



L I Z A R D

Landscape Design and Ecology

## ECOLOGICAL IMPACT ASSESSMENT

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**Land East of Mousdell Close, Ashington, West  
Sussex**

On Behalf of: Rocco Homes

**Planning Issue**

<b>Client:</b>	Rocco Homes			
<b>Project:</b>	Land East of Mousdell Close, Ashington, West Sussex			
<b>Reference:</b>	LLD3503-ECO-REP-001-02-EcIA			
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**Validity:**

This report is valid for 18 months from the date of the site visit. If works have not commenced by this date, an updated site visit should be carried out by a suitably qualified ecologist to assess any changes in the habitats present on site, and to inform a review of the conclusions and recommendations made.



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## SUMMARY

Lizard Landscape Design and Ecology (LLDE) has been commissioned by Rocco Homes to undertake an Ecological Impact Assessment of the proposed development of Land East of Mousdell Close, Ashington, West Sussex (*Grid Reference: TQ 1249 1635 – hereafter referred to as ‘the site’*). A Preliminary Ecological Appraisal of the site was undertaken on 4th of April 2025. Further assessment of local ponds was recommended for great crested newt (GCN) environmental DNA (eDNA) and was subsequently carried out. An assessment of the ecological impact of the proposals was then undertaken using this baseline data.

The main body of the site was dominated by bare ground, a habitat of low/negligible ecological value that is suitable for development. Higher value habitat of mixed scrub and hedgerow with trees were noted within the site boundary and UK priority habitat of Lowland Mixed Deciduous Woodland was noted adjacent to the south of the site. These habitats should be retained and protected where possible.

The proposed construction zone is located c. 0.8km from America & Gratwicke’s Wood LWS and c. 2.9km from Sullington Warren SSSI. Due to the intervening distances, impacts upon these sites is not expected. The site is within the Sussex North Water Supply Zone so water abstraction will have to be considered. To ensure no impacts upon these sites due to proposals, all construction should be undertaken in accordance with best practice guidelines with regards to control of dust, noise, and emissions.

The site also offers some limited suitable habitat within the boundary vegetation for reptiles, bats, common invertebrates and breeding birds. Habitats within the construction zone are mostly unsuitable for protected species and avoidance and mitigation measures have been built into the design to avoid potential and unlikely impacts of the scheme in accordance with the mitigation hierarchy and BS42020: 2013. Furthermore, opportunities for ecological enhancement have been provided to allow the ecological value of the site to be maximised. As this is a full planning application and no exemptions were found to apply, the development proposals shall be subject to the standard Biodiversity Gain Condition. Full Biodiversity Net Gain assessment of the site and proposals will accompany this application.

## 1.0 INTRODUCTION

- 1.1 Lizard Landscape Design and Ecology has been commissioned by Rocco Homes to undertake an Ecological Impact Assessment of the proposed development of Land East of Mousdell Close, Ashington, West Sussex (*Grid Reference: TQ 1249 1635 – hereafter referred to as 'the site'*).
- 1.2 The scope of this assessment has been determined with consideration of best-practice guidance provided by the Chartered Institute of Ecology and Environmental Management (CIEEM, 2018) and the Biodiversity: Code of practice for planning and development published by the British Standards Institute (BS 42020:2013).
- 1.3 An initial Preliminary Ecological Appraisal of the site was undertaken on 4th of April 2025. A number of ponds were noted within the surrounding area and so 2no. ponds where access was granted were tested for GCN eDNA.
- 1.4 A summary of the potential impacts of the proposals, and details of avoidance, mitigation and compensation measures have been detailed within this report. Residual impacts are then discussed once all mitigation and compensation measures have been considered.

### ***Site Information***

- 1.5 The site covers an area of c. 2.1ha, located towards the western edge of the developed area of Ashington in West Sussex. At the time of the initial site visit, the ground was recently cleared, and the site was dominated by bare ground. Hedgerows encompassed much of the site boundary with woodland along the southern edge. The site is bound by woodland to the south, residential properties to the west and east and further fields to the north, beyond Rectory Lane. The soil on site is described as slowly permeable seasonally wet slightly acid but base-rich loamy clayey soils.

***Surrounding Landscape***

1.6 The site is located within a rural setting, c. 8.5km east of Pulborough and c. 14km north of Worthing. Nearby is the A24 which provides good connection to further towns and cities. To the east is the majority of Ashington's developed area, including shops, community centres, a school, a church and residential estates. The surrounding landscape to the north, west and south is predominately arable fields, with some woodland parcels. These are well connected by mature treelines and hedgerow. The soil profile is described as slowly permeable seasonally wet and slightly acidic but base rich loamy and clayey soil.

***Development Proposals***

1.7 It is understood that the proposals are for a residential development of approximately 74no. homes with associated soft and hard landscaping.

***Biodiversity Gain Statement***

1.8 The proposed development does not qualify for any relevant exemption. As of 12th of February 2024, Biodiversity Net Gain is mandatory under Schedule 7A of the Town and Country Planning Act 1990 (as inserted by Schedule 14 of the Environment Act 2021). As such, the scheme shall be subject to the standard biodiversity gain planning condition.

1.9 As per the 'Accounting for degraded sites' section on page 50 of the BNG user guide (DEFRA, 2024) aerial imagery and remnant floral species found on site were used to estimate the habitats present, their conditions and their extent before the recent site clearance. This information was used as the baseline habitat information for the purposes of the BNG assessment.

### **Report Aims**

1.10 The aim of the baseline surveys and Ecological Impact Assessment has been:

- *To describe baseline conditions at the site;*
- *To determine the importance of features which may be impacted by the scheme;*
- *To identify impacts of the proposed development and set out appropriate avoidance, mitigation and compensation measures;*
- *To identify any residual impacts;*
- *To provide details of enhancements to be incorporated into the scheme; and*
- *To provide sufficient information to determine whether the project accords with relevant nature conservation policies and legislation, and where appropriate, to allow conditions or obligations to be proposed by the relevant authority.*

## **2.0 PLANNING POLICY AND LEGISLATION**

### ***Legislation***

2.1 Legislation relating to wildlife and biodiversity of particular relevance to this EclA includes:

- The Conservation of Habitats and Species Regulations 2017;
- The Wildlife and Countryside Act 1981 (as amended);
- The Natural Environment and Rural Communities (NERC) Act 2006; and
- The Environment Act 2021.

2.2 This above legislation has been addressed, as appropriate, in the production of this report.

***National Planning Policy***

2.3 The National Planning Policy Framework (NPPF) 2023 sets out the government planning policies for England and how they should be applied. 'Chapter 15: Conserving and Enhancing the Natural Environment' states that development should be 'minimising impacts on and providing net gains for biodiversity, including by establishing coherent ecological networks that are more resilient to current and future pressures.'

