



FIVE
ESTUARIES
OFFSHORE WIND FARM

FIVE ESTUARIES OFFSHORE
WIND FARM
ENVIRONMENTAL STATEMENT

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PRELIMINARY ENVIRONMENTAL
INFORMATION REPORT - HABITAT AND
HEDGEROW AND GREAT CRESTED
NEWT REPORTS

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- 1 Habitat and hedgerow survey report north of A120
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FIVE ESTUARIES OFFSHORE WIND FARM

Habitat & Hedgerow Survey: North of A120

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DEFINITION OF ABBREVIATIONS AND ACRONYMS

Term	Definition
ASNW	Ancient semi-natural woodland
CEnv	Chartered Environmentalist
CIEEM	Chartered Institute for Ecology and Environmental Management
EIA	Environmental Impact Assessment.
ES	Environmental Statement
DCO	Development Consent Order
GIS	Geographical Information System
GPS	Global Positioning System
GR	Grid Reference
HAP	Habitat Action Plans
NERC Act	Natural Environment and Rural Communities Act 2006
NSIP	Nationally Significant Infrastructure Project
PAWS	Plantation on Ancient Woodland Site
PEA	Preliminary Ecological Appraisal
VE OWFL	Five Estuaries Offshore Windfarm Limited.

1.0 Introduction

Five Estuaries Offshore Wind Farm (VE OWF) is a Nationally Significant Infrastructure Project (NSIP). An Environmental Impact Assessment (EIA) is being undertaken, the findings of which will be presented within an Environmental Statement (ES), which will accompany a Development Consent Order (DCO) application under the Planning Act 2008.

SLR Consulting was commissioned by GoBe Consultants, on behalf of Five Estuaries Offshore Wind Ltd, to undertake habitat survey and hedgerow assessment at the areas that may be affected by construction and operation of the onshore aspects of the VE OWF project comprising cable corridor and substation (hereafter referred to as “onshore infrastructure”), north of the A120¹. Initial identification and classification of areas of similar habitat (i.e., habitat polygons) had previously been undertaken primarily via interpretation of aerial imagery in 2021 and early 2022, as reported in the Preliminary Ecological Appraisal (PEA)². A recommendation of the PEA was that field survey of all areas within 100m of the onshore infrastructure options that were not previously accessible be undertaken, to be certain of habitat type and condition.

This report presents the findings of the habitat and hedgerow survey undertaken in summer and autumn 2022; the results supersede those reported in the PEA.

1.1 Survey area

The habitat survey area is shown on Drawing 1 and was initially undertaken to consider habitats within 100 m of the Red Line Boundary (RLB) that was under consideration at the time, for part of the scheme north of the A120.

1.2 Purpose of this report

This report presents the findings of the habitat survey and hedgerow assessment. The report seeks to establish baseline conditions and identify habitats that are important ecological features (irrespective of animal species they may support). The assessment of impacts resulting from VE is beyond the scope of this report however and will be covered in the Onshore Biodiversity and Nature Conservation chapters of the ES.

1.3 Evidence of technical competence and experience

This report has been authored by Jess Colebrook and Emily Drinkwater. Jess is a Principal Ecologist at SLR Consulting with over 20 years’ experience as a professional ecologist; she is a Chartered Environmentalist (CEnv) and a full member of CIEEM (MCIEEM). Jess is leading the onshore ecological work necessary to inform the EIA for the project, has been involved in the scoping and consultation process.

Emily Drinkwater and Ellen Miller undertook the habitat survey and condition assessment³. Emily is an Associate Ecologist and Ellen is a Senior Ecologist, both at SLR Consulting and Associate members of the Chartered Institute of Ecology and Environmental Management (CIEEM) (ACIEEM) and have over 10 years’ experience as

¹ Habitat and hedgerow survey for the remainder of the onshore infrastructure, and including the landfall area, has been undertaken by others and are detailed within separate reports.

² Five Estuaries Offshore Wind Farm: Preliminary Ecological Appraisal (Onshore), SLR Consulting, May 2022

³ Condition Assessment data collected in accordance with Defra Metric 3.0 is in the process of being processed and will be provided in an updated version of this report, to be submitted with the ES.

professional ecologists.

Hannah McBlain and Charlie Kempson undertook the hedgerow surveys. Both are skilled field ecologists with 3 years' experience of habitat, botanical and protected species survey. Both are working toward CIEEM membership and abide by its professional code of conduct.

Additional technical support and Quality Assurance review has been provided by Duncan Watson. Duncan is a Technical Director at SLR Consulting with over 23 years' professional ecological experience. He is also a Chartered Environmentalist (CEnv) and a full member of CIEEM (MCIEEM).

2.0 Methodology

2.1 Field survey

The field survey comprised three main elements:

- mapping of habitats - habitats were mapped using UKHab v1.1⁶, as agreed at the scoping stage, to capture the presence of Section 41 and Annex 1 habitat types. The presence of invasive non-native plant species was also recorded during the habitat survey;
- each polygon or line of habitat was subject to Condition Assessment in accordance with Defra Metric 3.1⁴; and
- sufficient detail was gathered to determine if hedgerows that could be breached by the proposed development meet the definition of “important” under the Hedgerow Regulations (1997).

2.1.1 Habitat survey

2021/2022 Initial mapping

Initial identification and classification of areas of similar habitat (i.e., habitat polygons) was undertaken primarily via interpretation of aerial imagery in 2021 and early 2022, as reported in the Preliminary Ecological Appraisal (PEA)⁵. Once more comprehensive access was gained to the survey area, each of the polygons identified from aerial interpretation was visited, and where necessary, remapped. Habitats at each were classified as follows using the UKHab Primary Habitat Hierarchy:

- Urban habitats were classified to Level 3 “Built up areas and gardens”, with the exception of buildings which were mapped to Level 5 “buildings”;
- Newly planted hedgerows were classified to Level 3 “Hedgerows” (h2), in conjunction with secondary code 56 “young trees – planted” to distinguish them from mature hedgerows, whereas hedgerows that were not accessible were also classified as h2 but without a secondary code;
- Ditches and ponds were classified to Level 3 “Standing open water and canals”, with the inclusion of the following secondary codes to denote their current conditions at the time of survey; 19 “Ponds (Priority Habitat)”, 39 “Freshwater – manmade”, 41 “Freshwater Natural”, 117 “Dry”, 162 “Temporary water bodies”, 191 “Ditch” and 362 “Artificial Lake or Pond”;
- All other habitats were classified to Level 4 (where applicable) including mandatory secondary habitat codes (numbers 10 – 41). Habitats were only further classified to Level 5 if they represented an Annex 1 habitat type;
- Each polygon or line of habitat was subject to Condition Assessment in accordance with Defra Metric 3.1; and

⁴ This data is being collated and will be appended to future iteration of this report.

⁵ Five Estuaries Offshore Wind Farm: Preliminary Ecological Appraisal (Onshore), SLR Consulting, May 2022.

- Boundary fences or walls were not mapped.

Additional secondary codes, photographs and notes were recorded for the majority of polygons; these have been retained in a Geographical Information System (GIS) and due to the amount of data are not presented in this report, they can however be supplied upon request. The most relevant/pertinent records are included here, in particular for habitats deemed to be important ecological features.

The field survey was undertaken by Emily Drinkwater ACIEEM and Ellen Miller ACIEEM over a period of 18 days in 2022; specific dates were as follows: .

- 24th May – 27th May 2022;
- 20th June – 24th June 2022;
- 27th June – 1st July 2022; and
- 11th – 14th July 2022.

2.1.2 Hedgerow assessment

During the habitat survey, summary notes were made about each hedgerow including the species present and the presence of trees, ditches, or walls. This information, along with the preliminary scheme design, was used to make a shortlist of hedgerows that could be breached by the onshore elements of VE, and which warranted more detailed survey, to determine if they were “important” under Schedule 1 Part II (6) of the Hedgerow Regulations 1997 (i.e., for wildlife and landscape reasons rather than archaeological or historical reasons).

