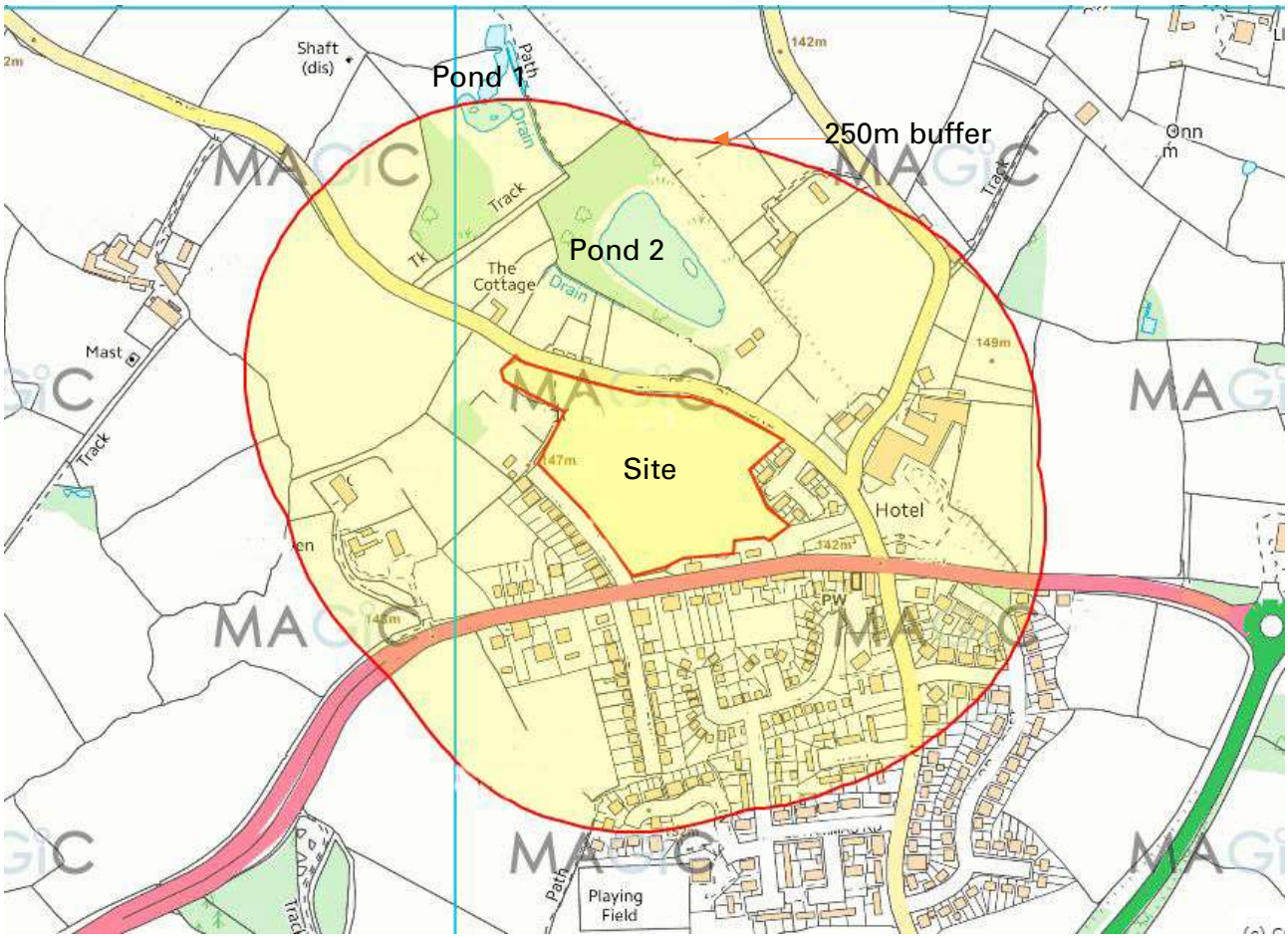
**Drawing 1:**
Site Location Plan & Pond Locations