2.4 The Government Circular 06/2005, which is referred to by the NPPF, provides further guidance in respect of statutory obligations for biodiversity and geological conservation and their impact within the planning system.

### 3.0 METHODOLOGY

#### 3.1 Desk Study

3.1.1 The Multi-Agency Geographical Information Centre (*MAGiC*) website was consulted for information regarding the location of waterbodies, priority habitats, statutory designated sites and existing wildlife mitigation licences, within a potential zone of influence of the site. Additionally, the Local Planning Authority (LPA) website was consulted for information regarding the location of non-statutory designated areas, and satellite imagery and historic mapping was used to inform an assessment of the recent land use changes and habitat types within the area. The following potential zones of Influence's have been used for the following potential ecological receptors during the desk study assessment:

**Table No. 01 – Zones of Influences for Ecological Receptors**

Potential Zone of Influence	Type of Record / Designation/s / Ecological Receptor
0.5km	<ul style="list-style-type: none"> <li>Ponds, ditches and other water bodies.</li> </ul>
2.0km	<ul style="list-style-type: none"> <li>Priority Habitats (UKBAP) (NERC, 2006);</li> <li>European Protected Species Mitigation Licences (EPSMLs);</li> <li>Local Nature Reserves (LNRs);</li> <li>National Nature Reserves;</li> <li>Sites of Special Scientific Interest (SSSIs); and</li> <li>Local Wildlife Sites (LWS) / Site of Nature Conservation Interest (SNCI).</li> </ul>
10.0km	<ul style="list-style-type: none"> <li>Special Protection Areas (SPAs);</li> <li>potential Special Protection Areas (pSPAs);</li> <li>Ramsars (Wetlands of International Importance);</li> <li>proposed Ramsars (pRamsar);</li> <li>Special Areas of Conservation (SACs); and</li> <li>possible Special Areas of Conservation (pSACs).</li> </ul>
12.0km	<ul style="list-style-type: none"> <li>Special Areas of Conservation (SACs) and possible Special Areas of Conservation (pSACs) designated for supporting Annex II bat species.</li> </ul>

- 3.1.2 All protected / notable species records within a 2.0km radius of the site were provided by Sussex Biological Records Centre (SxBRC) on the 9<sup>th</sup> of April 2025.
- 3.1.3 The Local Planning Authority website was also consulted to inform of additional relevant information to this assessment, including any Local Nature Recovery Strategies, Nature Improvement Areas (NIAs) and Biodiversity Opportunity Areas (BOAs) etc.
- 3.1.4 In accordance with Natural England's GCN Mitigation Guidelines (English Nature, 2001) a desktop search was undertaken to identify ponds within 500m and 250m of the site, which may have the potential to support breeding great crested newts (GCN) *Triturus cristatus*, using Ordnance Survey mapping, the MAGIC database and aerial photography.

## 3.2 Preliminary Ecological Appraisal

- 3.2.1 The initial field survey was undertaken on 4th of April 2025 by a Suitably Qualified Ecologist (Sam Hall, Consultant Ecologist, Lizard Landscape Design & Ecology). Weather conditions were warm (c.20°C), with a light wind (Beaufort Scale 2), 30% cloud cover and no rain.
- 3.2.2 The field survey comprised a walkover inspection of the site and immediately adjacent land and boundaries features, in which ecological features were noted and mapped in accordance with principles of the UKHabs-Professional Classification System (Butcher *et al*, 2023). A minimum mapping unit of 25m<sup>2</sup> was used and habitats were identified to at least level 4 wherever practicable.
- 3.2.3 A list of plant species was compiled, together with an estimate of abundance (*Table No. 13*). In addition, target notes (*Table No. 10*) were used to provide supplementary information on features which were particularly interesting or significant to specific construction proposals, or too small to map.

3.2.4 The survey methodology was extended to provide more detail in relation to the sites potential to support rare or protected fauna, as described by the *Chartered Institute of Ecology and Environmental Management's Guidelines for Preliminary Ecological Appraisal* (CIEEM, 2017). The assessment of habitat suitability for protected, rare or priority species is based on current good practice guidance such as those laid out in the table below. The possible presence of each taxon was summarised as either negligible, low, moderate, high or confirmed.

**Table No. 02 – Habitat Suitability Assessment References**

Fauna	Relevant Best Practice Guidance
Great Crested Newts	<i>Great Crested Newt Conservation Handbook</i> (Langton et al, 2001) & <i>Evaluating the Suitability of Habitat for the Great Crested Newt</i> (Oldham et al, 2000)
Reptiles	<i>Herpetofauna Workers' Manual</i> (Gent and Gibson, 2003)
Bats	<i>Bat Surveys for Professional Ecologists: Good Practice Guidelines (4<sup>th</sup> edition)</i> (Collins, 2023)
Dormice	<i>The Dormice Conservation Handbook</i> (English Nature, 2006)
Badger	<i>Survey Badgers</i> (Harris et al, 1989)
Water Vole	<i>The Water Vole Mitigation Handbook</i> (Dean et al, 2016)
Birds	<i>Guidance for Bird Surveys in Relation to Development</i> (NE, 2022)
Invertebrates	<i>Considering Terrestrial Invertebrates in Preliminary Ecological Appraisals</i> (Jukes, 2021) and <i>Organising Surveys to Determine Site Quality for Invertebrates</i> (English Nature, 2005)

3.2.5 Photographs were taken as evidence and to illustrate any notable ecological features on site. These have been provided within the body of the relevant parts of the Baseline Ecological Condition section, where appropriate.

### 3.3 Daytime Bat Walkover Survey

3.3.1 A Daytime Bat Walkover (DBW) survey was undertaken on 4th of April 2025 by a suitably experienced surveyor (Sam Hall Accredited Agent Under; Louise Barker (Bat Level 2 Class Licence; 2023-11422-CL18-BAT)). Weather conditions were warm (c.20°C), with a light wind (Beaufort Scale 2), 30% cloud cover and no rain.

3.3.2 The Daytime Bat Walkover (DBW) survey entailed a slow walkover of the site, during which time the surveyor identified any structures, trees and other features that could be suitable for bats to roost in, and any habitats which could be suitable for bats to commute, forage or swarm in.

3.3.3 During this survey any direct evidence of bats was searched for and recorded, such as grease marks, urine stains, bat droppings, feeding remains and dead / live bats. Furthermore, any structures or trees which offered features with the potential to support bats were noted. For trees this included the identification of features such as, but not limited to, cracks, crevices and holes naturally formed by trees. For structures this included the identification of features such as, but not limited to, slipped, missing or uneven tiles, gaps around the soffit / barge board, raised flashing.