For the purpose of the assessment a “hedgerow” is defined in Section 3(1) of the Hedgerow Regulations as follows:

any hedgerow growing in, or adjacent to, any common land, protected land, or land used for agriculture, forestry or the breeding or keeping of horses, ponies, or donkeys, if—

(a) it has a continuous length of, or exceeding, 20 metres; or

(b) it has a continuous length of less than 20 metres and, at each end, meets (whether by intersection or junction) another hedgerow.

“Important” hedgerows (for wildlife and landscape reasons) must be at least 30 years old and satisfy at least one of the criteria listed in Part II of Schedule 1 of the Hedgerow Regulations. To summarise, the criteria are a hedgerow that:

- Contains Schedule 1 birds, Schedule 5 animals or Schedule 8 plants species listed in the Wildlife and Countryside Act 1981 (as amended);
- Contains endangered, extinct, rare or vulnerable species, as listed in the British Red Data Books;
- Includes (on average, per 30m section) at least:
 - seven woody species (as defined within the Hedgerow Regulations 1997); or

- six woody species and three features in sub paragraph 4 (see below); or
- six woody species, including one of black-poplar *Populus nigra ssp betulifolia*, large-leaved lime *Tilia platyphyllos*, small-leaved lime *Tilia cordata* or wild service-tree *Sorbus torminalis*; or
- at least five woody species, and has associated with it at least four of the features specified in sub-paragraph 4 of Part II of Schedule 1 of the Hedgerow Regulations; or
- at least four woody species, at least two features in sub paragraph 4 and is adjacent to a bridleway or footpath, a road used as a public path, or a byway open to all traffic.

The features referred to in sub paragraph 4 of Part II of Schedule 1 of the Hedgerow Regulations are:

- a bank or wall which supports the hedgerow along at least one half of its length;
- gaps which in aggregate do not exceed 10% of the length of the hedgerow;
- where the length of the hedgerow does not exceed 50 m, at least one standard tree;
- where the length of the hedgerow exceeds 50 m but does not exceed 100 m, at least two standard trees;
- where the length of the hedgerow exceeds 100 m, such number of standard trees (within any part of its length) as would when averaged over its total length amount to at least one for each 50 m;
- at least three woodland species within one metre, in any direction, of the outermost edges of the hedgerow;
- a ditch along at least one half of the length of the hedgerow;
- connections scoring four points or more (a connection with another hedgerow scores one point and a connection with a pond or a woodland in which the majority of trees are broad-leaved trees scores two points); and
- a parallel hedge within 15 m of the hedgerow.

The shortlist for survey was based upon the minimum species requirements of the above; therefore, all hedgerows potentially breached by the onshore elements of VE, which were recorded as potentially supporting at least four woody species (in their entire length) were subject to further survey to gather the additional detail necessary for assessment. 29 hedgerows were subject to further survey and are identified on Figure 2.

The additional hedgerow survey was conducted on 17 and 18 August 2022 by Hannah McBlain and Charlie Kempson. The hedgerows requiring survey were marked on a plan in advance and divided into 30m sections. Global Positioning System (GPS) was used to determine the start and end of each section on the ground. For each section specific details were recorded on to a proforma to enable ease of reference against the regulations; if the hedge had less than four species (on average) within each 30 m section, or was less than 30 years old then no further details were recorded as it could not meet the threshold importance criteria.

2.1.3 Limitations

Surveys were all undertaken within the optimal period for habitat survey, but:

- it should be noted that some plant species may have been missed if not in evidence at the time of the survey due to their seasonal nature; and
- the surveys dates coincided with record breaking, prolonged periods of very hot and dry weather. This resulted in some vegetation suppression, wilting and dieback.

However, the above points are not considered to be a significant limitation as it was still possible to identify the majority of plants in order to assess the habitat types which were present.

Access permission was not granted for some areas of the survey area; therefore, these areas were not ground truthed and aerial mapping was used to identify the types of habitats present or likely to be present in these areas. The inaccessible areas constituted only a small proportion of the total survey area, unlikely to be of high conservation value based on aerial mapping interrogation and given the types of habitats recorded it is unlikely that high value habitats have been missed.

2.2 Determining Important Ecological Features

Ecological features can be important for a variety of reasons and the rationale used to identify them is explained below. Importance may relate, for example, to protected status, the quality or extent of the site or habitats therein; habitat and/ or species rarity; the extent to which such habitats and/or species are threatened throughout their range, or to their rate of decline.

Important habitats are considered here to be those which:

- match descriptions of habitats listed on Annex 1 of the Habitats Directive, so far as it applies to the UK and as transposed by The Conservation of Habitats and Species Regulations 2017 (as amended);
- match descriptions of habitats of principal importance for biodiversity under Section 41 of Natural Environment and Rural Communities (NERC) Act 2006;
- match Local Wildlife Site Selection Criteria⁶;
- match descriptions of habitats with Habitat Action Plans (HAPs) contained within Local Biodiversity Action Plans⁷;
- comprise irreplaceable habitats; such as (but not limited to) ancient woodland and veteran trees⁸; and/or
- comprise a significant habitat resource for an important species (see below).

⁶ <https://www.essexwtrrecords.org.uk/sites/default/files/LOCALWILDLIFESITESELECTIONCRITERIA2016.pdf>

⁷ Essex Biodiversity Action Plan "A Wild Future for Essex" (1999) does not appear to have been updated in recent years and can be found here <https://www.braintree.gov.uk/downloads/file/2436/e47-the-essex-biodiversity-action-plan-1999>

⁸ Referenced in Natural England Standing Advice for ancient woodland and veteran trees <https://www.gov.uk/guidance/ancient-woodland-ancient-trees-and-veteran-trees-advice-for-making-planning-decisions>)

3.0 Results

3.1 Habitats

Habitat types within the Survey Area are shown in Drawing 1 (Habitat Plan), with primary and mandatory secondary UKHab codes stated. Important habitats (as defined in Section 2.3) are shown in Drawing 2 (Important Habitats), including primary codes, mandatory secondary codes plus additional secondary codes as appropriate.

General descriptions for the various habitats encountered, including illustrative photographs, are provided below. More detailed descriptions are provided for specific areas of Priority Habitat, where there was potential for Annex 1 habitat, locally important or rare species to occur. Data for all habitat polygons, including (in almost all cases) photographs and dominant/characteristic species are stored in a GIS and can be made available upon request.

3.1.1 Cropland – arable and horticulture (c1)

The overwhelming majority of the area surveyed supports cropland comprising cereal crops such as wheat (as shown in Photograph 3.1), barley, maize and non-cereal crops; flax, oil-seed rape, potatoes, beetroot, onions, and peas.

Photograph 3.1: Cropland: Wheat fields south of Barns Lane, Lawford, Manningtree



Small rectangular strips or field corners planted with game bird cover (Photograph 3.2); maize interspersed with arable weeds, containing cereal feeders for pheasants and partridges and some pens, were regularly occurring throughout the survey area.

Photograph 3.2: Establishing game bird cover crop, south of the Ardleigh Road, Little Bromley



Some areas had been ploughed or cultivated. Other areas of bare ground exist within the cropland comprised of cross-field footpaths (Photograph 3.3), tracks (Photograph 3.4), and narrow strips surrounding the cropland allowing some common arable weeds and other herbs to establish in between. This included the following notable species (refer to Section 3.2 for further details);

- common cudweed *Filago vulgaris*;
- corn spurrey *Spergula arvensis* and
- sea holly *Eryngium maritimum*.

Additional species included:

- knotgrass sp.;
- black mustard *Brassica nigra*;
- redshank *Persicaria maculosa*;
- pale persicaria *Persicaria lapathifolia*;
- common fumitory *Fumaria officinalis*;
- wild radish *Raphanus raphanistrum* subsp. *Raphanistrum*;
- field bindweed *Convolvulus arvensis*;
- common field speedwell *Veronica persica*;

- scarlet pimpernel *Anagallis arvensis*;
- Shepherd's purse *Capsella bursa-pastoris*;
- small nettle *Urtica urens*;
- upright hedge parsley *Torilis japonica*;
- fat hen *Chenopodium album*;
- scentless mayweed *Tripleurospermum inodorum*;
- fool's-parsley *Aethusa cynapium*;
- sand spurrey *Spergularia rubra*;
- bird's-foot trefoil *Lotus corniculatus*;
- field madder *Sherardia arvensis*;
- toad rush *Juncus bufonius*;
- sun spurge *Euphorbia helioscopia* and
- mayweed.