**Drawing 2:**
Proposed Planning Layout (V23 07.04.21)

# Argoed View, Nr Mold
# Proposed Sketch Layout - 1:500

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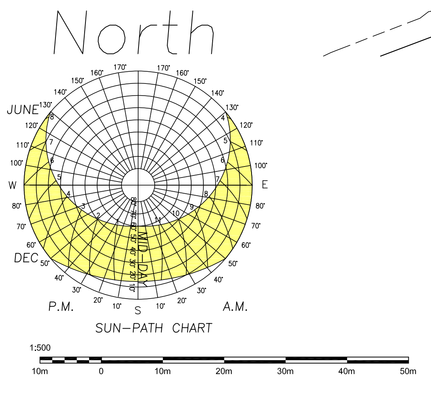
## Planning Layout Legend

- Proposed dwelling and Dwelling type.
- Proposed dwelling handing.
- 26 — Plot numbers.
- AF — Affordable Dwellings.
- Proposed dual aspect units.
- Proposed SUDS pond.
- Proposed indicative tree position. (refer to landscaping layout for detail )
- Existing Category A trees to be retained. (refer to tree report for detail )
- Existing Category B trees to be retained. (refer to tree report for detail )
- Existing Category C trees to be retained. (refer to tree report for detail )
- Trees to be removed. (refer to tree report for detail )
- Hard landscaping Treatment A — Alternative surface material
- Hard landscaping Treatment B — Block Paving Herringbone banded to shared surface, footpaths and private driveways Colour: TBC
- Soft Landscaping — Soft Landscaping. Refer to landscaping plans for schedule and detail.
- Boundary Treatment A — 1800mm high brick screen wall
- Boundary Treatment B — 1800mm high close boarded fence.
- Boundary Treatment C — 1200mm high 'stock proof' post and wire fence
- Application Site boundary.
- Existing Easement.


1:500
10m 0 10m 20m 30m 40m 50m

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## Schedule of Accommodation

| Mews Dwelling Types AFFORDABLE | | | SQFT | No | Total Sqft |
|---|---|---|---|---|---|
| AB | 5% | Aberlady Mid | 654 | 4 | 2616 |
| AB | 10% | Aberlady End | 657 | 8 | 5256 |
| AE | 4% | Aviemore Mid | 770 | 3 | 2310 |
| AE | 12% | Aviemore End | 770 | 10 | 7700 |
| Total dwellings and sqft | | | | 25 | 17,882 |

| 2/3 Bed Mews Dwelling Types | | | SQFT | No | Total Sqft |
|---|---|---|---|---|---|
| AL | 3% | Alnwick Mid | 713 | 2 | 1426 |
| BK | 5% | Berwick Mid | 866 | 3 | 2598 |
| BK | 22% | Berwick End | 869 | 13 | 11297 |
| CL | 19% | Cairnhill | 972 | 11 | 10692 |
| CU | 7% | Culcross | 1031 | 4 | 4124 |

| 3 Bed Detached Dwellings | | | SQFT | No | Total Sqft |
|---|---|---|---|---|---|
| CD | 7% | Castleford | 963 | 4 | 3852 |
| CM | 5% | Corningham | 1026 | 4 | 4104 |

| 4 Bed Semi Detached Dwellings | | | SQFT | No. | Total Sqft |
|---|---|---|---|---|---|
| DY | 7% | Dewsbury | 1141 | 4 | 4564 |

| 4 Bed Detached Dwellings | | | SQFT | No | Total Sqft |
|---|---|---|---|---|---|
| FH | 3% | Farnham | 1266 | 2 | 2532 |
| HR | 7% | Harris | 1382 | 4 | 5528 |
| KD | 4% | Kendal | 1493 | 3 | 4479 |