*Evaluation Criteria*

3.3.4 All suitable bat habitat was assessed in accordance best practice criteria (Collins, 2023), which is outlined herein. During the survey all trees within and immediately adjacent to the site were assessed using the following criteria:

**Table No. 03 – Criteria for Assessing the Bat Roosting Suitability of Trees**

Suitability	Description
<b>None</b>	Either no potential roosting features in the tree, or highly unlikely to be any.
<b>FAR</b>	Further assessment required to establish if potential roosting features are present in the tree.
<b>PRF</b>	A tree with at least one potential roosting feature present.

3.3.5 If it was possible to adequately assess a Potential Roosting Feature (PRF) from ground level then this was completed, and the feature classified as either:

- **PRF-I:** Feature only suitable for individual or very small numbers of bats, either due to size or lack of suitable surrounding habitat; or
- **PRF-M:** PRF is suitable for multiple bats and therefore has the potential to be used by a maternity colony.

3.3.6 Furthermore, all structures were assessed externally, and internally wherever possible for their potential to support bats, using the following criteria:

**Table No. 04 – Criteria for Assessing the Bat Roosting Suitability of Structures**

Potential Suitability	Description
<b>None</b>	No habitat features on site likely to be used by any roosting bats at any time of year.
<b>Negligible</b>	No obvious habitat features on site likely to be used by roosting bats. However, some small uncertainty remains, as bats can use small and apparently unsuitable features occasionally.
<b>Low</b>	A structure with one or more potential roost sites that could be used by individual bats opportunistically at any time of year. However, these do not provide enough shelter, space, protection, appropriate conditions or suitable surrounding habitat to be used on a regular basis or by larger numbers of bats.
<b>Moderate</b>	A structure with one or more potential roost sites that could be used by bats due to their size, shelter, protection, conditions and surrounding habitat, but unlikely to support a roost of high conservation status, irrespective of species conservation status.
<b>High</b>	A structure with one or more potential roost sites that are obviously suitable for use by larger numbers of bats on a more regular basis and potentially for longer periods of time due to their size, shelter, protection, conditions and surrounding habitat, with the potential to support high conservation status roosts irrespective of species conservation status.
<b>Confirmed</b>	Direct evidence of bats identified.

3.3.7 Finally, an assessment of the winter hibernation potential of the structures was made, in accordance with the following criteria:

**Table No. 05 – Criteria for Assessing the Winter Bat Roosting Suitability of Structures and Trees**

Potential Suitability	Description
<b>Low</b>	No or very limited potential winter roosting habitat
<b>Moderate</b>	Non classic site
<b>High</b>	'Classic sites', which offer stable humidity and consistent temperatures throughout the winter period, such as underground sites, cellars, tunnels etc.

### 3.4 Badger Walkover Survey

#### *Initial Survey*

3.4.1 The initial field survey was undertaken on 4<sup>th</sup> April 2025 by an experienced ecologist. The survey area covered the red line boundary of the site, and all land within a 30m radius (where access was available).

3.4.2 The survey area was systematically searched for any evidence of badger such as:

- *Setts.*
- *Latrines.*
- *Snuffle Holes.*
- *'Push-unders' through boundary fencing.*
- *Hair caught on fencing or sett entrances.*
- *Prints left in mud or sand.*
- *Mammal tracks.*

3.4.3 Any evidence was then mapped to allow the status and distribution of badger activity to be assessed.

### 3.5 Great Crested Newt Survey

- 3.5.1 Any ponds identified within or adjacent to the site were subject to a Habitat Suitability Index (HSI) assessment to determine their suitability to support GCN, in line with current guidance (Oldham *et al*, 2000). The HSI is a numerical index, between 0 and 1 (0 representing completely unsuitable habitat and 1 representing optimal habitat), calculated based on the suitability of 10 calculable indices.
- 3.5.2 HSI assessment is useful to aid in determining how suitable a given waterbody is for GCN, but it does not directly correlate with GCN presence or population numbers and serves as information only.
- 3.5.3 The 10 indices considered as part of the HSI assessment include geographic area, pond area, permanence of waterbody, water quality, shading, waterfowl presence, fish presence, number of ponds within 1.0km, suitability of terrestrial habitat and macrophyte cover, which were investigated during the field survey assessment.

#### eDNA survey

- 3.5.4 Access was gained for P3 and P6 and water samples were collected those ponds on the 29<sup>th</sup> of April 2025 and subsequently tested for traces of GCN environmental DNA.
- 3.5.5 20no. water samples were collected from the margin of each pond, with samples spaced as evenly as possible to collect a representative sample. All samples were collected using a sterile sampling kit as supplied by SureScreen Scientifics.
- 3.5.6 Each sample was stored in a refrigerator before return to SureScreen Scientifics for analysis. The results of the survey indicate the presence or absence of great crested newt environmental DNA within the water body.

### 3.6 Ecological Impact Assessment

3.6.1 The methodology for Ecological Impact Assessment (EIA) follows best practice guidelines set by the Chartered Institute of Ecology & Environmental Management (CIEEM): 'Guidelines for Ecological Impact Assessment' (CIEEM, 2018). This includes identifying the baseline conditions on the site and subsequently rating the potential effects of the development based on the sensitivity and value of the resource affected, combined with the magnitude, duration and scale of the impact (or change). This is initially assessed without mitigation measures, and then assessed again after allowing for the proposed mitigation measures; this provides the residual effects. The assessment is divided into construction effects and longer-term operational effects.

3.6.2 The CIEEM guidelines (2024) state that ecological features should be considered within a 'defined geographical context'. The geographical frame of reference used to determine ecological importance in this assessment is detailed below.

**Table No. 06 – Likely Importance Assessment Criteria**

Likely Importance Categories	Likely Importance Criteria
Negligible	Of no notable ecological value.
Site	Ecologically valuable within the context of the site
Local	Ecologically valuable within the context of the immediate surrounds, i.e., c. 1km <sup>2</sup>
District	Ecologically valuable within the context of the wider surrounds / LPA district, i.e., c. 10km <sup>2</sup>
County	Ecologically valuable within the context of the wider county, i.e., c. 100km <sup>2</sup>
Regional	Of ecological value within the region, i.e., south east, south west, midlands etc.
National	Of ecological value within the context of the United Kingdom, such as a SSSIs, NNR's etc.
International	Ecological value of global significance, such as SACs, SPAs etc.

3.6.3 Based upon CIEEM guidance, value was determined with reference to the following factors:

- Its inclusion as a Designated Site or other protected area;
- The presence of habitat types of conservation significance, e.g. Habitats of Principal Importance (NERC 2006);
- The presence (or potential presence) of species of conservation significance e.g. Species of Principal Importance (NERC 2006);
- The presence of other protected species e.g. those protected under The Wildlife and Countryside Act 1981;
- The sites social and economic value.

