



**LAND NORTH-WEST OF  
SOUTHWATER, HORSHAM**

**ENVIRONMENTAL STATEMENT,  
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2025 GREAT CRESTED NEWT eDNA  
AND HSI REPORT**

**FEBRUARY 2026**

**LAND NORTH-WEST OF SOUTHWATER, HORSHAM**

**2025 GREAT CRESTED NEWT HSI AND eDNA SURVEY REPORT**

**Prepared for Berkeley Strategic Land Ltd**

**by**

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HDA Document Control and Quality Assurance Record

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- A Great Crested Newt eDNA survey summary plan
- B Full HSI assessment results
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# 1 INTRODUCTION

## 1.1 Site location and summary description

1.1.1 This report describes a Habitat Suitability Index (HSI) assessment and eDNA sampling survey of waterbodies located in the vicinity of approximately 116ha of land west of Southwater, West Sussex, hereinafter referred to as 'the site'. The site centre is located by National Grid Reference TQ154274. The study was commissioned by Berkeley Strategic Land Ltd in April 2025.

1.1.2 The site is split into a main site and a smaller area. The main site lies on the north-western edge of the village of Southwater and comprises a series of arable fields, semi-improved grassland fields, and improved grassland fields bordered by hedgerows, treelines, and fence lines. Other habitats present include areas of broadleaved woodland, mixed woodland, and tall ruderal vegetation. In addition, Marpost Gill flows in a southerly direction through the west of this area and a second stream flows east to west through the north. The main site is bordered to the west by agricultural land and Two Mile Ash road and associated residential properties, beyond which are arable fields; to the north by the A24, industrial units, and agricultural land; to the south by Kirsty's Wood and Bonfire Hill road with associated residential properties and grassland fields; and to the east by Worthing Road, beyond which is the settlement of Southwater.

1.1.3 The smaller area of the site measures approximately 1.35ha in size and is located approximately 800m to the north-west of the main site near Christ's Hospital railway station. This area comprises part of a grassland field and is bordered to the north by woodland; to the east by grassland fields; to the west by a public right of way and access road with residential properties and Christ's Hospital station car park beyond, and to the south by residential properties.

1.1.4 This report relates to the Great Crested Newt Habitat Suitability Index (HSI) assessment and eDNA sampling surveys carried out in June 2025. The location and boundary of the site are shown in *Appendix A*.

## 1.2 Background and legislative context

1.2.1 Five species of amphibian are widespread in England: Common Frog *Rana temporaria*, Common Toad *Bufo bufo*; Smooth Newt *Lissotriton vulgaris*; Palmate Newt *Lissotriton helveticus*; and Great Crested Newt *Triturus cristatus*. Two further native species occur in the UK, namely the Natterjack Toad *Bufo calamita*, which has special habitat requirements that limit its range to certain sand dune and heathland sites, and the Pool Frog *Pelophylax lessonae* which is restricted to a limited number of sites in East Anglia.

- 1.2.2 Amphibians require aquatic habitat within which to breed and suitable terrestrial habitat to forage and hibernate. Suitable breeding ponds are usually well vegetated with still, shallow water that is not heavily shaded or very exposed. Terrestrial habitat includes woodland, scrub, field edges and gardens. Hibernation can occur under stone or log piles, in crevices or leaf litter and under the soil. Occasionally amphibians may be found hibernating in their breeding pools.
- 1.2.3 Over the last few decades all amphibians have suffered a decline in numbers. This is due to a combination of many factors, which include habitat destruction and fragmentation, loss of breeding pools through unsympathetic management and neglect, introduction of fish (which eat amphibian larvae) and pollution.
- 1.2.4 The Great Crested Newt is protected as a European Protected Species (EPS) under the 2017 Conservation of Habitats and Species Regulations (as amended). In relation to European Protected Species, the 2017 Regulations make it an offence to:
- Deliberately capture, injure or kill any wild animal of an EPS;
  - Deliberately disturb wild animals of any such species, in particular any disturbance which is likely to: (i) impair their ability to survive, to breed or reproduce, or to rear or nurture their young; or to hibernate or migrate; (ii) affect significantly the local distribution or abundance of the species to which they belong;
  - Damage or destroy a breeding site or resting place of such an animal; and/or
  - To (a) be in possession of, or to control; (b) to transport any live or dead animal or any part of an animal; (c) to sell or exchange or (d) offer for sale or exchange any live or dead animal or part of an animal of an EPS.
- 1.2.5 In addition, Great Crested Newts are protected under the 1981 Wildlife and Countryside Act (as amended). The Great Crested Newt is listed on Schedule 5 of the Act and is subject to the provisions of Sections 9.4b and 9.4c, which make it an offence to:
- Intentionally or recklessly disturb a Great Crested Newt while it is occupying a structure or place which it uses for shelter or protection; and/or
  - Intentionally or recklessly obstruct access to any structure or place used for shelter or protection by a Great Crested Newt.
- 1.2.6 Great Crested Newts and Common Toads are also identified as a Species of Principal Importance under Section 41 of the 2006 Natural Environment and Rural Communities (NERC) Act. Section 40 of the Act requires that these species are a material consideration in the planning process.

### **1.3 Development proposals**

1.3.1 Outline planning application, with all matters reserved (except for primary access to the highway) for a phased development comprising: the demolition of existing buildings and the construction of residential dwellings (including affordable housing) (Use Classes C2 and C3); a mixed-use neighbourhood centre (Use Classes E and F); education facilities (Use Class F1(a)); business and employment floorspace (Use Classes B2, B8 and E(g)); redevelopment of existing agricultural buildings including construction of a building for community use (Use Class F2); improvements to public rights of way; sports pitches; gypsy and traveller pitches/plots; public open space; landscaping, and associated infrastructure.

### **1.4 Scope and purpose of the report**

1.4.1 The desk study carried out in 2025 identified 61 records of Great Crested Newts, including one record relating to the main site itself, to the south of Great House Farm in the south of the site, dating from 2011. The UK Habitat Classification survey identified potential opportunities for breeding newts within the site in the form of ponds and suitable terrestrial habitats in the form of woodland, scrub, hedgerows, dry ditches and rough grasslands. A review of the OS 1:10,000 scale map and aerial photographs of the site's surrounds identified that a number of waterbodies are located within 300m of the site, of which thirteen are located within the site itself.

1.4.2 A Great Crested Newt HSI and eDNA survey was previously undertaken of the site by HDA in May and June 2022, which identified a population of Great Crested Newts in Waterbody 9 located off-site beyond the site boundary in the centre of the site (HDA, 2022).

1.4.3 In view of the presence of habitats suitable for aquatic and terrestrial phase Great Crested Newts within the site, the time that has elapsed since the previous survey, and the legislative context outlined in *Section 1.2* above, a series of updated Great Crested Newt surveys including Habitat Suitability Index (HSI) assessments and Great Crested Newt presence/absence eDNA surveys were subsequently undertaken. These surveys were undertaken by HDA in June 2025 and are the subject of this report. The aims of the study are:

- i. To establish the suitability of waterbodies within 300m of the site for Great Crested Newts;
- ii. To establish the likely presence/absence of Great Crested Newts breeding in suitable waterbodies within 300m of the site;
- iii. To determine requirements for any further survey work to estimate the size of any Great Crested Newt population potentially associated with the site; and
- iv. To predict likely impacts of the proposed development on Great Crested Newts and give recommendations for impact avoidance, minimisation and/or mitigation.