| 5 Bed Detached Dwellings | | | SQFT | No | Total Sqft |
|---|---|---|---|---|---|
| LE | 8% | Leven | 1630 | 5 | 8150 |
| Total dwellings and sqft - OMS | | | | 59 | 63,346 |
| Total dwellings and sqft - including Affordable | | | | 84 | 81,228 |

| | |
|---|---|
| Gross Site Area in Acres | 8.344 |
| POS | 1.118 |
| SUD's | 0.706 |
| Single Sided Road | 0.268 |
| Undevelopable | 0.507 |
| Net Site Area in Acres | 5.75 |
| Density (units per acre) | 15 |
| Density (units per hectare) | 36 |
| Nett Square foot / Acre | 14,139 |

| Rev | Details | Date | By | Chk |
|---|---|---|---|---|

| | | | |
|---|---|---|---|
| DATE | 03.2021 | DRAWN | DJM |
| SCALE | 1:500 | CHK | |

Argoed View, Nr Mold

Proposed Planning Layout

| Dwg No | SK296/NBM/PL50 | Rev |
|---|---|---|


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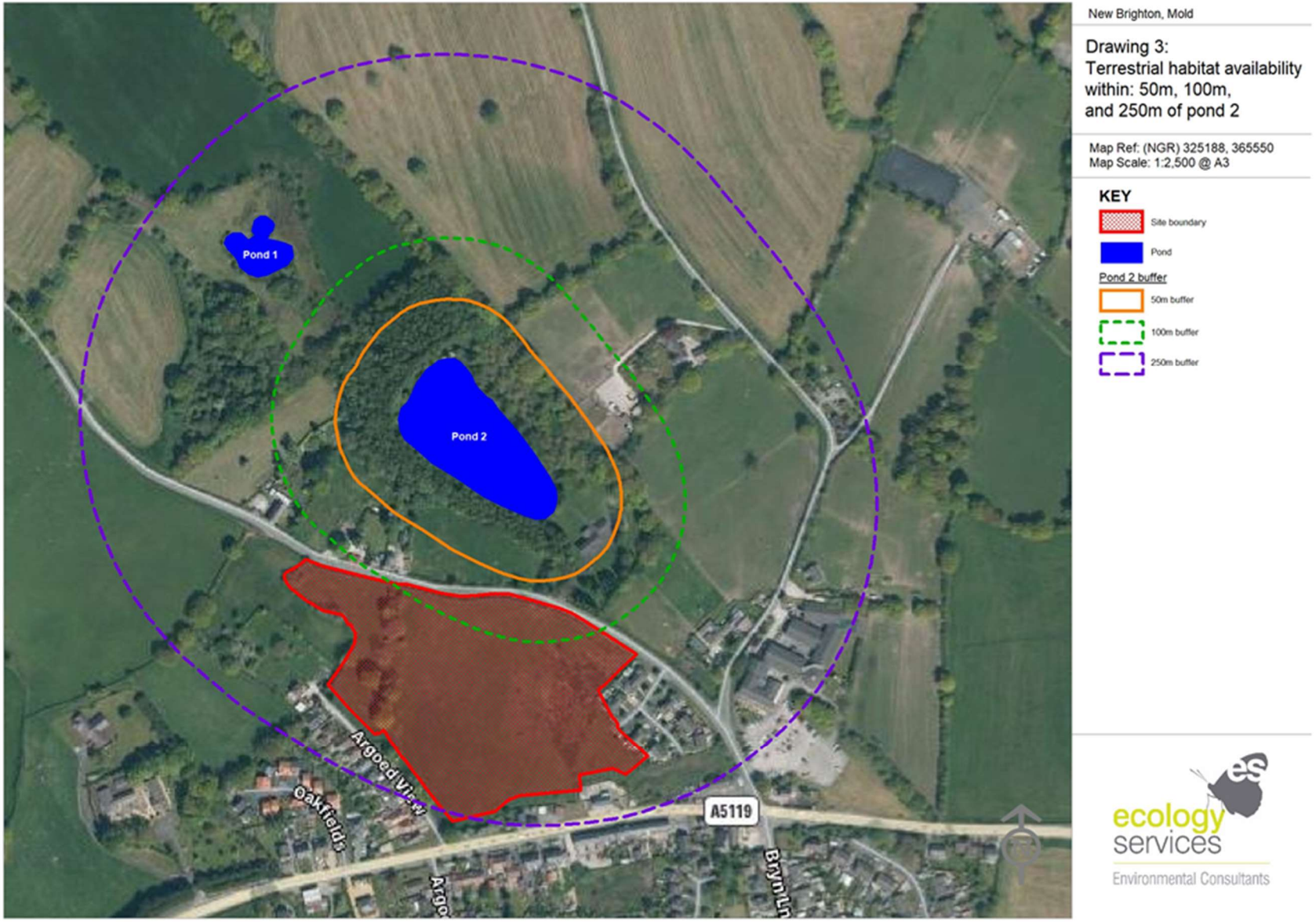
**Drawing 3:**
Terrestrial Habitat Availability within 50m, 100m and 250m of Pond 2