3.6.4 The ecological impacts resulting from the proposals were then described according to a defined set of characteristics as defined within '*Guidelines for Ecological Impact Assessment in the UK and Ireland*' (CIEEM, 2018). When describing impacts the assessment refers to characteristics such as the extent; magnitude; duration; frequency; and, reversibility of the impact in order to provide justification for any conclusions about the nature and likelihood of the impact described.

3.6.5 Where initial impacts have been identified as significant, avoidance, mitigation and compensation measures have been proposed to avoid, prevent or offset such effects. This assessment then considers residual impacts (*once all mitigation has been taken into account*), with any significant effects highlighted. A significant effect is defined as "*an effect which either supports or undermines biodiversity conservation objectives for 'important ecological features' or for biodiversity in general*". Enhancement has been proposed to ensure that the development represents a net gain in biodiversity in accordance with National Policy.

### 3.7 Constraints and Limitations

- 3.7.1 Due to the field survey consisting of only one site visit, certain species, particularly some of the flowering plants, may not have been visible and hence overlooked. These are accepted constraints associated with the standard Survey Methodology.
- 3.7.2 At the time of the initial site visit, the site had been recently cleared. Aerial imagery and some remnant floral species on site have been used to infer the habitats that occupied the site previously. Please note then that the site habitat plan (Figure No. 01) within this report shows the habitats observed on site and a precautionary approach has been applied to generate the habitats within the baseline habitat plan within the associated Biodiversity Gain Statement report.
- 3.7.3 It is understood that the site had been vacant for at least 2 years following the death of the landowner and the recent clearance was carried out to return the site to its long-term use of equine pasture. Further to this, LLDE was not involved with the recent clearance of the site vegetation, which may have removed potentially suitable habitat for protected species.
- 3.7.4 No other limitations were encountered, or assumptions made during either the desk study or the field survey and it is considered that with the access gained and recording undertaken an accurate assessment of the site's current ecological value has been made.

## 4.0 BASELINE ECOLOGICAL CONDITIONS

### 4.1 Designated Sites

#### **Statutory Protected Sites**

4.1.1 The following statutory protected sites are noted within the likely zone of influence of the proposed site:

**Table No. 07 – Statutory Designated Sites**

Site	Description	Location
<b><i>International Statutory Designated Sites within 10/12km</i></b>		
Arun Valley SPA	<p>The site is designated for the following Annex II species:</p> <ul style="list-style-type: none"> <li>Bewicks's Swan, <i>Cygnus columbianus bewickii</i></li> </ul> <p>Additionally, the site supports a significant waterbird assemblage.</p>	c. 6.5km W
Arun Valley SAC	<p>The site is designated for the following Annex II species as a primary reason:</p> <p>Ramshorn snail, <i>Anisus vorticulus</i></p>	c. 6.5km W
Arun Valley Ramsar	<p>The site is designated for meeting the following Ramsar criterion:</p> <p>Criterion 2 - The site holds seven wetland invertebrate species listed in the British Red Data Book as threatened. One, <i>Psuedoamnicola confusa</i>, is considered endangered. Additionally the site supports 4no. nationally scarce plant species.</p> <p>Criterion 3 – The site contains intersecting ditches with particularly diverse and rich flora. All five British <i>Lemna</i> species, all five <i>Rorippa</i> species, all three British milfoils (<i>Myriophyllum</i>), 6/7 British water dropworts (<i>Oenanthe</i>) and 2 thirds of the British pondweeds can be found on site.</p> <p>Criterion 5 – Internationally important waterfowl assemblage.</p>	c. 6.5km W
The Mens SAC	<p>The site is designated for the following Annex I habitats as a primary reason:</p> <ul style="list-style-type: none"> <li>Atlantic acidophilous beech forests with <i>Ilex</i> and sometimes also <i>Taxus</i> in the shrublayer (<i>Quercion robori-petraeae</i> or <i>Ilici-Fagenion</i>)</li> </ul> <p>The site is also designated for the following Annex II species as a qualifying feature:</p> <p>Barbastelle, <i>Barbastella barbastellus</i></p>	c. 11.7km NW

4.1.2 The site is located within the Impact Risk Zone of Sullington Warren SSSI and Chanctonbury Hill SSSI, whereby development including aviation infrastructure and/or livestock pollution over a set threshold would require consultation with Natural England. The current development proposals do not meet such a description. The site is within the Sussex North Water Supply Zone so water abstraction will have to be considered.

***Non-Statutory Protected Areas***

4.1.3 The following non-statutory designated areas were identified within 2.0km of the site.

***Table No. 08 – Non-Statutory Designated Areas***

Site	Location
H49 – America & Gratwicke’s Wood (Local Wildlife Site)	c. 0.8km WNW
Warminghurst Road Cutting, Ashington (Local Geological Site)	c. 0.9km E

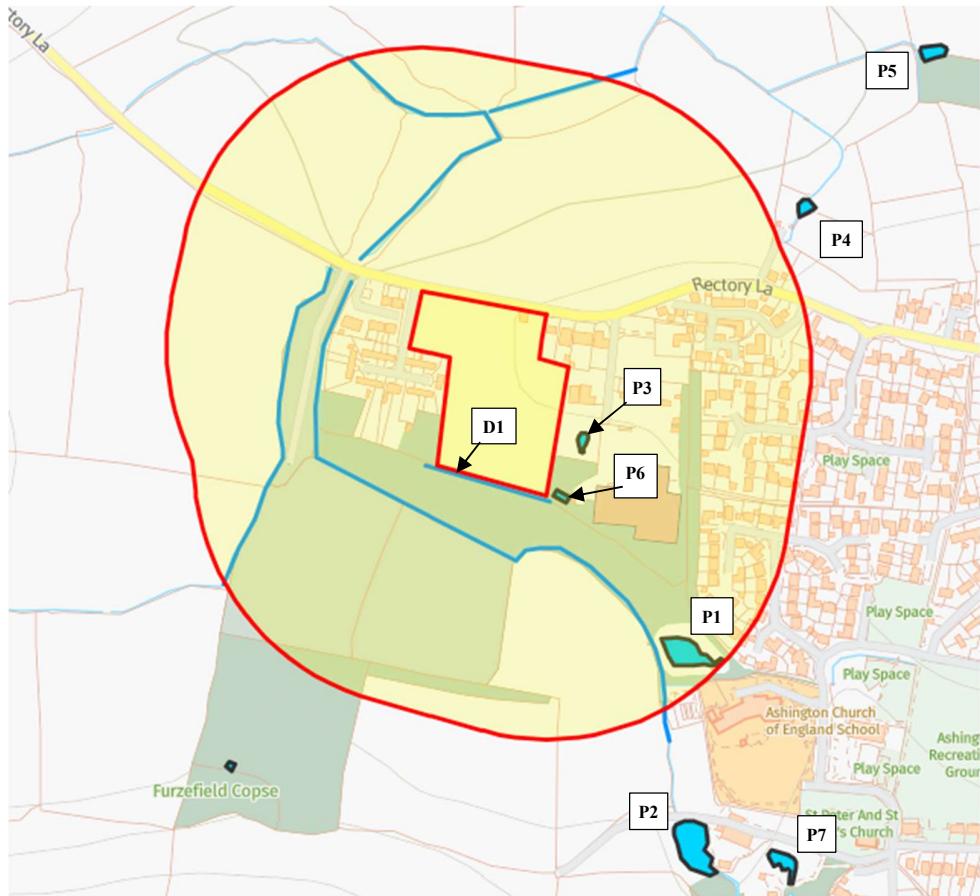
4.1.4 The site is comprised of distinctly different habitat to that of the above *Non-Statutory Protected Sites*, the site area provides no supporting habitat, and proposals would have no impact upon these areas due to the intervening distance.