## **2 METHODOLOGY**

### **2.1 Great Crested Newt Habitat Suitability Index (HSI) Assessment**

2.1.1 HSI assessments provide a mechanism by which the suitability of a pond to support Great Crested Newts can be objectively assessed in order to assist in the identification of ponds potentially supporting this species (Oldham *et al.*, 2000).

2.1.2 For the HSI assessment the locations of waterbodies within a 300m radius of the site were identified from online aerial photographs, a 1:10,000 scale Ordnance Survey map and from other waterbodies encountered during the survey. Where necessary, relevant landowners were contacted in advance of the survey in order to gain access to off-site waterbodies. Use of a 300m radius reflects the findings of studies into the movement of Great Crested Newts during terrestrial phases which indicate that the maximum routine migratory distance of Great Crested Newts away from breeding ponds during terrestrial phases is less than 250m (Cresswell and Whitworth, 2004).

2.1.3 HSI assessments of the site and surrounding area were conducted by Kate Thatcher and Neve Phillips of HDA on the 25<sup>th</sup> and 27<sup>th</sup> June 2025. During these surveys all accessible waterbodies identified within the survey area were visited and, where appropriate, assessed against each of the following ten suitability indices:

- i. Geographic location;
- ii. Pond area;
- iii. Pond permanence;
- iv. Water quality;
- v. Shading;
- vi. Presence of waterfowl;
- vii. Presence of fish;
- viii. Pond density in the area;
- ix. Terrestrial habitat quality; and
- x. Macrophyte cover in pond.

2.1.4 Details of the pond characteristics (depth, margin profile, etc.) and bankside, marginal and aquatic vegetation were also recorded during the assessment.

### **2.2 Great Crested Newt Environmental DNA (eDNA) Survey**

2.2.1 Great Crested Newt eDNA sampling surveys were conducted on all accessible waterbodies that had been identified as having suitability to support Great Crested Newts during the HSI survey. The eDNA survey methodology is recognised by Natural England as a reliable technique for determining the presence/likely absence of Great Crested Newts within a pond through detection of traces of Great Crested Newt DNA within the water.

2.2.2 The eDNA sampling surveys of the site and surrounding area were conducted by Kate Thatcher and Neve Phillips of HDA on the 25<sup>th</sup> and 27<sup>th</sup> June 2025. These field surveys involved taking samples of pond water at each of the surveyed waterbodies in line with the recognised methodology established by Biggs *et. al.* (2014). The samples were then despatched to a recognised laboratory for polymerase chain reaction (qPCR) analysis.

### **3 RESULTS**

3.1 Thirty-five waterbodies with potential to support Great Crested Newts were identified within the survey area during the desk study. The locations of the waterbodies are shown in *Appendix A*.

3.2 The results of the HSI assessments and eDNA surveys, together with descriptions of the surveyed waterbodies and any limitations encountered during the surveys, are provided below. Full findings of the HSI assessments and the laboratory results from the eDNA analysis are provided in *Appendices B* and *C* respectively.

#### **3.4 Waterbody 2**

*Location: 150m north-west of the site.*

*HSI assessment: 0.904 = 'excellent' suitability.*

3.3.1 Waterbody 2 comprises a pond located in the grounds of Christ's Hospital school to the north-west of the main site. The pond has an earth base with gently sloping earth banks supporting marginal plants. Habitats beyond the pond margins are dominated by woodland and grassland. The water quality was assessed as good and it is assumed the waterbody never dries.

3.3.2 The HSI score for Waterbody 2 indicated that the pond had 'excellent' suitability for Great Crested Newts.

3.3.3 Following the HSI survey, eDNA sampling of Waterbody 2 was carried out and the sample was sent to the Surescreen Scientifics laboratory for analysis. The analysis returned negative results for Great Crested Newt eDNA which indicates that Great Crested Newts were highly likely to have been absent from the waterbody at the time the survey was conducted. The results of the eDNA analysis are provided in *Appendix C*. An eDNA sampling survey of Waterbody 2 was previously conducted in 2022, which also returned a negative result for Great Crested Newt eDNA

3.3.4 No limitations specific to Waterbody 2 were encountered during the survey.

### **3.5 Waterbody 3**

*Location: 180m north-west of the site.*

*HSI assessment: 0.638 = 'average' suitability.*

3.4.1 Waterbody 3 comprises a lake in the grounds of Christ's Hospital school approximately 180m to the west of the main site. The waterbody has an earth base with earth banks that are steep in places supporting marginal and aquatic plants. Habitats beyond the lake margins are dominated by amenity grassland and mixed woodland. The water quality was assessed as poor and it is assumed the waterbody never dries.

3.4.2 The HSI score for Waterbody 3 indicated that the lake had 'average' suitability for Great Crested Newts.

3.4.3 Following the HSI survey, eDNA sampling of Waterbody 3 was carried out and the sample was sent to the Surescreen Scientifics laboratory for analysis. The analysis returned negative results for Great Crested Newt eDNA which indicates that Great Crested Newts were highly likely to have been absent from the waterbody at the time the survey was conducted. The results of the eDNA analysis are provided in *Appendix C*. An eDNA sampling survey of Waterbody 3 was previously conducted in 2022, which also returned a negative result for Great Crested Newt eDNA

3.4.4 No limitations specific to Waterbody 3 were encountered during the survey.

### **3.6 Waterbody 4**

*Location: On site.*

*HSI assessment: 0.676 = 'average' suitability.*

3.5.1 Waterbody 4 comprises a small pond located in a small copse in the north of the site. The pond has an earth base and has a stream inlet and outlet with slow running water. Habitats beyond the pond margins are dominated by arable fields, trees and hedgerows. The water quality was assessed as poor and it is assumed the waterbody rarely dries.

3.5.2 The HSI score for Waterbody 4 indicated that the pond had 'average' suitability for Great Crested Newts.

3.5.3 Following the HSI survey, eDNA sampling of Waterbody 4 was carried out and the sample was sent to the Surescreen Scientifics laboratory for analysis. The analysis returned negative results for Great Crested Newt eDNA which indicates that Great Crested Newts were highly likely to have been absent from the waterbody at the time the survey was conducted. The results of the eDNA analysis are provided in *Appendix C*. An eDNA sampling survey of Waterbody 4 was previously conducted in 2022, which also returned a negative result for Great Crested Newt eDNA.

3.5.4 No limitations specific to Waterbody 4 were encountered during the survey.

### **3.6 Waterbody 5**

*Location: On site.*

*HSI assessment: 0.378 = 'poor' suitability.*

3.6.1 Waterbody 5 comprises a slurry pit in the north of the site. It has steep concrete sides, the top of which are raised above ground level. It is set in a farmyard and surrounded by concrete, beyond which are grazed fields which have areas of scrub and hedgerows. The water quality was assessed as poor and it is assumed the waterbody never dries.

3.6.2 The HSI score for Waterbody 5 indicated that the pond had 'poor' suitability for Great Crested Newts.

3.6.3 Following the HSI survey, eDNA sampling of Waterbody 5 was carried out and the sample was sent to the Surescreen Scientifics laboratory for analysis. The analysis returned negative results for Great Crested Newt eDNA which indicates that Great Crested Newts were highly likely to have been absent from the waterbody at the time the survey was conducted. The results of the eDNA analysis are provided in *Appendix C*. An eDNA sampling survey of Waterbody 5 was not previously conducted in 2022, as it was considered unsuitable for Great Crested Newts.