New Brighton, Mold

Drawing 3:
Terrestrial habitat availability within: 50m, 100m, and 250m of pond 2

Map Ref: (NGR) 325188, 365550
Map Scale: 1:2,500 @ A3

**KEY**

- Site boundary
- Pond

Pond 2 buffer

- 50m buffer
- 100m buffer
- 250m buffer

ecology services
Environmental Consultants

# **Appendix 1:**
# 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 (*Tritrus cristatus*) environmental DNA 26th March 2014**

The field sampling equipment were supplied by SureScreen Scientific and used by Briggs et al (2014) has five components:

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

One sampling kit per pond is used up to an area of 1ha.

Kits can be stored at room temperatures before use and should be used within about two weeks of receipt.

***eDNA Sampling Methodology***
The following field water sampling protocol was strictly adhered too, Gloves are to be worn at all times during the sampling process, replacing gloves between sample collection for the pond and pipetting into the sterile sub-sample tubes.

Samples shall be collected without entering the water, i.e. the surveyor stands only on the bank or muddy pond edges, to prevent disturbance of the substrate and may limit cross contamination.

**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-Pack 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 30mL of pond water from around the pond using the ladle (fill the ladle) and empty each sample into the Whirl-Pack bag. At the end of the Whirl-Pack bag should be just under half full (600ml).

NB: 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 the pond bottom without disturbing the sediment on the bed of the pond. It is advisable not to sample very shallow water (less than 5-10cm deep).

**Step 4**
Once 20 samples have been taken, close the bag securely using the top tabs and shake the Whirl-Pack 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 c.15mL of water from the Whirl-Pak and pipette into a sterile tube containing 35mL of ethanol to preserve the eDNA sample (i.e. fill tube to the 500mL 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-Pack 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 to 4 degrees Celsius. 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 cost by requiring refrigerated transport. The length of time eDNA samples are stored in a refrigerator prior to analysis should be recorded and passed onto 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<br><br>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: | | % of pond sampled: (Min 80/90% desired) | |

| | | | |
|---|---|---|---|
| (20 samples required) | | | |
| Is the pond <1ha? | Yes/No | | |
| Notes of any constraints to sampling:<br>(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:<br>(Date and time) | | Signed into fridge by: | |
| Fridge temperature check: (Date & time) | | Fridge temperature checked: | |
| Collected by Courier:<br>(date) | | Collected by Courier:<br>(Staff responsible) | |
| eDNA sampling result | Positive/Negative | | |
| Other amphibian survey result: | Visit 00 of 4/6/Date/Method/Results | | |