Photograph 3.3: Sandy/gravel tracks with ephemeral vegetation, north of Manning Grove wood



Photograph 3.4: Cross -field footpath north of Manning Grove wood



Priority habitat Arable Field Margins sown with wild flowers or pollen and nectar mixes occur in a few locations throughout the survey area (Photograph 3.5, Photograph 3.6 and Photograph 3.7). These areas vary from having 80% herb and 20% grass cover, 50% herbs and 50% grass cover, areas which have recently begun to establish with bare ground and an area which includes a non-native seed mix. Species noted to occur in these areas include;

- chicory *Cichorium intybus* (considered notable, refer to Section 3.2 for details);
- oxeye daisy *Leucanthemum vulgare*;
- field madder *Sherardia arvensis*;
- hawkweed sp.;
- common poppy *Papaver rhoeas*;
- wild carrot *Daucus carota*;
- musk mallow *Malva moschata*;
- Yorkshire fog *Holcus lanatus*;
- red clover *Trifolium pratense*;
- curled dock *Rumex crispus*;
- self-heal *Prunella vulgaris*;
- meadow vetchling *Lathyrus pratensis*;
- tufted vetch *Vicia cracca*;
- sainfoin *Onobrychis viciifolia*;
- black medick *Medicago lupulina*;
- field bindweed;
- creeping thistle *Cirsium arvense*;
- scarlet pimpernel;
- creeping bent *Agrostis stolonifera*;
- crested dog's-tail *Cynosurus cristatus*;
- greater bird's-foot trefoil *Lotus pedunculatus*;
- bristly oxtongue *Helminthotheca echioides*;

- scentless mayweed;
- common knapweed *Centaurea nigra*;
- lady's bedstraw *Galium verum*;
- red fescue *Festuca rubra*;
- common hogweed *Heracleum sphondylium*;
- timothy grass *Phleum pratense*;
- white campion *Silene latifolia*;
- common nettle *Urtica dioica*;
- ribwort plantain *Plantago lanceolata*;
- perforate St john's wort *Hypericum perforatum*;
- rose sp.;
- rosebay willowherb *Chamaenerion angustifolium*;
- cut-leaved geranium *Geranium dissectum*;
- bird's-foot trefoil;
- hedge bedstraw *Galium mollugo*;
- cleavers *Galium aparine*;
- perennial ryegrass *Lolium perenne*;
- creeping buttercup *Ranunculus repens*;
- yarrow *Achillea millefolium*;
- rough meadow grass *Poa trivialis*;
- soft brome *Bromus hordeaceus*;
- cow parsley *Anthriscus sylvestris*;
- common bent *Agrostis capillaris*;
- hairy tare *Vicia hirsute*;
- cock's-foot *Dactylis glomerata*;
- common sorrel *Rumex acetosa*;
- wild oat-grass *Avena fatua*;
- slender meadow foxtail *Alopecurus myosuroides*;
- scented mayweed *Matricaria chamomilla*;
- lady's mantle sp.;
- perennial sow-thistle *Sonchus arvensis*;
- white clover *Trifolium repens*;
- common ragwort *Jacobaea vulgaris*;
- salsify *Tragopogon porrifolius*;
- creeping soft grass *Holcus mollis*;
- field horse tail *Equisetum arvense*;
- Shepherd's purse;
- wild mustard *Sinapis arvensis*;
- burdock *Arctium minus*;
- melilot *Melilotus officinalis*;
- fat hen;
- lucerne *Medicago sativa*;
- pineapple weed *Matricaria discoidea*;
- common nettle;
- red dead nettle *Lamium purpureum*;
- field speedwell;
- salad burnet *Sanguisorba minor*;
- greater plantain *Plantago major*;
- black horehound *Ballota nigra*;

- common fumitory *Fumaria officinalis*;
- mugwort *Artemisia vulgaris*;
- phacelia *Phacelia tanacetifolia*;
- broad-leaved willowherb *Epilobium montanum*;
- groundsel *Senecio vulgaris*;
- prickly sow-thistle *Sonchus asper*;
- field pansy *Viola arvensis*; and
- prickly lettuce *Lactuca serriola*.

Photograph 3.5: Flower forage abundant field within Bounds Farm east of Hungerdown Lane



Photograph 3.6: Flower forage abundant field west of Barlon Road, Little Bromley, Manningtree



Photograph 3.7: Herbaceous strip within cropland field west of Barlon Road, Little Bromley, Manningtree



3.1.2 Neutral grassland (g3) and modified grassland (g4)

Two areas of lowland meadow (g3a) were noted within the survey area providing nectar and pollen vital for bees, and food for other beneficial insects and birds. Most comprise of a diverse range of species within an abundance of finer grasses, herbs with fewer undesirable species. A comprehensive list was not gathered in both instances,

however, the most diverse of the two areas (Photograph 3.8) contained a notable population of pyramidal orchids *Anacamptis pyramidalis* (approximately 30 individuals within survey area) plus creeping softgrass, cock's-foot, yarrow, bristly oxtongue, creeping bent, Yorkshire fog, ribwort plantain, small amount of bramble *Rubus fruticosus* towards the roadside, red fescue, oxeye daisy, common centaury *Centaureum erythraea*, hawkweed sp., black medick, white clover, white campion, common ragwort, hairy tare, curled dock, fairy flax, common vetch *Vicia sativa*, soft brome, sweet vernal grass, common knapweed, common poppy, common mouse ear *Cerastium fontanum*, red fescue hybrid sp., lady's bedstraw, self-heal, Perforate St John's wort, crested dog's-tail, salsify, cow parsley, meadow buttercup *Ranunculus acris*, common sorrel, meadow fescue *Festuca pratensis*, sheep's fescue *Festuca ovina*, ribbed melilot, creeping thistle, willow seedlings *salix* sp., rough meadow grass, smooth meadow grass *Poa pratensis*. A skylark was recorded flying and landing within the meadow and numerous invertebrates were also observed, including ant hills.

Photograph 3.8: Pyramidal orchard at lowland meadow south of Barlon Road, Little Bromley, Manningtree



The second lowland meadow (Photograph 3.9) species included; fairy flax *Linum catharticum*, Yorkshire fog, false oat grass *Arrhenatherum elatius*, meadow fescue, red fescue, hawkweed sp., cock's-foot, black medick, salsify, common dandelion *Taraxacum officinale*, hairy tare, common vetch, tall fescue *Festuca arundinacea*, white clover, creeping thistle, common ragwort, common knapweed, common sorrel, oak *Quercus robur* seedlings, ribwort plantain, common hogweed, common mouse-ear *Cerastium fontanum*, sheep's sorrel, common centaury, creeping bent, perforate St John's wort, sweet vernal grass, perennial rye-grass, yarrow, broad-leaved dock and oxeye daisy. The field was noted to have been cut and bailed by surveyors carrying out bat surveys in August.

Photograph 3.9: Lowland meadow south of Norman's Farm



Neutral grassland (g3c) is the most numerous grassland habitat across the survey area, which occurs around fields edges, hedgerows, woodland, ditches and standing open water. It is predominantly tall and tussocky around field edges and mown at roadsides (Photograph 3.10).