3.6.4 No limitations specific to Waterbody 5 were encountered during the survey.

### **3.7 Waterbody 8**

*Location: On site boundary.*

*HSI assessment: 0.556 = 'below average' suitability (in 2022)*

3.7.1 Access to undertake a survey of Waterbody 8 was not granted in 2025, however the waterbody was surveyed by HDA in 2022 where it was described as a small pond located on the western boundary of the site. The pond had an earth base with sloping earth banks supporting aquatic and marginal plants. Habitats beyond the pond margins comprise semi-improved grassland and hedgerows. The water quality was assessed as moderate and it was assumed the waterbody rarely dries. The HSI score for Waterbody 8 in 2022 indicated that the pond had 'below average' suitability for Great Crested Newts and the eDNA sampling of Waterbody 8 returned negative results for Great Crested Newt eDNA.

### **3.8 Waterbody 9**

*Location: 25m beyond the site boundary in the centre of the site*

*HSI assessment: 0.760 = 'good' suitability.*

3.8.1 Waterbody 9 comprises a small pond located off-site approximately 25m beyond the site boundary in the centre of the site. The pond has a clay base with sloping earth banks supporting aquatic and marginal plants. Habitats beyond the pond margins comprise a residential garden which include grass, hedges and trees, beyond which were arable fields with hedgerows. The water quality was assessed as good and it is assumed the waterbody never dries.

3.8.2 The HSI score for Waterbody 9 indicated that the pond had 'good' suitability for Great Crested Newts.

3.8.3 Following the HSI survey, eDNA sampling of Waterbody 9 was carried out and the sample were sent to the Surescreen Scientifics laboratory for analysis. The analysis returned positive results for Great Crested Newt eDNA which indicates that Great Crested Newts are highly likely to have been present within the waterbody at the time the survey was conducted. The results of the eDNA analysis are provided in *Appendix C*. An eDNA sampling survey of Waterbody 9 was not previously conducted in 2022, however a Great Crested Newt was encountered within the site during subsequent surveys which was considered to be associated with a population within Waterbody 9.

3.8.4 No limitations specific to Waterbody 9 were encountered during the survey.

### **3.9 Waterbody 12**

*Location: 200m west of site.*

*HSI assessment: 0.646 = 'average' suitability (in 2022).*

3.9.1 Access to undertake a survey of Waterbody 12 was not granted in 2025, however the waterbody was surveyed by HDA in 2022 where it was described as a small pond located in grassland fields to the west of the site. The pond had an earth base with earth banks with limited vegetation. Habitats beyond the pond margins were dominated by grassland and hedgerows. The water quality was assessed as moderate and it was assumed the waterbody rarely dries. The HSI score for Waterbody 12 indicated that the pond had 'average' suitability for Great Crested Newts and the eDNA sampling of Waterbody 12 returned negative results for Great Crested Newt eDNA.

### **3.10 Waterbody 15**

*Location: On site boundary.*

*HSI assessment: 0.577 = 'below average' suitability (in 2022).*

- 3.10.1 Access to undertake a survey of Waterbody 15 was not granted in 2025, however the waterbody was surveyed by HDA in 2022 where it was described as a small pond located on the western site boundary. The pond had an earth base with steep earth banks supporting marginal plants. Habitats beyond the pond margins were dominated by arable fields and hedgerows. The water quality was assessed as moderate and it was assumed the waterbody sometimes dries. The HSI scores for Waterbody 15 indicated that the pond had 'below average' suitability for Great Crested Newts and the eDNA sampling of Waterbody 15 returned negative results for Great Crested Newt eDNA.

### **3.11 Waterbody 16**

*Location: On site.*

*HSI assessment: 0.569 = 'below average' suitability.*

- 3.11.1 Waterbody 16 comprises a small pond located in a small patch of woodland in the south-west of the site. The pond has an earth base with earth banks. Habitats beyond the pond margins are dominated by woodland and grassland. The water quality was assessed as poor and it is assumed the waterbody dries annually.
- 3.11.2 The HSI scores for Waterbody 16 indicated that the pond had 'below average' suitability for Great Crested Newts.
- 3.11.3 Following the HSI survey, eDNA sampling of Waterbody 16 was carried out and the sample was sent to the Surescreen Scientifics laboratory for analysis. The analysis returned negative results for Great Crested Newt eDNA which indicates that Great Crested Newts were highly likely to have been absent from the waterbody at the time the survey was conducted. The results of the eDNA analysis are provided in *Appendix C*. An eDNA sampling survey of Waterbody 16 was previously conducted in 2022, which also returned a negative result for Great Crested Newt eDNA.

- 3.11.4 No limitations specific to Waterbody 16 were encountered during the survey.

### **3.12 Waterbody 17**

*Location: On site.*

*HSI assessment: 0.612 = 'average' suitability.*

- 3.12.1 Waterbody 17 comprises a moderate sized pond located in a wooded copse in the south of the site. The pond has an earth base with earth banks which support aquatic and marginal plants. The surface of the pond is covered in algae. Habitats beyond the pond margins are dominated by a wooded copse and beyond this, grassland with cows grazing

and hedgerows. The water quality was assessed as poor and it is assumed the waterbody dries annually.

3.12.2 The HSI scores for Waterbody 17 indicated that the pond had 'average' suitability for Great Crested Newts.

3.12.3 Following the HSI survey, eDNA sampling of Waterbody 17 was carried out and the sample was sent to the Surescreen Scientifics laboratory for analysis. The analysis returned negative results for Great Crested Newt eDNA which indicates that Great Crested Newts were highly likely to have been absent from the waterbody at the time the survey was conducted. The results of the eDNA analysis are provided in *Appendix C*. An eDNA sampling survey of Waterbody 17 was not previously conducted in 2022, as it was dry.

3.12.4 No limitations specific to Waterbody 17 were encountered during the survey.

### **3.13 Waterbody 19**

*Location: On site boundary.*

*HSI assessment: 0.643 = 'average' suitability (in 2022).*

3.13.1 Access to undertake a survey of Waterbody 19 was not granted in 2025, however the waterbody was surveyed by HDA in 2022 where it was described as a small pond located adjacent to a farmyard in the south of the site. The pond had an earth base with earth banks. Habitats beyond the pond margins were dominated by grassland and hedgerows. The water quality was assessed as good and it was assumed the waterbody never dries. The HSI scores for Waterbody 19 indicated that the pond had 'average' suitability for Great Crested Newts and the eDNA sampling of Waterbody 19 returned negative results for Great Crested Newt eDNA.

### **3.14 Waterbodies 20 and 21**

*Location: 8m beyond the site boundary in the south of the site*

*HSI assessment: 0.727 = 'good' suitability.*

3.14.1 Waterbodies 20 and 21 comprises a pond located in a residential garden approximately 8m beyond the site boundary in the south of the site. The pond has an earth base and banks and supports aquatic and marginal plants. Habitats beyond the pond margins are dominated by a residential garden comprising primarily of grass with shrubs and trees, beyond which are semi-improved grassland fields with hedgerows. The water quality was assessed as poor and it is assumed the waterbody sometimes dries. During the 2022 survey, this feature was dry and was assumed to be two ponds (Waterbodies 20 and 21); in 2025 however, it was apparent that Waterbodies 20 and 21 are one waterbody.