# **Appendix 2:**
# Correspondence Regarding Ponds 1 and 2 Access

**From:** Richard Baker <Richard.Baker@fishergerman.co.uk>
**Sent:** 12 May 2021 10:52
**To:** Sophie Doyle <SDoyle@StewartMilne.com>
**Subject:** [EXTERNAL] FW: Land at New Brighton, Nr Mold - Mr R Bletcher

**Please note that this Email originated outside of the organisation.**

---

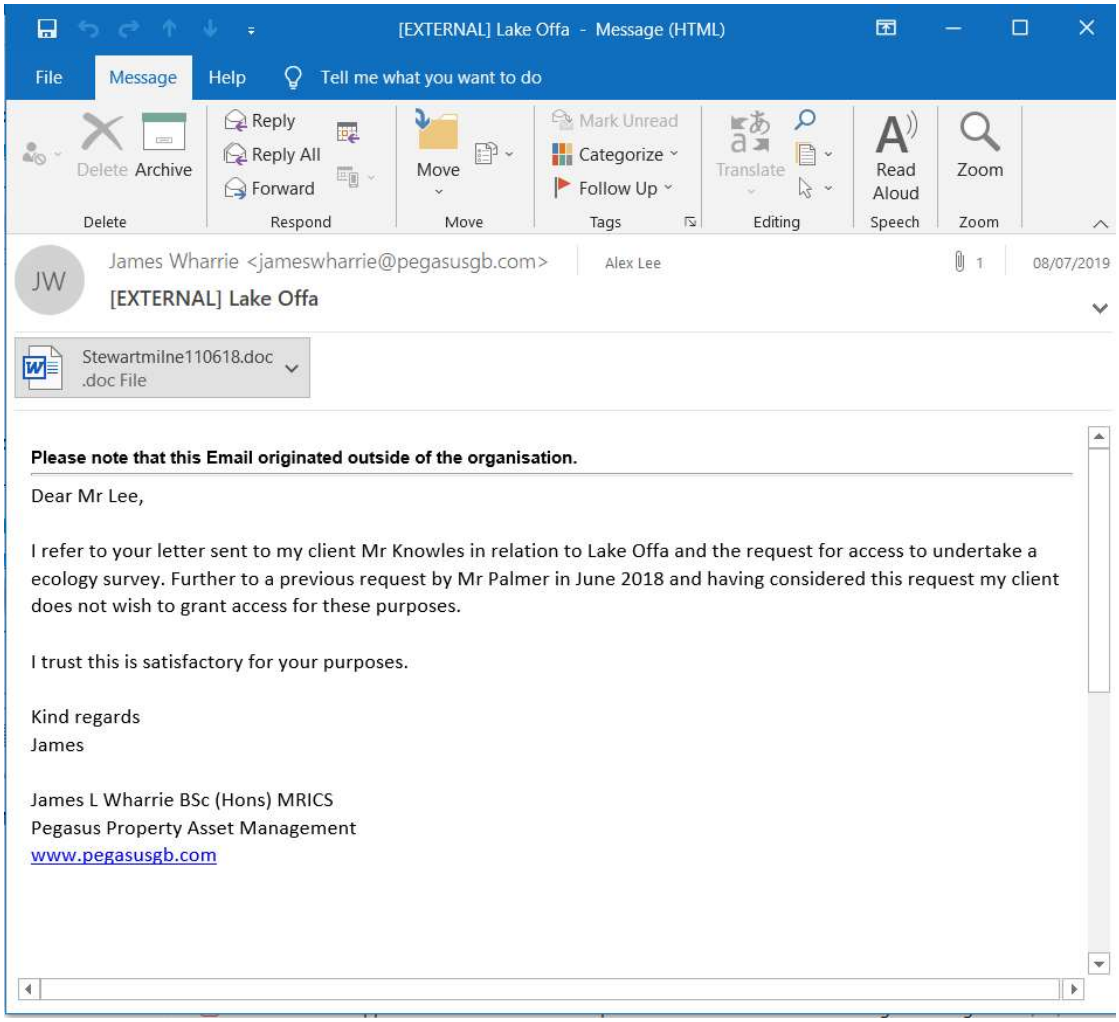
Sophie,

I understand that you have written to my client Mr Roger Bletcher with regard to an access requirement for ecological surveys on land at Sychdyn which he owns. My client has stated that he is not prepared to grant any access on this occasion and trust that you understand.