***Priority Habitat***

4.1.5 In accordance with the MAGIC dataset, within a 2.0km search radii of the site there were UKBAP Priority Habitats (NERC, 2006) of Lowland Mixed Deciduous Woodland (some of which was categorised as ancient) and Traditional Orchards.

***Pond Study***

4.1.6 3no. ponds and multiple ditches were identified within 250m of the site, based on OS mapping and satellite imagery. Numerous other waterbodies were noted within 500m of the site and are highlighted in Figure No. 01 below.



**Figure No. 01 – Pond Plan.** Buffer zone of 250m from the site boundary shown as well as all waterbodies in blue. Data taken from MAGIC. Contains OS Data © Crown Copyright and database rights 2025.

### **European Protected Species Mitigation Licence (EPSML) Search**

4.1.7 In accordance with the MAGIC dataset, within a 2.0km search radii of the site, the following records for existing European Protected Species Mitigation Licences (EPSMLs) were returned:

**Table No. 09 – EPSMLs within Potential Zone of Influence**

Date	Species	Licence Permission	Distance and Direction from Site
05/05/2017	Brown long eared <i>Plecotus auritus</i>	Damage to a breeding site	c. 0.75km SW
10/01/2019	Dormouse <i>Muscardinus avellanarius</i>	Destruction of a resting and breeding site	c. 0.8km E
06/01/2016	Brown long eared <i>Plecotus auritus</i> & common pipistrelle <i>Pipistrellus pipistrellus</i>	Destruction of a resting site	c. 0.9km NW

## **4.2 Existing Habitat Assessment**

4.2.1 Habitats within and adjacent to the site include:

- Bare ground
- Mixed scrub
- Species-rich native hedgerow with trees
- Lowland Mixed Deciduous Woodland (*Priority Habitat*)

### *Bare ground*

4.2.2 At the time of the initial site visit, the site had been cleared leaving little but bare earth across much of the site. However, historical aerial imagery suggests that the site was dominated by rough grassland with scattered scrub and mixed scrub habitat extending into the site from the boundaries. Bracken stems were noted across the site and a small remnant of grass on the west site boundary was noted as dominated by Yorkshire fog *Holcus lanatus* with occasional forbs such as dandelion *Taraxacum officinale* agg. and common nettle *Urtica dioica*. This habitat is of **site level** value.



Photograph No. 01 – View across the site from west to east.

#### *Mixed scrub*

4.2.3 An area of mixed scrub was noted to the northeast of the site. Species composition included holly *Ilex aquifolium*, elder *Sambucus nigra*, bramble *Rubus fruticosus* and hawthorn *Crataegus monogyna*. This habitat is of limited extent and is of **site level** value.



Photograph No. 02 – View of mixed scrub along the western site boundary.

*Species-rich Native hedgerow with tree*

4.2.3 Hedges with trees were noted along the northern site boundary and a small section of the eastern boundary close to the southeastern corner. Hedgerow species included hawthorn and blackthorn *Prunus spinosa* and tree species included oak *Quercus robur*, ash *Fraxinus excelsior*, sycamore *Acer pseudoplatanus* and a willow species *Salix* sp. The shrub canopy is sparse with regular gaps in the hedge although there is little evidence of regular management. This habitat is of **site level** value.



Photograph No. 03 – View eastward along the northern site boundary.

*Lowland Mixed Deciduous Woodland (Priority Habitat)*

4.2.4 Although not included as priority habitat within the government priority habitats inventory as shown on MAGIC maps, the woodland adjacent to the south of the site was assessed as Lowland Mixed Deciduous Woodland. Observations were made of the woodland from within the site and species included mature oak and ash trees as well as hazel *Corylus avellana* and hawthorn within the shrub layer and sparse ground vegetation including dog's mercury *Mercurialis perennis*. A single oak was noted that had fallen into the site. This habitat is of **local level** value.



*Photograph No. 03 – View eastward along the southern site boundary*

Ditch

4.2.5 A wet ditch was noted close to the southern site boundary within the adjacent woodland. The ditch was heavily shaded by the woodland with very little vegetation. This habitat is of **site level** value.

*Invasive Species*

4.2.6 No Schedule 9 invasive species:

<https://www.legislation.gov.uk/ukpga/1981/69/schedule/9> were identified on site.

**Table No. 10 – Target Notes**

<b>Target Note</b>	<b>Feature</b>	<b>Description</b>
TN01	Snuffle hole	Badgers snuffle hole noted off site



## Figure No. 02 - Site Habitat Plan



# LIZARD

Landscape Design and Ecology

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## Client

Rocco Homes

### Project Title & Location

## Land East of Mou

Drawn by      Approved by      Rev      Date

SH COR 01 08/07/23

N 1:1,500

## 4.3 Protected Species Assessment

### **Amphibians**

#### *Desk Study*

4.3.1 SxBRC returned 4 no. records for Great crested newt *Triturus cristatus* from within the search area, The closest of which was located c. 1.1km east of the site beyond the A24. SxBRC also returned records for common toad *Bufo bufo*, smooth newt *Lissotriton vulgaris*, palmate newt *Lissotriton helveticus*, and common frog *Rana temporaria*.

#### *Site Assessment*

4.3.2 No waterbodies were identified on site. Construction activity had begun on the site adjacent to the east and pond P6 was not present there. Ditch D1 and Pond P1 were subject to HSI assessment, a summary of which is provided below.