3.14.2 The HSI scores for Waterbodies 20 and 21 indicated that the pond had 'good' suitability for Great Crested Newts.

3.14.3 Following the HSI survey, eDNA sampling of Waterbodies 20 and 21 was carried out and the sample was sent to the Surescreen Scientifics laboratory for analysis. The analysis returned negative results for Great Crested Newt eDNA which indicates that Great Crested Newts were highly likely to have been absent from the waterbody at the time the survey was conducted. The results of the eDNA analysis are provided in *Appendix C*.

3.14.4 No limitations specific to Waterbodies 20 and 21 were encountered during the survey.

### **3.15 Waterbody 29**

*Location: 275m to the east of the site.*

*HSI assessment: 0.683 = 'average' suitability.*

3.15.1 Waterbody 29 comprises an artificial pond located to the east of the site. The pond has an earth base with vertical sides and supports some aquatic vegetation. Habitats beyond the pond margins are dominated by developed land and amenity grassland. The water quality was assessed as poor and it is assumed the waterbody never dries.

3.15.2 The HSI scores for Waterbody 29 indicated that the pond had 'average' suitability for Great Crested Newts.

3.14.3 Following the HSI survey, eDNA sampling of Waterbody 29 was carried out and the sample was sent to the Surescreen Scientifics laboratory for analysis. The analysis returned negative results for Great Crested Newt eDNA which indicates that Great Crested Newts were highly likely to have been absent from the waterbody at the time the survey was conducted. The results of the eDNA analysis are provided in *Appendix C*. An eDNA sampling survey of Waterbody 29 was previously conducted in 2022, which also returned a negative result for Great Crested Newt eDNA.

3.14.4 No limitations specific to Waterbody 29 were encountered during the survey.

### **3.15 Waterbody 30**

*Location: 275m east of site.*

*HSI assessment: 0.510 = 'below average' suitability.*

3.15.1 Waterbody 30 comprises a small pond located in a small patch of woodland in the village of Southwater. The pond has an earth base with steep earth banks supporting marginal and aquatic plants. Habitats beyond the pond margins are dominated by developed land and woodland. The water quality was assessed as poor and it is assumed the waterbody sometimes dries.

3.15.2 The HSI scores for Waterbody 30 indicated that the pond had 'below average' suitability for Great Crested Newts.

3.15.3 Following the HSI survey, eDNA sampling of Waterbody 30 was carried out and the sample was sent to the Surescreen Scientifics laboratory for analysis. The analysis returned negative results for Great Crested Newt eDNA which indicates that Great Crested Newts were highly likely to have been absent from the waterbody at the time the survey was conducted. The results of the eDNA analysis are provided in *Appendix C*. An eDNA sampling survey of Waterbody 30 was previously conducted in 2022, which also returned a negative result for Great Crested Newt eDNA.

3.15.4 No limitations specific to Waterbody 30 were encountered during the survey.

### **3.16 Waterbody 33**

*Location: 200m south of the Christ's Hospital car park site.*

*HSI assessment: 0.536 = 'below average' suitability.*

3.16.1 Waterbody 33 comprises an artificial pond located in a housing development 200m from the Christ's Hospital car park section of the site. The pond has vertical brick sides and supports some marginal and aquatic plants. Habitats beyond the pond margins are dominated by buildings and hardstanding. Fish are present in the pond and the water quality was assessed as moderate. It is assumed the waterbody never dries.

3.16.2 The HSI scores for Waterbody 33 indicated that the pond had 'below average' suitability for Great Crested Newts.

3.16.3 Following the HSI survey, eDNA sampling of Waterbody 33 was carried out and the sample was sent to the Surescreen Scientifics laboratory for analysis. The analysis returned negative results for Great Crested Newt eDNA which indicates that Great Crested Newts were highly likely to have been absent from the waterbody at the time the survey was conducted. The results of the eDNA analysis are provided in *Appendix C*. An eDNA sampling survey of Waterbody 33 was previously conducted in 2022, which also returned a negative result for Great Crested Newt eDNA.

3.16.4 No limitations specific to Waterbody 33 were encountered during the survey.

### **3.17 Waterbody 35**

*Location: On western site boundary*

*HSI assessment: 0.643 = 'average' suitability.*

3.17.1 Waterbody 35 comprises a small pond located in a residential garden to the west of the site. The pond has a liner with large rocks round the margins and supports predominantly ornamental aquatic and marginal plants. The pond is set in a residential garden which includes shrubs, grass and hedges, beyond which are fields dominated by grassland and hedgerows. The water quality was assessed as moderate and it is assumed the waterbody sometimes dries.

3.17.2 The HSI score for Waterbody 35 indicated that the pond had 'average' suitability for Great Crested Newts.

3.17.3 Following the HSI survey, eDNA sampling of Waterbody 35 was carried out and the sample were sent to the Surescreen Scientifics laboratory for analysis. The analysis returned positive results for Great Crested Newt eDNA which indicates that Great Crested Newts are highly likely to have been present within the waterbody at the time the survey was conducted. The results of the eDNA analysis are provided in *Appendix C*. This waterbody was not identified as present in 2022.

3.17.4 No limitations specific to Waterbody 35 were encountered during the survey.

### **3.18 Other Waterbodies**

3.18.1 Letters requesting access to off-site waterbodies 1, 6, 7, 8, 12, 13, 14, 15, 19, 23, 25 and 32 were posted in advance of the survey, which when no response was received were followed up by in-person visits, however no permission to survey these ponds was granted.

3.18.2 Waterbodies 10, 11, 18, 22, 24, 26, 27, 28, 31 and 34 were found to be dry or absent at the time of survey, and therefore unsuitable for HSI assessment or eDNA sampling.

## **4 EVALUATION**

4.1 There are two waterbodies within the wider area that returned a positive result for Great Crested Newts during the eDNA survey, and this species is therefore expected to be present within the site during terrestrial phases. Within the site, the hedgerow bases, woodland, grassland and scrub habitats at the site provide areas of suitable terrestrial habitat for Great Crested Newts, and piles of brash and debris provide suitable opportunities for refuge.

4.2 Use of a 300m radius for identification of potential breeding waterbodies around the site reflects the findings of studies into the movement of Great Crested Newts during terrestrial

phases which indicate that the maximum routine migratory distance of Great Crested Newts away from breeding ponds during terrestrial phases is less than 250m (Cresswell and Whitworth, 2004), and more recent studies suggest that 95% of newt summer refuges are within 63m of breeding ponds.

4.3 In view of the above, this document sets out a precautionary approach to mitigation that demonstrates how the proposed development can mitigate effects reflecting a reasonable worst-case scenario of a large population of Great Crested Newts being present in off-site Waterbodies 9 and 35. This demonstrates that the development proposals are able to protect any individual newts present during construction works and maintain the favourable conservation status of any local Great Crested Newt population. The measures set out below should be implemented unless otherwise agreed with the planning authority and/or Natural England.

## **5 MITIGATION AND SITE SAFEGUARDING**

5.1 This section identifies measures to be implemented during development of the site in order to avoid and mitigate potential impacts on Great Crested Newts. These measures should be implemented unless otherwise agreed with the planning authority and/or Natural England.