Kind regards

Richard Baker MRICS FAAV

For and on behalf of Fisher German LLP
01244409662
07786336925



PEGASUS
PROPERTY ASSET MANAGEMENT

11th June 2018

Phillip Palmer Esq.
Stuart Milne Homes
Harrier House
2 Lumsdale Road
Cobra business Park
Trafford Park
Manchester
M32 0UT

Via Email
PRIVATE AND CONFIDENTIAL

Dear Phillip,

**Lake Offa, New Brighton – Pond Access**

I act on behalf of my client, Mr Knowles who has provided me with a copy of your letter dated 4th June in connection to the above property.

I note your request to undertake a non-intrusive water sample survey of the private lake, known as Lake Offa, to assist with your forthcoming planning application. Having considered the merits or otherwise, at this time my client will not grant you consent to undertake the survey or access of any kind to the property.

I trust this suffices for your records. I would be grateful if further correspondence relating to this matter could be directed to me.

Yours sincerely

James L Wharrie BSc (Hons) MRICS

Property Director: James Wharrie BSc (Hons) MRICS

Pegasus Property Asset Management Woodside House Ashton Hayes Chester CH3 8AE

Email: jameswharrie@pegasusgb.com Web: www.pegasusgb.com

# Appendix 2:
SureScreen Scientifics Technical Reports

SureScreen Scientifics

Folio No: E3568
Report No: 1
Order No: 18081
Client: ECOLOGY SERVICES LTD
Contact: Charlotte Wood
Contact Details: charlotte.wood@ecologyservices.co.uk
Date: 11/07/2018

## TECHNICAL REPORT

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

**Date sample received at Laboratory:** 29/06/2018
**Date Reported:** 11/07/2018
**Matters Affecting Results:** None

**RESULTS**

| Lab Sample No. | Site Name | O/S Reference | SIC | DC | IC | Result | Positive Replicates |
|---|---|---|---|---|---|---|---|
| 3082 | 18081 | SJ250659 | 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.

**RESULTS INTERPRETATION**

Forensic Scientists and Consultant Engineers
SureScreen Scientifics Division Ltd, Morley Retreat, Church Lane, Morley, Derbyshire, DE7 6DE
UK Tel: +44 (0)1332 292003 Email: scientifics@surescreen.com
Company Registration No. 08950940

SureScreen Scientifics

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.

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

SureScreen Scientifics

different buildings at our premises for added

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:** Sam Humphrey **Approved by:** Derry Hickman

End Of Report

Forensic Scientists and Consultant Engineers
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UK Tel: +44 (0)1332 292003 Email: scientifics@surescreen.com
Company Registration No. 08950940

SureScreen Scientifics

Folio No: E5886
Report No: 1
Order No: 19079
Client: ECOLOGY SERVICES LTD
Contact: Ben Meadows
Contact Details: ben.meadows@ecologyservices.co.uk
Date: 15/07/2019

# TECHNICAL REPORT

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

**Date sample received at Laboratory:** 02/07/2019
**Date Reported:** 15/07/2019
**Matters Affecting Results:** None

## RESULTS

| Lab Sample No. | Site Name | O/S Reference | SIC | DC | IC | Result | Positive Replicates |
|---|---|---|---|---|---|---|---|
| 4080 | Pond 1, New Brighton | SJ 25029 45899 | Pass | Pass | Pass | Negative | 0 |

## SUMMARY

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SureScreen Scientifics

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SureScreen Scientifics

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**Reported by:** Chris Troth **Approved by:** Sarah Evans

End Of Report

# **Appendix 3:**
Data Search Plan