**Table No. 11 – Summary of HSI Results**

HSI Criteria	P1		D1	
Location	1	Zone A	1	Zone A
Pond Area	0.95	1,000m <sup>2</sup>	0.2	120m <sup>2</sup>
Permanence	0.9	Never dries	0.1	Dries annually
Water Quality	0.67	Moderate	0.01	Bad
Shade	1	< 50% shade	0.2	100% shade
Waterfowl	0.01	Major	1	Absent
Fish	0.67	Possible	1	Absent
Pond Count	0.9	3.82	0.9	3.82
Terrestrial Habitat	1	Good	1	Good
Macrophytes	0.3	0% cover	0.3	0% cover
<b>HSI Score</b>	<b>0.5</b>	<b>Below Average</b>	<b>0.32</b>	<b>Poor</b>

4.3.3 It is accepted that, unless connected by highly suitable habitat, most great crested newts tend to stay within 250m of breeding ponds (Langton *et al.*, 2001). The suitable terrestrial habitat was limited to boundary vegetation. The site has been assessed as offering **low** suitability for GCN and other amphibians within boundary vegetation and **negligible** suitability across the main site area.

### eDNA results

4.3.4 eDNA analysis of water samples taken from ponds P1 and P2 returned negative results for GCN environmental DNA in both tested ponds, suggesting the likely absence of this species within these waterbodies and in the local area. See Appendix A.

### **Reptiles**

#### *Desk Study*

4.3.5 SxBRC returned records for three species of reptiles, including records for slow worm *Anguis fragilis*, common lizard *Zootoca vivipara*, and grass snake *Natrix Helvetica*. The closest records were for slow worm and located 100m east of the site.

#### *Site Assessment*

4.3.6 Reptiles require a mosaic of habitats to persist in a landscape, including vegetative cover for refuge opportunities, open areas for basking and a diverse flora to support viable invertebrate prey throughout the year. The suitable habitat was limited to boundary vegetation. The site has been assessed as offering **low** suitability for reptiles within boundary vegetation and **negligible** suitability across the main site area.

### **Bats**

#### *Desk Study*

4.3.7 SxBRC returned records for 7no. species of bat, including records for brown long-eared *Plecotus auritus*, noctule *Nyctalus noctule*, serotine *Eptesicus serotinus*, Brandt's *Myotis brandtii*, Nathusius' pipistrelle *Pipistrellus nathusii*, common pipistrelle *Pipistrellus pipistrellus* and soprano pipistrelle *Pipistrellus pygmaeus*. This includes records of roost sites and observations of bats in the field. The closest record is for a serotine located c. 100m east of the site and recorded in 2020.

### **Daytime Bat Walkover - Trees**

4.3.8 Various trees were identified as offering some level of bat roost suitability during the ground level assessment. A summary of these features is illustrated in the table below. The tree reference numbers are illustrated on *Figure No. 01 – Site Habitats Plan*.

**Table No. 12 – Preliminary Bat Roost Assessment Results - Trees**

Tree Ref	Description	Category
T01	Oak tree located within the northern site boundary. No cavities noted but moderate ivy to stem may provide some small roosting opportunities.	PRF-I
TG02	Off-site oaks of a size that might be expected to contain PRF. As these trees are off-site access was not possible for a 360-degree inspection.	FAR
T03	Off-site oak tree that has fallen into the site. No cavities noted but moderate ivy to stem may be obscuring small features.	PRF-I
T04	Off-site oak of a size that might be expected to contain PRF. Impossible to rule out from the ground.	FAR

### **Buildings**

4.3.9 No buildings were identified.

### **Foraging and Commuting Suitability**

4.3.10 The site was dominated by bare ground of little value to any species other than those which are gap tolerant, such as common pipistrelle and noctule. However, relatively small lengths of linear habitat are present towards the north, northwest and south of the site which is also connected with further suitable habitat in the wider landscape. The site has been assessed as offering **low** suitability for foraging and commuting bats.

4.3.11 The scrub and rough grassland that previously occupied the site would likely have been of **moderate** value as a foraging resource for a range of bats, including light averse species.

#### *Winter Roosting Potential*

4.3.11 Given the results of the preliminary roost assessment and in consideration of the potential presence of hibernation features in off-site trees only, it was determined that the site offered **negligible** winter roosting potential.

#### **Dormouse**

##### *Desk Study*

4.3.12 SxBRC returned 32no. records of dormouse *Muscardinus avellanarius* from within the search area. The closest record is located 320m northeast and dated 2021.

##### *Site Assessment*

4.3.13 Suitable floral species to support dormice were noted in the boundary vegetation and adjacent woodland, although it is of note that the boundary hedgerow to the north of the site was limited in extent and gappy. The suitable habitat was limited to boundary vegetation. The site has been assessed as offering **low** suitability for this species within boundary vegetation and **negligible** suitability across the main site area.

#### **Badger**

##### *Desk Study*

4.3.14 Badger records have been treated as sensitive and have not been included in this report.

##### *Site Assessment*

4.3.15 The habitats on site offer low value potential foraging opportunities and woodland was noted adjacent to the south of the site. A potential snuffle hole was noted immediately off-site beyond the southern site boundary. towards the southeast corner of the site. The site is of **low** value to badgers, and proposals should be mindful of their likely presence in the local area.

## **Birds**

### *Desk Study*

4.3.16 SxBRC returned records for 60 no. species of birds from within the search area. This included records for species listed on Schedule 1 (Wildlife and Countryside Act, 1981) (as amended), as well as records for species of birds listed on the Birds of Conservation Concern (BoCC) Red List (Stanbury *et al*, 2021). This includes records for birds associated with grassland, woodland and scrub habitat, such as woodcock *Scolopax rusticola* and cuckoo *Cuculus canorus*. A single record for Bewick's swan of 6 individuals was returned c. 1.0km northeast of the site from 2005.

### *Site Assessment*

4.3.17 Suitable habitat for birds was limited to the boundary vegetation. The site has been assessed as offering **low** suitability for this species group within boundary vegetation and **negligible** suitability across the main site area.

4.4.18 At 6.5km from the Arun Valley SPA it is possible that the site could provide supporting habitat for Bewick's swan *Cygnus columbianus bewickii*, for which the SPA is designated. However, the existing site conditions do not meet the wintering habitat requirements for this species such as flooded pasture or others listed in the 'International Single Species Action Plan for the Conservation of the Northwest European Population of the Bewick's Swan' (WWT, 2012). Adjacent residential development further diminishes the suitability for the Bewick's swan, and it is unlikely to make use of the site.

4.4.19 The scrub and rough grassland that occupied the site previously would likely have created an enclosed environment unsuitable for taking off and landing and with long vegetation that would have been broadly unsuitable for Bewick's swan grazing.

**Invertebrates***Desk Study*

4.3.20 SxBRC returned records for species of protected / notable invertebrates from within the search area, including records for Stag beetle *Lucanus cervus* and purple emperor butterfly *Apatura iris*.

*Site Assessment*

4.3.21 The habitats and individually recorded floral species on site are common and widespread within the local area and surrounding landscape and are unlikely to support any notable assemblage of invertebrate species. The site is of **low** suitability for common and widespread invertebrates only.