5.2 The areas of the site in the vicinity of Waterbodies 9 and 35, are dominated by arable and grazed fields which provide poor-quality habitat for terrestrial phase Great Crested Newts. Habitats of higher value to terrestrial phase newts are however present around the margins of these fields in the form of hedgerow bases, ditches, treelines and areas of tall ruderal vegetation. It is considered that if Great Crested Newts are breeding within Waterbodies 9 and 35 then terrestrial phase Great Crested Newts would be likely to use the site on a regular basis. In view of this, unless otherwise agreed with Natural England it would therefore be necessary to:

- Protect individual Great Crested Newts through removal and exclusion from affected habitat within the site prior to and during construction works; and
- Provide replacement habitat within the landscape scheme of the proposed development to ensure that opportunities remain at the site for Great Crested Newts, thereby protecting the favourable conservation status of the local population in the long-term.

5.3 Unless covered under a Natural England District Licence, these works should be carried out under a Natural England European Protected Species (EPS) licence. It is anticipated that the mitigation strategy described below would provide the basis for a detailed Method Statement to be submitted to Natural England as part of an EPS licence application unless an alternative approach is agreed with Natural England at an appropriate stage.

#### 5.4 **District Licence approach**

5.4.1 With regards to the above, District Licensing involves subscription to a wider Natural England licence covering the entire district, which allows impacts on Great Crested Newts within a development site to be compensated through creation and long-term management of breeding and terrestrial habitats in the wider landscape. It should be noted however that even where this approach is taken, measures to protect individual newts may be required during construction of the site albeit at a substantially lower level of effort than that which may be required under a full EPS licence (see below). Notwithstanding the focus of the District Licensing approach being on off-site habitat creation, where possible consideration should still be given to the retention of opportunities for Great Crested Newts within the site itself in accordance with the mitigation hierarchy, planning policy and the 2006 NERC Act. This is also likely to reduce costs associated with entering the District Licence.

#### 5.5 **Full European Protected Species Licence approach**

5.5.1 In the event that it is preferred to carry out works under a bespoke European Protected Species (EPS) licence specific to the site, then it would be necessary to accompany any licence application made to Natural England with a method statement detailing measures to:

- Protect individual Great Crested Newts through removal and exclusion from areas of works prior to and during construction; and
- Provide replacement habitat within the landscape scheme of the proposed development to ensure that opportunities remain at the site for Great Crested Newts following development, thereby protecting the favourable conservation status of the local population in the long-term.

5.5.2 The detailed method statement would need to be informed by a full population estimate survey to provide an indication of the number of Great Crested Newts using the site and inform proportionate levels of capture effort and habitat provision and therefore a full population estimate survey would need to be carried out prior to the application being made<sup>1</sup>. It should also be noted that the population estimate survey would likely be required prior to determination of a planning application if the EPS licensing route is to be taken. A mitigation strategy is described below which takes a precautionary approach assuming that a large metapopulation of Great Crested Newts is identified in association with the on- and off-site waterbodies. This demonstrates that the development proposals are able to protect any individual newts present during construction works and can maintain the favourable

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<sup>1</sup> Population estimate surveys require 6 survey visits carried out between mid-March and mid-June, with at least 3 of the visit undertaken between mid-April and mid-May.

conservation status of any local Great Crested Newt population should a bespoke European Protected Species (EPS) licence specific to the site be pursued<sup>2</sup>.

*EPS Licence approach: Protection of Great Crested Newts during construction*

5.5.3 Proposals for the site include construction of residential homes, neighbourhood centre, education facilities and an employment area and associated infrastructure, which is expected to result in loss of a proportion of the area of suitable Great Crested Newt terrestrial habitat in the vicinity of Waterbodies 9 and 35, including hedgerow, ditch, tall ruderal and arable habitats. In order to protect individual Great Crested Newts in accordance with nature conservation legislation it will be necessary to translocate Great Crested Newts from affected areas of suitable habitat up to 250m from Waterbodies 9 and 35 to a receptor area prior to the commencement of works. Approximately 12.3ha of the site falls within 250m of Waterbodies 9 and 35 (see *Appendix A*). The translocation should follow the procedure outlined below:

*Identification and preparation of the receptor site*

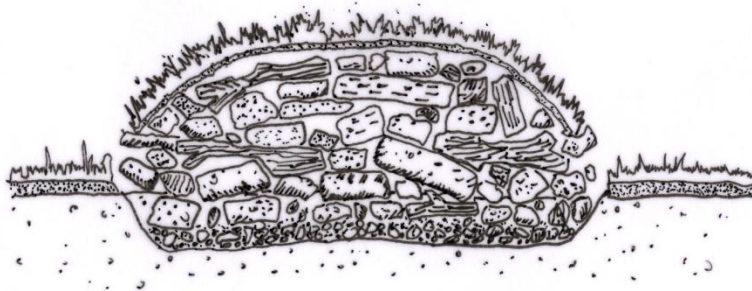
- A suitable area of the site in the vicinity of Waterbodies 9 and 35 should be identified as a receptor site. The emerging development proposals indicate that much of the land to the south of Waterbody 9 will constitute informal greenspace and would therefore be suitable for establishment of a receptor site.
- Habitat creation and management of the receptor area should comprise:
  - High quality terrestrial habitat for Great Crested Newts should be established within the receptor area such as rough grassland and scrub.
  - Once established the rough grassland should be managed by cutting selected areas once every 2-3 years in order to establish a tussocky grassland sward.
  - The extent of scrub establishment within the area should be reviewed each winter and cut back where appropriate to create a mosaic of scrub and rough grassland habitats at a ratio of approximately 40% scrub and 60% rough tussocky grassland. Techniques for scrub control, i.e. cutting back with or without stump treatment, should be implemented as and where deemed necessary. Such work should be undertaken in the winter months outside of the bird nesting season.
  - Arisings from the management both within the receptor area and wider site should be used to create and maintain habitat piles.

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<sup>2</sup> Due to the need to carry out a Great Crested Newt population estimate survey prior to applying for an EPS licence, the measures outlined assume a higher level of breeding interest associated with the waterbodies than is probable. If a lower level of breeding interest across the ponds is recorded during subsequent population estimate surveys, the approach to mitigation outlined in *Section 5.5* should be revised.

- These works are expected to result in an enhancement to an area of the site that is currently of low suitability for amphibians, being dominated by arable farmland<sup>3</sup>.
- A purpose-built hibernaculum (see *Figure 1* below) and minimum of 8 refuge piles should be included within the receptor area to provide opportunities for hibernating amphibians.

**Figure 1:** Example cross-section through hibernaculum



5.5.4 In order to allow for the translocation of any Great Crested Newts from the development area and prevent newts from dispersing into the development area during construction, exclusion fencing should firstly be erected around affected habitat within up to 250m of Waterbodies 9 and 35. Pitfall traps should then be installed at 10m intervals along the inside of the fencing and artificial refugia placed along the fence line and within suitable terrestrial habitat (ditches, hedgerow bases, rough grassland etc) within the interior of the exclusion zone at a density of 50-100 refugia per hectare. Trapping and translocation should follow the methodology set out below unless otherwise agreed with Natural England:

*Installation of fencing and pitfall traps*

- Fencing and pitfall traps should only be installed under the supervision of a suitably qualified ecologist at a time of year when night time temperatures are consistently above 5°C;
- The proposed fenceline should first be searched and cleared of amphibians. Any amphibians found should be translocated to the receptor site outside of the exclusion area (detailed above);
- Any vegetation that requires removal for fence installation should be removed using hand tools in a two stage cutting regime (firstly cut down to 15cm above ground level, followed by a second cut to ground level), taking care to avoid risk of injury to resting amphibians;
- Pitfall traps should be installed flush with the newt fencing and ensure no top lip is present above ground level;
- Each pitfall trap should remain closed until the translocation commences; and

<sup>3</sup> In some instances, it may be more appropriate to release newts at the site boundary where this coincides with suitable terrestrial habitat and proximity to the likely breeding pond.