Photograph 3.10: Tall sward and mown neutral grassland along the Grange Road



The majority of this grassland is relatively species poor, containing an abundance of competitive grass species such as false oat-grass and cock's-foot. One notable species was recorded - field scabious *Knautia arvensis* - plus false oat-grass, cock's-foot, perennial ryegrass, creeping bent, common bent, meadow foxtail *Alopecurus pratensis*, red fescue, rough meadow grass, couch *Elymus repens*, common nettle, creeping thistle, cow parsley, greater willowherb, burdock, common ragwort, bristly oxtongue, prickly lettuce, smooth sow thistle *Sonchus oleraceus*, hedge mustard *Alliaria petiolate*, soft brome, timothy, wild oat-grass, common hogweed, common mallow *Malva sylvestris*, red dead nettle, curled dock, nipplewort *Lapsana communis*, cleavers, white clover, yarrow, black horehound, mugwort, poppy, lemon balm *Melissa officinalis*, white campion, slender meadow foxtail, barren brome *Bromus sterilis*, fat hen, bramble, love in a mist *Nigella damascena*, wild radish, common sorrel, scentless mayweed, knotgrass sp., cut-leaved geranium, hedge woundwort *Stachys sylvatica*, hawkweed sp., rosebay willowherb, broad-leaved willowherb, meadowsweet *Filipendula ulmaria*, common knapweed, wall barley *Hordeum murinum*, comfrey sp., meadow vetchling, viper's bugloss *Echium vulgare*, dove's-foot cranesbill *Geranium molle*, annual meadow-grass *Poa annua*, , agrimony *Agrimonia eupatoria*, yellow toadflax *Linaria vulgaris*, rough hawkbit *Leontodon hispidus*, common foxglove *Digitalis purpurea* and borage *Borago officinalis*, Bracken occurs infrequently.

Some of the grassland was classified as Arrhenatherum neutral grassland (g3c5) where false oatgrass dominates (Photograph 3.11).

Photograph 3.11: Arrhenatherum neutral grassland to the north of Red House Farm, Little Bromley



Grassland also occurs within private gardens and churchyards (both mapped as u1), along tracks adjacent to arable fields where fertilisers are frequently used, around fishing lakes (Photograph 3.12) and newly seeded areas lacking in species diversity (mapped as g4).

Photograph 3.12: Mown modified grassland around angling lake west of Clacton Road B1035



This grassland is typically dominated by a few species and in this instance notably; perennial ryegrass and cock's-foot and Yorkshire fog with other species occurring sporadically with the main sward and more frequently at the margins; ribwort plantain, creeping bent, common nettle, white dead nettle *Lamium album*, rough meadow grass, pineapple weed, cut-leaved geranium, timothy-grass, smooth sow thistle, common hogweed, soft brome, barren brome, common dandelion, cleavers, greater plantain, common groundsel, creeping buttercup, white clover, cow parsley, wall barley, common fumitory, common mallow, annual meadow-grass, creeping thistle, black medick, wild oat-grass and occasional mosses.

The remaining modified grassland (g4) provides habitat and food for grazing livestock including sheep and horses (Photograph 3.13 and Photograph 3.14). These areas are species poor with palatable grasses and herbs including; ryegrass, timothy-grass, Yorkshire fog, cock's-foot, creeping bent, ribwort plantain, white clover, broad-leaved dock *Rumex obtusifolius*, crested dog's-tail *Cynosurus cristatus* and common dandelion.

Photograph 3.13: Modified grassland: sheep grazed to the west of St Mary's Church, Little Bromley,



Photograph 3.14: Modified grassland: horse paddock northwest of Bentley Road, Little Bromley



3.1.3 Hedgerows (h2) and scrub (h3)

Hedgerows are present across the Survey Area and commonly classified as Priority Habitat (h2a) (Photograph 3.15 and Photograph 3.16). Most are nevertheless species-poor, dominated by a mixture of hawthorn *Crataegus monogyna*, blackthorn *Prunus spinosa* and bramble, though even these hedgerows often included mature oak

trees *Quercus* spp. The most species-rich hedgerows also included species such as (but not limited to) wych elm *Ulmus glabra*, hazel *Corylus avellana*, sycamore *Acer pseudoplatanus*, holly *Ilex aquifolium*, rose *Rosa* sp. bird cherry *Prunus padus* and ash *Fraxinus excelsior*. Further information in respect of the most species-rich hedges which may be breached by the scheme is included in Section 4.

Photograph 3.15: Less intensively managed hedgerow, North of Little Bromley Road



Photograph 3.16: Managed hedgerow along Grange Road



Other hedgerows (h2b) include non-native and ornamental hedgerows (i.e., *leylandii*, cherry laurel *Prunus laurocerasus*) (Photograph 3.17) as well as newly planted hedgerows; hedging whips planted in two rows (Photograph 3.18). The newly planted hedgerows comprise of a mixture of; blackthorn, hawthorn, hazel, dogwood *Cornus sanguinea*, field maple *Acer campestre* and oak.

Photograph 3.17: Ornamental hedgerow West of Payne’s Lane



Photograph 3.18: Newly planted hedgerow along Grange Road



The majority of the hedgerows are managed on the roadsides leaving the field side less intensively managed. Many field boundaries were also noted to retain remnant sections of hedge, which due to their sparsity could no longer be technically termed hedgerow.

Scrub habitat (h3) occurs in patches across the survey area and most commonly comprises a single species stands or mixtures of brambles, blackthorn and hawthorn with occasional hazel, elder *Sambucus nigra*, rose sp., field maple, holly, elm *Ulmus* sp., and willow *Salix* sp. Some of the above noted sections of remnant hedge have been classified as scrub.

3.1.4 Standing open water (r1)

Lakes, ponds and ditches are present across the area surveyed. It is worth highlighting here that all waterbodies have been mapped as r1, those considered to meet the pond Priority Habitat definition have the additional secondary code 19.

The majority of waterbodies appeared to be of man-made origin, or have been modified, based upon the regular shape of the waterbody and the landscape context (i.e., garden pond, angling lakes, agricultural reservoirs and ditches).

Nine ponds or lakes occur either entirely or partially within the survey area and these were identified by a combination of aerial mapping and ground truthing. These comprise of three semi-natural ponds (two are shown on Photograph 3.19 and Photograph 3.20) and six man made ponds and lakes (Photograph 3.21, Photograph 3.22 and Photograph 3.23). One of the manmade ponds was within a private garden and inaccessible, but from aerial mapping it is shown as situated within a well-maintained garden setting.

Photograph 3.19 Woodland pond south of Moorhouse Farms Ltd, Little Bromley Road



Photograph 3.20: Hedgerow pond adjacent to Mulberry Lane, Manningtree



Photograph 3.21: Newly created pond adjacent to the Barlon Road, Little Bromley, Manningtree



Photograph 3.22: Recently created lake to the west of Clacton Road B1035



Photograph 3.23: Angling lake to the west of Clacton Road B1035



The types of vegetation present varies greatly between ponds. Two of the semi-natural ponds (Photograph 3.19 and Photograph 3.20) are heavily shaded by woodland and mature hedgerow and therefore aquatic vegetation and marginal vegetation has been suppressed as few wetland plants tolerate shade, however, they did contain leaf litter, fallen dead wood and tree roots providing food and shelter for invertebrates. The woodland pond (Photograph 3.19) was completely covered with duckweed *Lemna* sp. Of the semi-natural ponds this is the only

one that has partial engineering works along the side closest to the road, with a solid retaining wall with hessian bags filled with wet concrete and pinned together. The third semi-natural pond is shaded by trees and scrub over one half and contains marginal vegetation of common bullrush *Typha latifolia* and branched bur reed *Sparganium erectum* but with no aquatic vegetation present. All three semi-natural ponds have connections with drainage ditches.

The newly created pond (Photograph 3.20) is lined, connected to a pump, is highly turbid and currently contains no submergent or emergent aquatic vegetation. It is completely open with no trees or scrub with establishing neutral grassland around the perimeter.

Four lakes; i.e., bodies of water bigger than 1 acre (4,000 m²) are also present and are likely to be used for angling. Two of the lakes were stocked with large carp and it likely that the other lakes are also artificially stocked with fish. Riparian and marginal vegetation around the lakes include; scattered grasses, scattered rushes, reeds *Phragmites australis* and scattered trees including willow *Salix sp.* Filamentous algae occurred in one of the lakes. One of the lakes (Photograph 3.22) had been recently created, connected to a pump and aquatic and marginal vegetation has yet to establish.