**Water Vole***Desk Study*

4.4.22 SxBRC returned a single record of water vole *Arvicola amphibius* from within the search area, dated 2014.

*Site Assessment*

4.4.23 No suitable habitat was identified on site and the ditch noted close to the southern boundary was heavily shaded with very little vegetation and a low water level. The site is therefore of **negligible** value to this species.

**Others**

4.4.24 Considering the suburban setting, proposals should also be mindful of the potential presence of hedgehogs.

## 5.0 ASSESSMENT OF EFFECTS

5.0.1 Using the Guidelines for Ecological Impact Assessment (IEEM, 2006 & updated by CIEEM, 2018), the assessment set out below considers the potential impacts of the scheme prior to mitigation. Detailed avoidance, mitigation and compensation measures are then discussed, with residual impact identified once these measures have been taken into account. Wherever possible mitigation measures have been designed into the scheme as this gives greater certainty over deliverability and ensures the correct application of the 'Mitigation Hierarchy' (as advocated by BS42020:2013, Defra 2019 and CIEEM, CIRIA & IEMA 2016).

5.0.2 Protected species for which the site offers negligible suitability have been scoped out of further assessment.

### 5.1 Designated Sites

#### *Potential Impacts*

5.1.1 The site is not located within the Impact Risk Zone of Sullington Warren SSSI and Chanctonbury Hill SSSI, whereby development including aviation infrastructure and/or livestock pollution over a set threshold would require consultation with Natural England. The current development proposals do not meet such a description. The site is within the Sussex North Water Supply Zone so water abstraction should be considered.

5.1.2 Impacts to local designated sites such as Sullington Warren SSSI (2.9km) and America & Gratwicke's Wood LWS (c. 0.8km) are unlikely to occur due to the intervening distance. Although America & Gratwicke's Wood LWS may be intersected by public footpaths, it is not open access like Sullington Warren SSSI. Moreover, whilst Sullington Warren is open access it is notable that Horsham District Council Habitat Regulations Assessment of the local plan ruled out recreational pressure upon Waltham Brooks SSSI due to a minimum distance of 2.5km to the closest settlement.

*Mitigation and Compensation*

5.1.3 To ensure no impacts upon these sites due to proposals, all construction should be undertaken in accordance with best practice guidelines with regards to control of dust, noise, and any other potential emissions.

*Residual Impacts*

5.1.4 Once mitigation measures have been considered, there shall be **no likely significant effect** upon any designated site as a result of this development.

**5.2 Habitats***Potential Impacts*

5.2.1 Development proposals will predominantly result in the loss of bare ground. This habitat is of low ecological value, the loss of which would be of minor impact magnitude.

5.2.2 Construction traffic and other activities could impact the adjacent woodland and retained boundary vegetation through physical damage and / or pollution events. The proposed access route through the northern boundary will also result in the loss of a relatively short length of hedgerow with trees. This habitat is of moderate ecological value but considering the lower quality of this length of hedgerow and the limited extent of the loss it is of minor impact magnitude.

*Mitigation and Compensation*

5.2.3 Works during the construction phase will be undertaken in accordance with best practise guidelines to control any excess dust creation and other potential pollution events which may impact retained and adjacent habitats. Measures should include but not be limited to dampening down of dust with water sprays in dry weather and limiting the height of load tipping. All re-fuelling and chemical storage shall take place in a bunded enclosure with appropriate containment measures in place and spill kits available.

5.2.4 In order to compensate the loss of habitat scheduled for removal, all new areas of soft landscaping will be designed to maximise the biodiversity value of the site. This will be done by creating new individual trees, hedgerows and species rich grassland with a strong preference for native species. A full Biodiversity Net Gain assessment will be provided to detail how these compensation measures can contribute towards the +10% net gain mandate detailed under the *Environment Act (2021)* and will be prepared in line with best practice guidance (CIEEM, 2021).

5.2.5 Measures such as tree protective barriers should be incorporated into the construction phase to protect individual trees from physical damage and impacts upon the ground within their root protection areas in line with BS 5837:2012 *Trees in relation to design, demolition and construction: Recommendations*.

#### *Residual Impacts*

5.2.6 Provided mitigation and protection measures are followed, no priority or other important habitats or plant species will be substantially affected by this development, the impact of which is **not significant**.

### **5.3 Reptiles and Amphibians**

#### *Potential Impacts*

5.3.1 The proposed construction zone is unlikely to support reptiles and/or amphibians. Therefore, future development here would be unlikely to impact reptiles/amphibians.

#### *Mitigation and Compensation*

5.3.2 If vegetation is allowed to grow then it should be regularly managed and the sward kept as short as possible to ensure that the site does not become suitable for reptiles, amphibians or any other protected species. As a precautionary approach, any boundary vegetation that requires removal shall be cut using hand tools only such as strimmers and in the unlikely event that a reptile or newt species is discovered, all works shall cease, and the project ecologist contacted. Compensatory grassland and scrub should be included in the final design of the scheme.

*Residual Impacts*

5.3.3 Once mitigation measures are considered, the impact of the scheme shall be **negligible** and shall ensure the creation of suitable reptile/amphibian habitat in the long-term.

**5.4 Bats***Potential Impacts*

5.4.1 In the absence of mitigation impacts could include the disruption of commuting corridors and foraging/roosting habitat through inappropriate lighting. Due to the high suitability of the woodland to the south of the site, impacts would be of moderate significance and likely to occur.

5.4.2 The proposed site access may result in the removal of tree T01 along the northern boundary.

*Mitigation and Compensation*

5.4.3 Artificial light spill upon the woodland to the south of the site and retained mature trees shall be avoided throughout construction and within the scheme design to allow the use of this area as a foraging/roosting resource and commuting route for bats. Any external lighting designs should comply with best practice standards in regard to external lighting and bats (BCT & ILP, 2023).

5.4.4 Any trees to be removed within the northern boundary should be soft felled under ecological supervision and compensatory bat boxes provided nearby, prior to felling. Further to this, exclusion fencing should be used during construction to create a buffer area around those existing trees that were identified as offering some level of potential roosting value for bats.

5.4.5 Compensatory grassland and scrub should be included in the final design of the scheme.

*Residual Impacts*

5.4.6 The overall impact of the scheme will be **negligible**.

## 5.5 Badger and Hedgehog

### *Potential Impacts*

5.5.1 In the absence of mitigation, impacts could include the trapping of badgers / hedgehogs in footings / trenches, fragmentation of habitat, and disruption of commuting corridors. Future impacts would be of low significance and likely to occur.