- Pitfall traps should be complemented by installation of carpet tile refugia (or similar) along the fencelines and within the interior of the capture area, installed at a density of 50 - 100 mats per hectare of suitable habitat.

#### *Translocation exercise*

- Translocation works should be carried out in suitable climatic conditions between March and early November inclusive;
- Once the translocation has started, the pitfall traps should be opened and equipped with a mammal ramp, float and vegetation for trapped newts to hide under;
- Each pitfall trap should be checked daily before 11am and weather conditions monitored and traps closed if necessary;
- Refugia should be lifted and any amphibians beneath captured;
- All amphibians captured should either be translocated to the pre-established receptor area outside of the exclusion fencing or, where appropriate, released at the site boundary where this coincides with suitable terrestrial habitat and proximity to the likely breeding pond.
- The pitfall trapping and artificial refugia searches should be undertaken on a minimum of 90 occasions<sup>4</sup> (with 5 clear days at the end of the translocation) unless trapping results suggest other as appropriate (in agreement with Natural England);
- Once capture rates decline in any given capture compartment within the exclusion area, ground level vegetation should be progressively removed in order to encourage movement of newts and increase chances of capture; and
- Fencing should be monitored on a weekly basis and any damaged repaired until all newts have been cleared from the area of search.
- Fencing should remain in place until completion of works in the area, after which it should be removed under the supervision of a suitably qualified ecologist at a time of year when Great Crested Newts are active (generally mid-March to mid-October).

5.5.6 Once capture rates decline, vegetation manipulation described below would commence to encourage capture of any remaining newts. Any remaining naturally occurring refugia should also be dismantled by hand and searched for newts. In the event that vegetation clearance is likely to be counterproductive to the capture of newts (e.g. by encouraging newts to go to ground) this work should not be carried out.

#### *Vegetation manipulation*

- Vegetation manipulation to encourage capture of newts would require clearance of remaining vegetation to no less than 100mm by hand;

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<sup>4</sup> This assumes a worst case scenario that a large population is present within Waterbodies 9 and/or 35.

- Vegetation should be cut using hand tools under the supervision of a suitably qualified ecologist;
- Again, any newts encountered within the area of works during vegetation clearance should be captured and moved to the receptor site outside the exclusion area; and
- A further 5 days with night time temperatures of >5°C should then be allowed to elapse to enable capture of any remaining newts prior to a destructive search (see below).

#### *Destructive search*

- In order to be certain that no newts are present within the area of works, once five suitable trapping nights have passed with no captures from terrestrial habitats, vegetation should be cleared from areas of suitable habitat to ground level and topsoil disturbed by scraping back to a depth of up to 10cm where the possibility of newts being present remains. This should be carried out under the direct supervision of a suitably qualified ecologist where it is expected Great Crested Newts might still be present. Topsoil should not be stripped from the root protection areas of retained trees, scrub and hedgerows, or where translocation results and vegetation clearance to ground level allows satisfactory inspection to confirm likely absence of newts.
- In the event that the destructive search is delayed following newt capture, the vegetation should be maintained at ground level until the destructive search is carried out.

5.5.7 Once the translocation, vegetation clearance and destructive search measures have been completed in accordance with the methodology outlined above, development works would be able to go ahead within the cleared area.

## **5.6 Reasonable Avoidance Measures (RAMs)**

5.6.1 Subject to review and agreement with a suitably qualified ecologist, where only small areas of suitable terrestrial habitat are affected by works (e.g. installation of a bench or paths within an otherwise undisturbed area of habitat), it may be possible to avoid a full translocation. In order to ensure that no Great Crested Newts are present or harmed during such works it is recommended that a two-stage approach to clearance of the affected area is undertaken. This is also in keeping with measures to avoid unlawful killing/ injuring of reptiles which also have potential to be present within similar habitat as described in the 2025 Reptile Survey Report (HDA, 2026). This would involve:

- i. Manipulation of suitable habitat within the construction zone to reduce suitability for newts.
- ii. Destructive search of areas of cleared habitat to ensure the absence of newts.

5.6.2 Each of the above stages is described below:

Vegetation manipulation:

- Vegetation within areas of suitable terrestrial habitat located within the proposed area of works should be cleared using hand held machinery to a height of no less than 100mm in order to reduce suitability of the habitat present to encourage the dispersal of any animals present away from affected areas.
- Vegetation should be cut using handheld tools under the supervision of a suitably qualified ecologist who will inspect affected areas prior to and during works.
- In addition, any refuge opportunities present should be dismantled by hand and searched prior to being removed.
- Vegetation removal works should be carried out at a time of year when amphibians are active (generally March to October inclusive), ideally during the newt breeding season (mid-March to mid-June inclusive) when newts are even less likely to be present at the site.

Destructive search:

- In order to be certain that no amphibians are present within the area of works, once five suitable days/ nights have passed since vegetation manipulation works have been completed, the remaining vegetation would be cleared from areas of suitable habitat to ground level and where necessary topsoil disturbed by scraping back to a depth of up to 100mm where the possibility of animals being present remains.
- This should be carried out under the direct supervision of a suitably qualified ecologist.
- Topsoil should not be stripped from the root protection areas of retained trees, scrub and hedgerows, or where vegetation clearance to ground level allows satisfactory inspection to confirm likely absence.
- In the event that the destructive search is delayed following vegetation manipulation, the vegetation should be maintained at ground level until the destructive search is carried out.
- Destructive search works should be carried out at a time of year when amphibians are active (generally March to October inclusive), ideally during the newt breeding season (mid-March to mid-June inclusive) when newts are even less likely to be present at the site.

5.6.3 In the unlikely event that during any of the works on site a Great Crested Newt is encountered outside the area covered under the Great Crested Newt licence, works should stop, a suitable qualified ecologist contacted and Natural England consulted.

## **5.7 Further survey**

5.7.1 It is recommended that the need to update the Great Crested Newt survey work undertaken to date is periodically reviewed by a suitably qualified ecologist in order to ensure effective assessment of effects of the proposed development on this species and to identify appropriate avoidance and mitigation measures.

## **5.8 Site safeguards and habitat creation**

5.8.1 The exclusion fencing should be retained and maintained until completion of works and the condition of the fencing monitored and repairs made as required. Following the completion of the development the exclusion fencing should be removed allowing newts to disperse back into newly created habitat within the site.

5.8.2 Development proposals should also seek to maintain and where possible enhance opportunities for Great Crested Newts at the site. This could be achieved through the retention, enhancement and creation of Great Crested Newt habitats as part of the landscape strategy for the site in addition to those required for any receptor site provision in relation to the development works. Consideration should be given to:

- Creation of new open water wetland habitats suitable for breeding amphibians planted with a range of native aquatic and marginal vegetation, either as standalone features or as part of the site surface water drainage strategy;
- Inclusion of high quality terrestrial habitats within the landscape scheme in the form of woodland, scrub, hedgerows, swales and ditches and rough, meadow and wet grassland;
- Provision of opportunities for hibernation and refuge through provision of log/brush piles and purpose built hibernacula; and
- Securing the long-term integrity of new and retained Great Crested Newt habitats through inclusion within a long-term management plan.