Whilst the survey area includes a network of arable drainage ditches, the vast majority held no water and were dominated by neutral grassland, denoting that they had been dry for a long period of time, and were therefore mapped as such (Photograph 3.24).

Photograph 3.24: Typical arable drainage ditch habitat, near Mulley's wood



Few ditches in the survey area contained water and where they did, this wasn't along the entire ditch, in short sections or sporadic pooling. Some of the roadside drainage ditches were culverted, no other artificial pipework was observed elsewhere. Ditches which had indicators for holding water, albeit containing water normally for less than four months of the year; i.e., bare ground at the base and presence of riparian species such as watercress sp., hemlock and greater willowherb, were mapped as temporary waterbodies (Photograph 3.25).

Photograph 3.25: Ditch containing water culverted at either end, along the Ardleigh Road



3.1.5 Rivers, streams (r2b)

One watercourse with obviously flowing water along its length was recorded (Photograph 3.26), this is Holland Brook which is fed by ditches starting near the junction between Shop Road and Bentley Road, and just north of Welham's Farm. Whilst these ditches started dry, closer to the A120 Holland Brook contained flowing water of approximately 0.2-0.3m depth. The bed material was silt and little to no aquatic vegetation was contained within the stream flow itself; typical bank face vegetation comprised common nettle, greater willowherb, bramble and hemlock, similar in structure to seasonally dry ditches recorded onsite, as well as scattered mature willow trees in the southerly portion.

Photograph 3.26:Section of Holland Brook near the A120



3.1.6 Reedbeds (f2e) and aquatic marginal vegetation (f2d)

The largest and therefore mappable stand of reedbed is located along the banks of an angling lake to the north of Welham's Farm, Little Bentley (Photograph 3.27). Less extensive stands of reeds and other aquatic marginal habitat occur along the ditch network; however, these were not mapped due to being smaller than the minimum mapping units. Species included; great willowherb, meadowsweet, water-cress *Rorippa nasturtium-aquaticum*, water mint *Mentha aquatica*, tansy *Tanacetum vulgare*, broad-leaved dock, common figwort *Scrophularia nodosa*, hemlock *Conium maculatum*, couch, hard rush *Juncus inflexus*, soft rush *Juncus effusus*, common hogweed, marsh bedstraw *Galium palustre*, branched bur reed and bullrush.

No evidence of hog's fennel *Peucedanum officinale* was encountered during the surveys.

Photograph 3.27: Reed bed along the bank of an angling lake to the north of Welham's Farm, Little Bentley



3.1.7 Urban (u1)

Urban areas within the Survey Area include (but are not limited to) agricultural buildings, plant nursery/horticulture, an electricity substation, church, and small settlements. These constitute a very small proportion of the surveyed area and were not subject to detailed survey.

Although detailed inspections of buildings were outside the scope of the surveys, the following ad hoc notes were recorded.

- The door to a small agricultural building (Photograph 3.28) to the south of the Ardleigh Road, Little Bromley at Grid Reference: TM 07985 28395, was open during the survey, and a brief look inside found evidence of a small number of old bat droppings on the surfaces inside and a couple of mud nests (likely swallow *Hirundo rustica*) at the apex of the roof.
- A colony of honey bees was recorded swarming around a cavity on the northeast aspect stone wall of St Mary's church at Grid Reference: TM 09163 27828 (Photograph 3.29).

Photograph 3.28: Small agricultural building, south of the Ardleigh Road, Little Bromley



Photograph 3.29: Honey bee colony in stone wall cavity in St Mary's Church, Little Bromley



3.1.8 Woodland and forest, including lines of trees (w1)

Woodland is relatively scarce within the survey area, and the majority is plantation or secondary woodland. Three stands of lowland mixed deciduous woodland (w1f, a Priority Habitat) are present. The main woodland areas are described below in Table 3.1 and identified on the habitat plan.

Table 3.1
Main woodlands within Survey Area

Woodland Name and Location	Description
<p>Unnamed woodland to the west of Hungerdown Lane.</p> <p>Grid Reference (GR): TM 07131 29226</p>	<p>Broadleaved mixed and yew woodland, plantation (w1 36)</p> <p>Not accessible. From aerial mapping going back to 2000 tree planted in rows can be clearly seen, interspersed with a few older trees.</p>
<p>Unnamed woodland to the north of Moorhouse Farms Ltd.</p> <p>GR: TM 07189 29089</p>	<p>Other woodland, mixed, plantation (w1h6 36)</p> <p>A narrow strip of planted woodland in rows containing, conifers (mostly), cypress sp., conifer sp., silver birch <i>Betula pendula</i>, field maple, hazel, ash, cherry, poplar sp. Conifers make up most of the tree cover. Trees are young - semi-mature with diameters at breast height (DBH) between 10-45cm. Understorey comprises predominantly bare ground with a few scattered grasses and bramble. Pheasant pen present.</p>
<p>Unnamed woodland to the north of Moorhouse Farms Ltd</p> <p>GR: TM 07163 29055</p>	<p>Other woodland, mainly broadleaved, secondary (w1h5 38)</p> <p>This area appears to have once been part of a landscaped garden, bordered by mature trees with a few individual trees in the centre. Broad-leaved trees make up most of the tree cover. Species include; hazel, sycamore, cypress sp., horse chestnut <i>Aesculus hippocastanum</i>, pine/conifer sp., silver birch, sweet chestnut <i>Castanea sativa</i>, oak, yew <i>Taxus baccata</i>, goat willow <i>Salix caprea</i>, gorse <i>Ulex europaeus</i>, elm sp., blackthorn, bramble, and hawthorn. Dead wood is present on the ground. The clearings comprise of grasses and scrub. Species include; Yorkshire fog, rough meadow-grass, perennial rye-grass, false oat-grass, Perforate St John's wort, white dead nettle, common nettle, common bent, common ragwort, toadflax, white campion, garlic mustard <i>Alliaria petiolata</i> and bramble.</p>
<p>Unnamed woodland to the west of Moorshour Farms Ltd.</p> <p>GR: TM 07106 29058</p>	<p>Lowland mixed deciduous woodland, secondary (w1f7 38)</p> <p>Small triangular block of woodland containing mature oak, holm oak <i>Quercus ilex</i>, with young sycamore, holly, and rose sp. The understorey is predominantly bare ground with occasional grasses and herbs; Yorkshire fog, cock's-foot, honeysuckle, cleavers, ivy and bramble. Connected to a hedgerow on either side with a dry ditch running through it closest to the roadside.</p>
<p>Unnamed woodland to the south of Moorhouse Farms Ltd</p>	<p>Other woodland, mixed, mainly broadleaved, secondary (w1h5 38)</p> <p>The woodland contains mature oaks >1m DBH, horse chestnut,</p>

Woodland Name and Location	Description
GR: TM 07238 28828	standing deadwood, sycamore, elder, ash, cypress sp., silver birch, holly, hazel, sweet chestnut, lime, willow sp., some standing dead wood and a few piles of brash and compost heaps presumably taken in from the footpath off the main road and discarded there. The understorey and ground flora included; bramble, elder, tree seedlings, garlic mustard, broad-leaved willowherb, common nettles, hedge woundwort, ivy and non-native small balsam. Broad-leaved trees make up most of the tree cover. A dry ditch runs through the woodland and connects to a pond in the northwest corner.
Unnamed woodland to the south of Moorhouse Farms Ltd. GR: TM 07285 28876	Other woodland, mixed, mainly broadleaved, plantation (w1h5 36) Planted woodland in rows with an older line of trees along the western side, which appears to have been a hedgerow before the woodland was planted. Based on aerial images trees were planted post 2000 and species include; ash, oak, sycamore, sweet chestnut, spruce sp., blackthorn, silver birch, hazel. Broad-leaved trees make up most of the tree cover. The understorey includes; bramble, tree seedlings, ivy, bare ground, cow parsley, common ragwort, common nettle, cleavers, rose sp. and occasional grasses including; cock's-foot, false oat-grass and Yorkshire fog.
Unnamed woodland to the west of Hungerdown Lane GR: TM 07542 29374	Other woodland, mixed, mainly broadleaved, plantation (w1h5 36) and other lowland mixed deciduous woodland, coppice (w1f7 51) This comprises of a series of adjoining planted woodland strips including a small section of planted hazel coppice at one end. Based on aerial images they were planted up post 2000 and before that there were only a few lines of poplar sp. trees and hedgerow. Species include; cherry, silver birch, cypress/pine sp., oak, ash, rose sp., blackthorn, bramble, field maple, dog rose <i>Rosa canina</i> , hawthorn, hazel, blackthorn, sycamore, horse chestnut, oak, poplar sp., Prunus sp., and ash. Most of the trees are between 15-25cm DBH, with fewer trees 30-40 DBH. The understorey is sparse, and the ground flora is mainly grassy tracks. Species include; cow parsley, common nettle, red fescue, cock's-foot, rough meadow-grass, false oat-grass, and common ragwort. Very few seedlings/saplings. Broad-leaved trees make up most of the tree cover.
Unnamed woodland south of the Ardleigh Road GR: TM 08231 28135	Other woodland, mixed, mainly broadleaved, secondary (w1h5 38) Very small triangular area of woodland which was previously two parallel hedgerows and trees have self-set in the middle creating a small woodland. Species include mature oak, silver birch, blackthorn, elder, conifer sp., hazel, and bramble. The ground flora includes cock's-foot, false oat-grass, Yorkshire fog, common nettles, and