### *Mitigation and Compensation*

5.5.2 In order to ensure that potential impacts to badgers and hedgehogs are avoided, the following Reasonable Avoidance Measures (RAMs) shall be incorporated into the construction phase as follows:

- All contractors should be given a toolbox talk to make them aware of the potential presence of these species in the area;
- All trenches and / or excavations should be covered overnight or have a broad and shallow ramp installed to prevent badgers or other mammals becoming trapped;
- Any exposed pipework greater than 200 mm diameter should be blocked to prevent badgers gaining entry;
- Any loose material stockpiled on site for an extended period should be fenced to prevent badger access, as they readily build setts in loose, easy-to-excavate material;
- Short-term stockpiles should be checked daily for any signs of digging and fenced if appropriate.

### *Residual Impacts*

5.5.3 Once mitigation measures are taken into account, the overall impact of the scheme will be **negligible**.

## 5.6 Breeding Birds

### *Potential Impacts*

5.6.1 In the absence of avoidance / mitigation, the development could result in the damage / destruction of a bird nest.

### *Mitigation and Compensation*

5.6.2 Any dense scrub, or trees scheduled for removal will be removed outside the nesting season (*season: March-August, although pigeons may nest all year*) or shall be checked prior to removal by a suitably qualified ecologist. The use of seed and fruit bearing shrub and tree species such as cherry, rowan, birch and crab apple should be selected within the scheme to provide a foraging resource for birds and invertebrates.

### *Residual Impacts*

5.6.3 The overall impact of the scheme will be **negligible**.

## 5.7 Invertebrates

### *Potential Impacts*

5.7.1 In the absence of mitigation, small areas of suitable habitat for common widespread invertebrates would be lost. Due to the common and widespread floral species and current levels of disturbance on site, the impacts would be of minor impact magnitude but certain to occur.

### *Mitigation and Compensation*

5.7.2 Opportunities within soft landscaping should be maximised for their suitability for UK wildlife including invertebrate species and a focus on providing new species rich scrub and grassland where possible.

### *Residual Impacts*

5.7.3 The overall impact of the scheme will be **negligible**.

## 6.0 CONCLUSIONS

- 6.1 The existing woodland to the south of the site and hedgerow with trees to the north are of moderate ecological value and the woodland and most of the hedgerow with trees will be retained and protected within the scheme.
- 6.2 America & Gratwicke's Wood LWS and Sullington Warren At 0.8km and 2.9km from the site (respectively), are the closest designated sites and are unlikely to be impacted by the development. Precautionary measures have been recommended for the construction phase. The site is within the Sussex North Water Supply Zone so water abstraction will have to be considered.
- 6.3 The boundary vegetation also offers some suitable low value habitat for reptiles, GCN, bats, common invertebrates and breeding birds. Habitats within the construction zone are broadly unsuitable for protected species and avoidance and mitigation measures have been built into the design to avoid potential, unlikely impacts of the scheme in accordance with the mitigation hierarchy and BS42020: 2013.
- 6.4 Opportunities for ecological enhancement have been provided to allow the ecological value to the site to be maximised. As a full planning application with no BNG exemptions, the development proposals shall be subject to the standard Biodiversity Gain Condition. A full Biodiversity Net Gain assessment of the site, and proposed habitat creation measures should accompany this application.

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**Table No. 13 – Species Lists for Habitat Parcels****Bare ground**

Common Name	Scientific Name	DAFOR
Bramble	<i>Rubus fruticosus</i>	<b>F</b>
Common nettle	<i>Urtica dioica</i>	<b>LO</b>
Cleavers	<i>Galium aparine</i>	<b>LO</b>
Dandelion	<i>Taraxacum officinale agg.</i>	<b>LO</b>
Moss species	<i>Bryophyta sp.</i>	<b>LO</b>
Stinking iris	<i>Iris foetidissima</i>	<b>LO</b>
Thistle species	<i>Cirsium sp</i>	<b>LO</b>
Yorkshire fog	<i>Holcus lanatus</i>	<b>LD</b>

**Mixed scrub**

Common Name	Scientific Name	DAFOR
Bramble	<i>Rubus fruticosus</i>	<b>O</b>
Cherry	<i>Prunus sp</i>	<b>R</b>
Elder	<i>Sambucus nigra</i>	<b>O</b>
Hawthorn	<i>Crataegus monogyna</i>	<b>F</b>
Holly	<i>Ilex aquifolium</i>	<b>O</b>

**Native hedgerow with trees**

Common Name	Scientific Name	DAFOR
Ash	<i>Fraxinus excelsior</i>	<b>F</b>
Bramble	<i>Rubus fruticosus</i>	<b>O</b>
Blackthorn	<i>Prunus spinosa</i>	<b>F</b>
Hawthorn	<i>Crataegus monogyna</i>	<b>F</b>
Sycamore	<i>Acer pseudoplatanus</i>	<b>O</b>
Willow	<i>Salix sp</i>	<b>R</b>

**Woodland**

Common Name	Scientific Name	DAFOR
Ash	<i>Fraxinus excelsior</i>	<b>F</b>
Dog's mercury	<i>Murcurialis perennis</i>	<b>LA</b>
Elder	<i>Sambucus nigra</i>	<b>O</b>
Hazel	<i>Corylus avellana</i>	<b>O</b>
Hawthorn	<i>Crataegus monogyna</i>	<b>O</b>
Holly	<i>Ilex aquifolium</i>	<b>O</b>
Ivy	<i>Hedera helix</i>	<b>LA</b>
Oak	<i>Quercus robur</i>	<b>D</b>

**D – Dominant; A – Abundant; F – Frequent; O – Occasional; R – Rare; L – Locally**

**APPENDIX A. – GCN eDNA results**

**Folio No:** 1070-2025  
**Purchase Order:** LLD 3503  
**Contact:** Lizard Landscape Design & Ecology  
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**Received Date:** 02.05.2025

# GCN Report

Technical Report

Folio No: 1070-2025  
Purchase Order: LLD 3503  
Contact: Lizard Landscape Design & Ecology  
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# 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 2341	Mousdell CI - P1	TQ 12693 16086	Pass	Pass	Negative	0/12
GCN25 2356	Mousdell CI - P2	TQ 12705 15889	Pass	Pass	Negative	0/12

Matters affecting result: none

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## 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

<b>Sample Integrity Check:</b>	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.
<b>Degradation Check:</b>	<b>Pass/Fail.</b> 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.
<b>Inhibition Check:</b>	<b>Pass/Fail.</b> 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.
<b>Result:</b>	<b>Presence of GCN eDNA (Positive/Negative/Inconclusive)</b> <b>Positive:</b> 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. <b>Positive Replicates:</b> 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. <b>Negative:</b> 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. <b>Inconclusive:</b> Controls indicate inhibition or degradation of the sample, resulting in the inability to provide conclusive evidence for GCN presence or absence.