5.8.3 In order to protect any newts entering the entire site during the operational phase of the development, any gully pots within 250m of Waterbodies 9 and 35 or areas of targeted Great Crested Newt habitat creation works should be suitably designed with a stand-off from the kerb and/or through use of 'wildlife friendly' kerbs<sup>5</sup> to avoid entrapment of any newts and other wildlife passing over hard landscaped areas. Consideration should also be given to installation of 'escape ladders' in drains. Where appropriate, dropped kerbs should also be used where Great Crested Newts are likely to cross roads and other areas of hardstanding. Detailed drainage and infrastructure proposals for the development should be reviewed at appropriate design stages by a suitably qualified ecologist.

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<sup>5</sup> Wildlife friendly kerbs - [www.wildlifefencing.co.uk/product.php?productid=477&cat=68&page=1](http://www.wildlifefencing.co.uk/product.php?productid=477&cat=68&page=1)

## 6 CONCLUSION

6.1 Subject to implementation of the mitigation works described in *Section 5* above to protect Great Crested Newts associated with off-site Waterbodies 9 and 35, the development would be expected to maintain compliance with nature conservation legislation and planning policy, and ensure that opportunities for Great Crested Newts are maintained at the site in the long-term thereby ensuring that the favourable conservation status of the local Great Crested Newt population is maintained.

6.2 Furthermore, the emerging development proposals provide opportunity to substantially enhance terrestrial habitats in the vicinity of the off-site Waterbodies 9 and 35 in addition to the enhancement of existing ponds and creation of new ponds suitable for breeding Great Crested. This would be expected to enhance opportunities currently provided by the site for Great Crested Newts and other amphibians in accordance with the 2006 NERC Act and the 2024 National Planning Policy Framework.

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KPK (2026) *Land North West of Southwater: Landscape Parameter Plan*.

KPK (2026) *Land North West of Southwater: Land Use Parameter Plan*.

## HDA Document Control and Quality Assurance Record

Project Title: Land North-West of Southwater, Horsham  
Project Reference: 2090.78  
Document Title: 2025 Great Crested Newt HSI and eDNA Survey Report  
Commissioning Party: Berkely Strategic Land Ltd

Issue	Description	Date of Issue	Signed
1	2025 Great Crested Newt HSI and eDNA Survey Report	January 2026	

	Personnel	Position
Author	Kate Thatcher	Assistant Ecologist
Approved for issue	Clare Bird MCIEEM	Associate Ecologist

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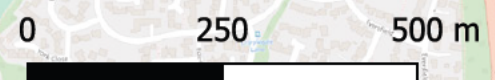
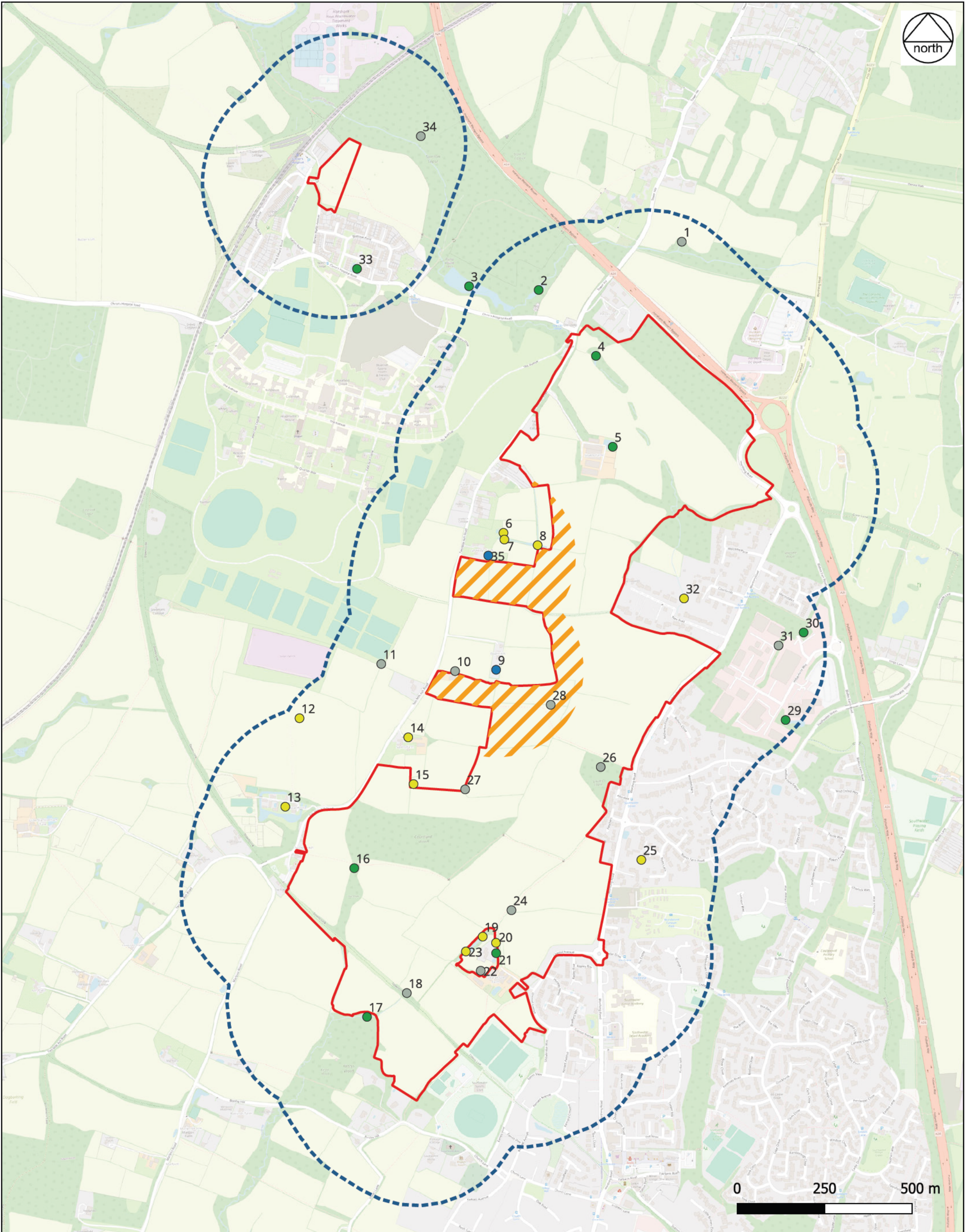
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**APPENDIX A**

**Great Crested Newt eDNA survey summary plan**



**KEY**

-  Site boundary
-  300m Site Buffer
-  250m pond buffer
-  Waterbody dry/ no longer present
-  Waterbody tested negative during eDNA survey
-  Waterbody tested positive during eDNA survey
-  Access to waterbody not granted

CLIENT:  
Berkeley Strategic land Ltd

PROJECT:  
Land North-West of Southwater, Horsham

TITLE:  
2025 Great Crested Newt eDNA Survey Summary Plan

SCALE AT A3:  
See scale bar

DATE:  
January 2026

2090.78 / 27

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Landscape Architecture  
Masterplanning  
Ecology