Woodland Name and Location	Description
	bracken. Broad-leaved trees make up most of the tree cover.
Unnamed woodland adjacent to the Grange Road GR: TM 08260 29325	Other lowland mixed deciduous woodland, secondary (w1f7 38) Small area of woodland with trees only just reaching the 5m height criteria. Species include; cheery, elm, hawthorn and elder. The understorey and ground flora include; bramble, rough meadow-grass, cock's-foot, Yorkshire fog, common nettle, cow parsley and grape hyacinth <i>Muscari</i> sp. Woodland present on aerial maps in 2000, unknown beforehand.
Unnamed woodland adjacent to Church Road GR: TM 09445 27807	Other woodland, mixed, mainly broadleaved, secondary (w1h5 38) Very small block of woodland. There was no access, but it was small enough to view from the outside. Species include; ash, conifer sp., oak, prunus sp., hawthorn, lime sp., sycamore, horse chestnut, sweet chestnut seedling and bramble. Tree ages range from 10-50cm DBH. Broad-leaved trees make up most of the tree cover.
Unnamed woodland east of the Bentley Road GR: TM 10935 27749	Other woodland; broadleaved, young trees self-set (w1g7 57) Small area of woodland along an arable field edge with young trees self -set. Species include; hawthorn, field maple, ash, and cherry laurel. Ground flora includes grasses and a few herbs including foxglove. The area is used for game rearing.
Unnamed woodland east of the Bentley Road GR: TM 11129 27631	Other woodland; broadleaved, secondary (w1g7 38) Small area of woodland along the northern boundary of a semi-natural pond and on an arable field edge. Species include goat willow in the pond with ash and oak on the northern boundary which resembles a hedgerow. To the east is a section of blackthorn.
Unnamed woodland north of the A120 at an intersection of the Holland Brook. GR: TM 11728 27349	Other woodland; broadleaved, secondary (w1g7 38) Small area of woodland in between ditch (north) & hedgerow. Species include; hawthorn, elm, oak and elder with bramble understorey.
Unnamed woodland adjacent to a tributary of the Holland Brook. GR: TM 11106 27429	Other woodland; broadleaved, secondary (w1g7 38) Small group of trees with elder, bramble, holly, field maple, ash, and oak with a common nettle understorey. Possibly a result of two merging hedgerows.

Woodland Name and Location	Description
Mulley's Wood GR: TM 11025 28358	Other woodland; broadleaved, plantation (w1g7 36) Mulley's Wood consists primarily of planted mature hybrid poplar trees in the centre, surrounded by a diversity of trees in the perimeter including mature pedunculate oak, ash, holly, willow, and alder. The understorey was predominantly comprised of common nettle and bramble, with occasional laurel bushes also present. A large pheasant pen is located in the centre.
Unnamed woodland to the east of Mulley's Wood GR: TM 11283 28353	Other woodland; broadleaved, plantation (w1g7 36) This area consists of small blocks of planted semi-mature aspen <i>Populus tremula</i> and ash with obvious planting lines. The understorey comprised predominantly tall ruderal species such as common nettle and cow parsley.

Lines of trees occur occasionally across the survey area and include two lines of poplar *Populus* sp. (Photograph 3.30) and a line of young trees with; ash, oak and field maple (Photograph 3.31). Semi-natural lines of trees are however the most numerous and are frequently associated with remnant hedgerow where the hedgerows have become damaged or removed leaving behind mature trees; predominantly oak (Photograph 3.32). Other species recorded include; mature hawthorns, holly, ash, field maple and willow *salix* sp. There is also a short section of three mature hazel trees which qualified as it was at least 20 m in length. All lines of trees are indicated on the habitat plan at Drawing 1.

Photograph 3.30: Line of mature poplar trees *Populus sp.*, East of Hungerdown Lane



Photograph 3.31: Recently planted line of trees, North of Grange Road



Photograph 3.32: Line of mature oak and ash trees, South of Mulley’s Wood



3.2 Species

3.2.1 Notable species

Whilst detailed surveys for specific species have not been undertaken (though special attention was made to seek hogs fennel), a small range of notable plant species has been recorded within the Survey Area during the field survey. The species are mainly associated with lowland meadows and arable field margins habitats; specific records are shown in Table 3.2, but should not be considered exhaustive.

Table 3.2
Notable plant species

Species	Habitat	Location Coordinates	Status
Common cudweed	Cropland	TM 08229 27659	GB Red listing based on 2001 IUCN guidelines (from 2018): Near Threatened, England Red listing based on 2001 IUCN guidelines (from 2014): Near Threatened
Corn spurrey	Cropland	TM 07983 29070	GB Red listing based on 2001 IUCN guidelines (from 2018): Vulnerable, England Red listing based on 2001 IUCN guidelines (from 2014): Vulnerable

Species	Habitat	Location Coordinates	Status
Sea holly	Cropland	TM 07888 27225	England Red listing based on 2001 IUCN guidelines (from 2014): Near Threatened Essex Red List
Chicory	Cropland	TM 08528 27626	England Red listing based on 2001 IUCN guidelines (from 2014): Vulnerable
Field scabious	Neutral grassland	TM 08199 30155	England Red listing based on 2001 IUCN guidelines (from 2014): Near Threatened
Pyramidal orchid	Lowland meadow	TM 08910 27487	Essex Red List

3.2.2 Invasive Non-native Species

Occasional neophytes and garden escapes were recorded throughout the survey, including:

- Snowberry *Symphoricarpos albus* within a hedgerow at TM 09460 27804 adjacent to Church Road;
- Small balsam *Impatiens parviflora* within woodland south of Moorhouse Farms Ltd at TM 07250 28815; and
- An ornamental St John's wort *Hypericum sp.* shrub was recorded within scrub adjacent to the Barlon Road at TM 08777 27420.

Two invasive species listed on Schedule 9 of the Wildlife and Countryside Act 1981 (as amended) were recorded:

- Water fern *Azolla filiculoides* within one of the ditches to the south of Ardleigh Road with the highest concentration at TM 08268 28225; and
- A species of rhododendron *Rhododendron sp* (not considered to be *R. ponticum*, but potentially a hybrid) at TM 09485 27729 along the vegetated garden perimeter of the Old Rectory, Church Road.

No other non-native invasive species were recorded during the habitat survey, although it is possible that additional species may be present in areas which were not accessible during the survey or died-off due to the prolonged periods of hot and dry weather.

4.0 Hedgerow assessment

None of the assessed hedgerows were found to be “Important” on wildlife and landscape ground (irrespective of animal species they may support⁹). Further details are provided below.

A total of 29 hedgerows were subject to further assessment initially comprising an estimation of age and species present within 30 m as detailed in Table 4.1, with locations shown on Drawing 3. Only one hedgerow warranted detailed assessment, the results of which are shown in Table 4.2.