**APPENDIX B**

**Full HSI assessment results**

Pond ID		Waterbody 2		Waterbody 3		Waterbody 4		Waterbody 5	
SI ref	Description of Index	Measure / Comment	SI score	Measure / Comment	SI score	Measure / Comment	SI score	Measure / Comment	SI score
S11	Geographic location	A	1	A	1	A	1	A	1
S12	Pond area m <sup>2</sup>	500	1	2300	Omit	130	0.2	55	0.1
S13	Pond permanence	ND	0.9	ND	0.9	RD	1	ND	0.9
S14	Water quality	Good	1	Poor	0.33	Poor	0.33	Bad	0.01
S15	Shading %	20%	1	25%	1	80%	0.6	10%	1
S16	Presence of waterfowl	Minor	0.67	Minor	0.67	Absent	1	Minor	0.67
S17	Presence of fish	Minor	0.67	Minor	0.67	Absent	1	Absent	1
S18	Pond density in area	>15	1	>15	1	>15	1	>15	1
S19	Terrestrial habitat quality	Good	1	Poor	0.33	Good	1	Poor	0.33
S110	Macrophyte cover in pond	90%	0.9	10%	0.4	20%	0.5	0%	0.3
HSI	Overall HSI for pond:		0.904		0.638		0.676		0.378
	Overall Suitability	Excellent		Average		Average		Poor	

Pond ID		Waterbody 9		Waterbody 16		Waterbody 17		Waterbody 20 and 21	
SI ref	Description of Index	Measure / Comment	SI score	Measure / Comment	SI score	Measure / Comment	SI score	Measure / Comment	SI score
SI1	Geographic location	A	1	A	1	A	1	A	1
SI2	Pond area m <sup>2</sup>	85	0.2	9	0.5	265	0.5	380	0.7
SI3	Pond permanence	ND	0.9	DA	0.1	DA	0.1	SD	0.5
SI4	Water quality	Good	1	Poor	0.33	Poor	0.33	Poor	0.33
SI5	Shading %	40%	1	70%	0.8	60%	1	30%	1
SI6	Presence of waterfowl	Minor	0.67	Absent	1	Minor	0.67	Minor	0.67
SI7	Presence of fish	Absent	1	Absent	1	Poss	0.67	Absent	1
SI8	Pond density in area	>15	1	>15	1	>15	1	>15	1
SI9	Terrestrial habitat quality	Moderate	0.67	Moderate	0.67	Good	1	Moderate	0.67
SI10	Macrophyte cover in pond	50%	0.8	10%	0.4	80%	1	100%	0.8
HSI	Overall HSI for pond:		0.569		0.569		0.612		0.727
	Overall Suitability	Below Average		Below Average		Average		Good	

Pond ID		Waterbody 29		Waterbody 30		Waterbody 33		Waterbody 35	
SI ref	Description of Index	Measure / Comment	SI score	Measure / Comment	SI score	Measure / Comment	SI score	Measure / Comment	SI score
SI1	Geographic location	A	1	A	1	A	1	A	1
SI2	Pond area m <sup>2</sup>	900	1	30	0.05	400	0.8	3	0.1
SI3	Pond permanence	ND	0.9	SD	0.5	ND	0.9	SD	0.5
SI4	Water quality	Poor	0.33	Poor	0.33	Moderate	0.67	Moderate	0.67
SI5	Shading %	35%	1	90%	0.4	15%	1	50%	1
SI6	Presence of waterfowl	Minor	0.67	Absent	1	Minor	0.67	Minor	0.67
SI7	Presence of fish	Poss	0.67	Poss	0.67	Major	0.01	Absent	1
SI8	Pond density in area	>15	1	>15	1	>15	1	>15	1
SI9	Terrestrial habitat quality	Poor	0.33	Average	0.67	Moderate	0.67	Moderate	0.67
SI10	Macrophyte cover in pond	20%	0.5	100%	0.8	60%	0.9	100%	0.8
HSI	Overall HSI for pond:		0.683		0.510		0.536		0.643
	Overall Suitability	Average		Below Average		Below Average		Average	

**APPENDIX C**

**eDNA sampling analysis results**

**Folio No:** 3601-2025  
**Purchase Order:** 2090.78  
**Contact:** Hankinson Duckett Associates  
**Issue Date:** 18.07.2025  
**Received Date:** 04.07.2025

# GCN Report

Technical Report



SureScreen Scientifics

Folio No: 3601-2025  
 Purchase Order: 2090.78  
 Contact: Hankinson Duckett Associates  
 Issue Date: 18.07.2025  
 Received Date: 04.07.2025

# GCN eDNA Analysis

## 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 analyzing water samples, we can detect these small traces of environmental DNA (eDNA) to confirm GCN habitation or establish GCN absence.

## Results

Lab ID	Site Name	OS Reference	Degradation Check	Inhibition Check	Result	Positive Replicates
GCN25 1760	Southwater - P4	TQ 15631 28640	Pass	Pass	Negative	0/12
GCN25 1761	Southwater - 17	TQ 14975 26750	Pass	Pass	Negative	0/12
GCN25 1764	Southwater - P33	TQ 14947 28888	Pass	Pass	Negative	0/12
GCN25 1765	Southwater - P2	TQ 15467 28828	Pass	Pass	Negative	0/12
GCN25 1766'	Southwater - P5	TQ 15679 28379	Pass	Pass	Negative	0/12
GCN25 1769	Southwater - 16	TQ 14939 27177	Pass	Pass	Negative	0/12
GCN25 1773	Southwater - D1	TQ 14755 27111	Pass	Pass	Negative	0/12
GCN25 1782	Southwater - P30	TQ 16234 27855	Pass	Pass	Negative	0/12
GCN25 1794	Southwater - 9	TQ 15345 27743	Pass	Pass	Positive	6/12
GCN25 1796	Southwater - P21	TQ 15346 26934	Pass	Pass	Negative	0/12

Folio No: 3601-2025  
Purchase Order: 2090.78  
Contact: Hankinson Duckett Associates  
Issue Date: 18.07.2025  
Received Date: 04.07.2025

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GCN25 1799	Southwater - P3	TQ 15253 28854	Pass	Pass	Negative	0/12
GCN25 1803	Southwater - P29	TQ 16173 27598	Pass	Pass	Negative	0/12
GCN25 8538	Southwater - P35	TQ 15323 28070	Pass	Pass	Positive	12/12

---

Matters affecting result: none

Reported by: Amy Bermudez

Approved by: Consuela Sopronyi



## Methodology

The samples detailed above have been analyzed 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 tube which then undergoes DNA extraction. The extracted sample is then analyzed 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 attention to detail to prevent the 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 analytical security.

SureScreen Scientifics Ltd is ISO9001 accredited and participates in Natural England's proficiency testing scheme for GCN eDNA testing.

## Interpretation of Results

### Sample Integrity Check:

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. Any samples which fail this test are rejected and eliminated before analysis.

### 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.

### Inhibition Check:

**Pass/Fail.** The presence of inhibitors within a sample is assessed using a DNA marker. If inhibition is detected, samples are purified and re-analyzed. 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 the WC1067 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.

**Inconclusive:** Controls indicate inhibition or degradation of the sample, resulting in the inability to provide conclusive evidence for GCN presence or absence.