Table 4.1
Hedgerows subject to assessment

Survey Date	Hedgerow Number on Plan	At least 30 years old?	4 woody species present in 30m	Detailed assessment required?
2022-08-18	417	No	No	No
2022-08-18	418	No	No	No
2022-08-18	415	No	Yes	No
2022-08-18	262	Yes	No	No
2022-08-18	286	Yes	No	No
2022-08-18	410	No	No	No
2022-08-18	411	No	No	No
2022-08-18	412	No	No	No
2022-08-18	409	No	No	No
2022-08-18	413	No	No	No
2022-08-18	414	Yes	Yes	Yes
2022-08-18	298	Yes	No	No
2022-08-18	408	Yes	No	No
2022-08-18	299	No	No	No
2022-08-18	416	Yes	No	No
2022-08-18	297	No	No	No
2022-08-18	302	Yes	No	No
2022-08-18	303	No	No	No
2022-08-18	304	No	No	No

⁹ A review of the findings of ongoing protected species field surveys will be undertaken once they are completed, so as to identify any additional hedgerows that are of known value to protected species, and which may therefore qualify as “Important” under that criterion. If necessary, this report will be updated accordingly.

Survey Date	Hedgerow Number on Plan	At least 30 years old?	4 woody species present in 30m	Detailed assessment required?
2022-08-18	416	Yes	No	No
2022-08-17	335	Yes	No	No
2022-08-17	336	Yes	No	No
2022-08-17	374	Yes	No	No
2022-08-17	339	Yes	No	No
2022-08-17	355	Yes	No	No
2022-08-17	379	Yes	No	No
2022-08-17	384	Yes	No	No
2022-08-17	359	Yes	No	No
2022-08-17	350	Yes	No	No

Table 4.2
Hedgerow 414 Detailed Assessment Results

Hedgerow feature		Survey Result
Dimensions	Height (m)	10
	Width (m)	2
	Length (m)	150
	Number of 30m sections	5
Features Present	Bank or wall present?	No
	% length that comprises a gap	10
	Number of trees in total length	6
	Ditch along at least one half present?	Yes
	Public Right of Way adjacent?	No
	Parallel hedge within 15m?	Yes
Schedule 3 species average per 30m section	Section 1	Beech, blackthorn, pedunculate oak
	Section 2	Ash, beech, blackthorn, field maple, pedunculate oak
	Section 3	Beech, blackthorn, field maple, pedunculate oak
	Section 4	Beech, blackthorn, field maple, pedunculate oak

Hedgerow feature		Survey Result
	Section 5	Beech, blackthorn, field maple, pedunculate oak
Schedule 2 species present in entire length of hedgerow		None
Number of connections		3
Signs of notable fauna		None

To summarise, hedgerows 414 supports an average of four Schedule 3 species per 30 m section and four features as follows:

- Gaps which aggregate do not exceed 10% of length of hedgerow;
- Where the length of the hedgerow exceeds 100m, such number of standard trees (within any part of its length) as would when averaged over its total length amount at least one for each 50 m;
- A ditch along at least one half of the length of the hedgerow; and
- A parallel hedge within 15 m of the hedgerow.

It therefore does not qualify as an “important” under the Hedgerow Regulations 1997.

5.0 Important ecological features

5.1.1 Habitats

Whilst the majority of the Survey Area comprises agricultural crop land used for growing cereal and which is of limited ecological importance, the following habitats of Principal Importance (i.e., those included under Section 41 of Natural Environment and Rural Communities (NERC) Act 2006) are confirmed to be present and are shown on Drawing 2. In all cases the reference definition for each habitat type has been taken from UK Biodiversity Action Plan Priority Habitat Descriptions¹⁰.

- None of the woodland within the Survey Area is listed as ancient woodland (ASNW and/or PAWS), in the Ancient Woodland Inventory. Three large stands (described in Table 3.1) have been identified that are considered to meet the definition of lowland mixed deciduous woodland.
- Hedgerows (h2a) – most of the hedgerows within the Survey Area meet the S41 definition, which states:

“A hedgerow is defined as any boundary line of trees or shrubs over 20m long and less than 5m wide, and where any gaps between the trees or shrub species are less than 20m wide. Any bank, wall, ditch or tree within 2m of the centre of the hedgerow is considered to be part of the hedgerow habitat, as is the herbaceous vegetation within 2m of the centre of the hedgerow. All hedgerows consisting predominantly (i.e. 80% or more cover) of at least one woody UK native species are covered by this priority habitat, where each UK country can define the list of woody species native to their respective country”.

None of the hedgerows were assessed as “important” according to the ecological requirements of the Hedgerow Regulations 1997.

- Lowland Meadows (g3a) – Two species-rich grassland habitats were identified during the UK Habitat Survey that meet the S41 definition⁷. The definition for this habitat type is:

“A wide-ranging approach is adopted in this plan to lowland grasslands treated as lowland meadows. They are taken to include most forms of unimproved neutral grassland across the enclosed lowland landscapes of the UK. In terms of National Vegetation Classification plant communities, they primarily embrace each type of Cynosurus cristatus - Centaurea nigra grassland, Alopecurus pratensis - Sanguisorba officinalis floodplain meadow and Cynosurus cristatus - Caltha palustris flood-pasture. The plan is not restricted to grasslands cut for hay, but also takes into account unimproved neutral pastures where livestock grazing is the main land use. On many farms in different parts of the UK, use of particular fields for grazing pasture and hay cropping changes over time, but the characteristic plant community may persist with subtle changes in floristic composition”.

- Reedbeds (f2e) – one small reedbed on the banks of an angling lake to the north of Welham’s Farm, Little Bentley, as well as in limited locations within the ditch/drain network elsewhere and are considered to meet the S41 description:

“Reedbeds are wetlands dominated by stands of the common reed Phragmites australis, wherein the water table is at or above ground level for most of the year. They tend to incorporate areas of open water

¹⁰ Section 41 Habitat definitions align with the UK Biodiversity Action Plan Priority Habitat Descriptions published in 2008 and updated in 2011, available at <https://data.jncc.gov.uk/data/2728792c-c8c6-4b8c-9ccd-a908cb0f1432/UKBAP-PriorityHabitatDescriptions-Rev-2011.pdf>

and ditches, and small areas of wet grassland and carr woodland may be associated with them.”

- Arable field margins (c1a6 and c1a8) – This habitat was identified at several locations during the habitat survey; some may have been managed to specifically provide benefit to wildlife¹¹Error! Bookmark not defined.. The definition for this S41 habitat type is:

“Arable field margins are herbaceous strips or blocks around arable fields that are managed specifically to provide benefits for wildlife. The arable field must be in a crop rotation which includes an arable crop, even if in certain years the field is in temporary grass, set-aside or fallow. Arable field margins are usually sited on the outer 2-12m margin of the arable field, although when planted as blocks they occasionally extend further into the field centre.”

Given the above definition, it is possible this habitat may be lost from its current locations, and/or be found in new locations in future, depending on agricultural management practice.

- Rivers (r2b) – The Holland Brook and its tributaries within the site are considered likely to meet the definition by virtue of supporting other protected and/or Section 41 species such as water vole rather than for habitat type/quality *per se*. Remaining water courses within the survey area are not considered to meet the definition, but if S41 41 or protected species are later found to use them then this report will be updated accordingly; and
- Ponds (UKHab secondary code 19); Ponds, for the purpose of priority habitat classification, ponds are defined as permanent and seasonal standing water bodies up to 2 ha in extent which meet one or more criteria pertaining to notable or protected species. Most ponds in the area are likely to meet the Section 7 definition by supporting Section 41 or Red Data Book species; all ponds are shown on Figure 3 and are concluded to be important ecological features.

None of the habitats are part of, or assist towards, Natural England Habitat Network Areas¹¹.

5.1.2 Plant species

A range of notable plant species has been recorded within the Survey Area during the field survey; the species are mainly associated with two areas of lowland meadow and several cropland margins, which are already identified as important ecological features. The species are:

- Common cudweed;
- Corn spurrey;
- Sea holly;

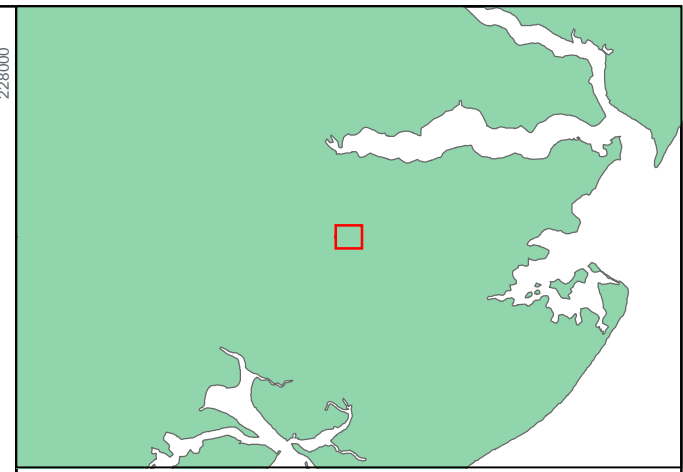
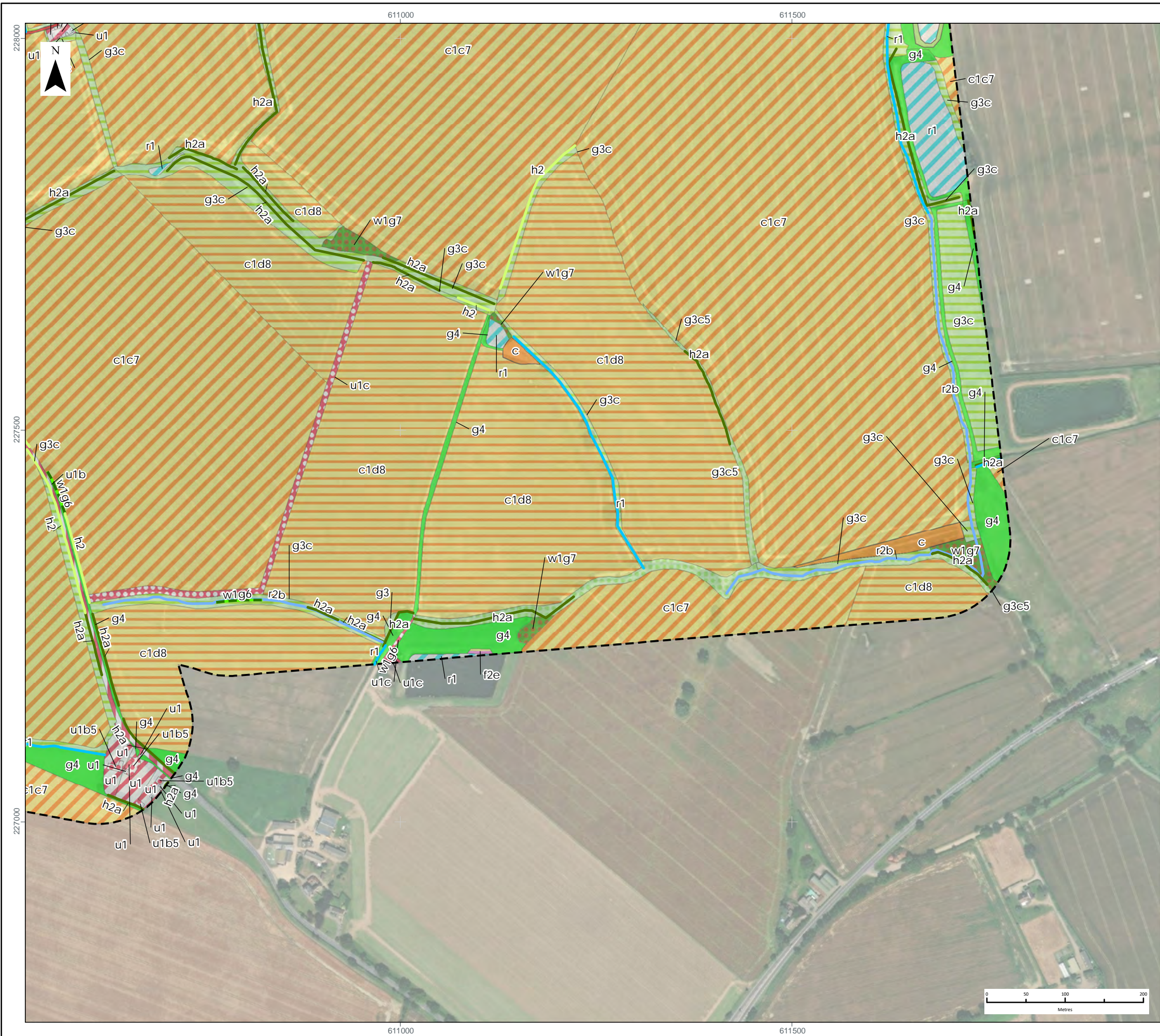
¹¹ Habitat Networks (England) is a spatial dataset that describes the geographic extent and location of Habitat Networks for 18 priority habitats based primarily, but not exclusively, on the priority habitat inventory with additional data added in relation to habitat restoration-creation, restorable habitat, plus fragmentation action, and network enhancement and expansion zones. The Habitat Network Maps provide spatial guidance to plan and develop local ecological networks and may be used to help target action to build greater ecological resilience for habitats across England.

- Chicory;
- Field scabious; and
- Pyramidal orchid.

Two invasive non-native species listed on Schedule 9 were recorded; water fern was recorded in one of the ditches to the south of Ardleigh Road and a species of Rhododendron at a vegetated garden perimeter of the Old Rectory, Church Road. Other invasive species, neophytes or garden escapes not listed on Schedule 9 have also been recorded at a limited number of locations within the survey area, but in no locations were found to dominate.

FIGURE 1

Habitat Plan



LEGEND

Habitat Survey Area (2022)
 UKHab Classification: Linear Features

- h2 - Hedgerow
- h2a - Hedgerow (Priority Habitat)
- r1 - Standing Open Water and Canals
- r2b - Other River and Stream
- w1g6 - Line of Trees

UKHab Classification: Area Features

- c - Cropland
- c1c7 - Other Cereal Crops
- c1d8 - Other Non-Cereal Crops
- f2e - Reedbeds
- g - Grassland
- g3 - Neutral Grassland
- g3c - Other Neutral Grassland
- g3c5 - Arrhenatherum Neutral Grassland
- g4 - Modified Grassland
- r1 - Standing Open Water and Canals

UKHab Classification: Linear Features

- u1 - Built-up Areas and Gardens
- u1b - Developed Land, Sealed Surface
- u1b5 - Buildings
- u1c - Artificial Unvegetated Unsealed Surface
- u1e - Built Linear Features
- w1g7 - Other Broadleaved Woodland Types

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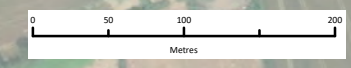
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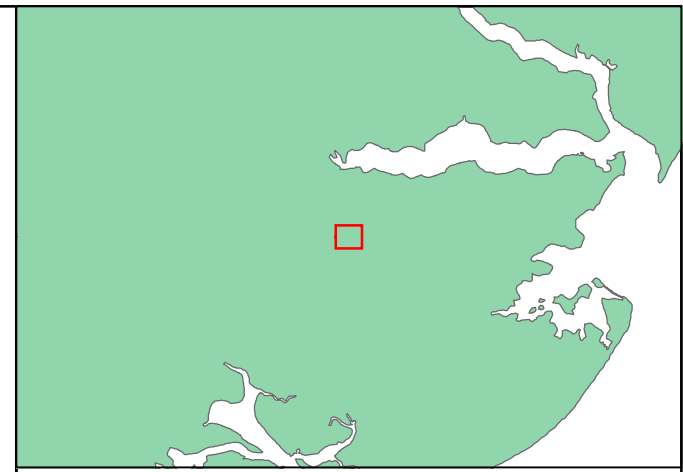
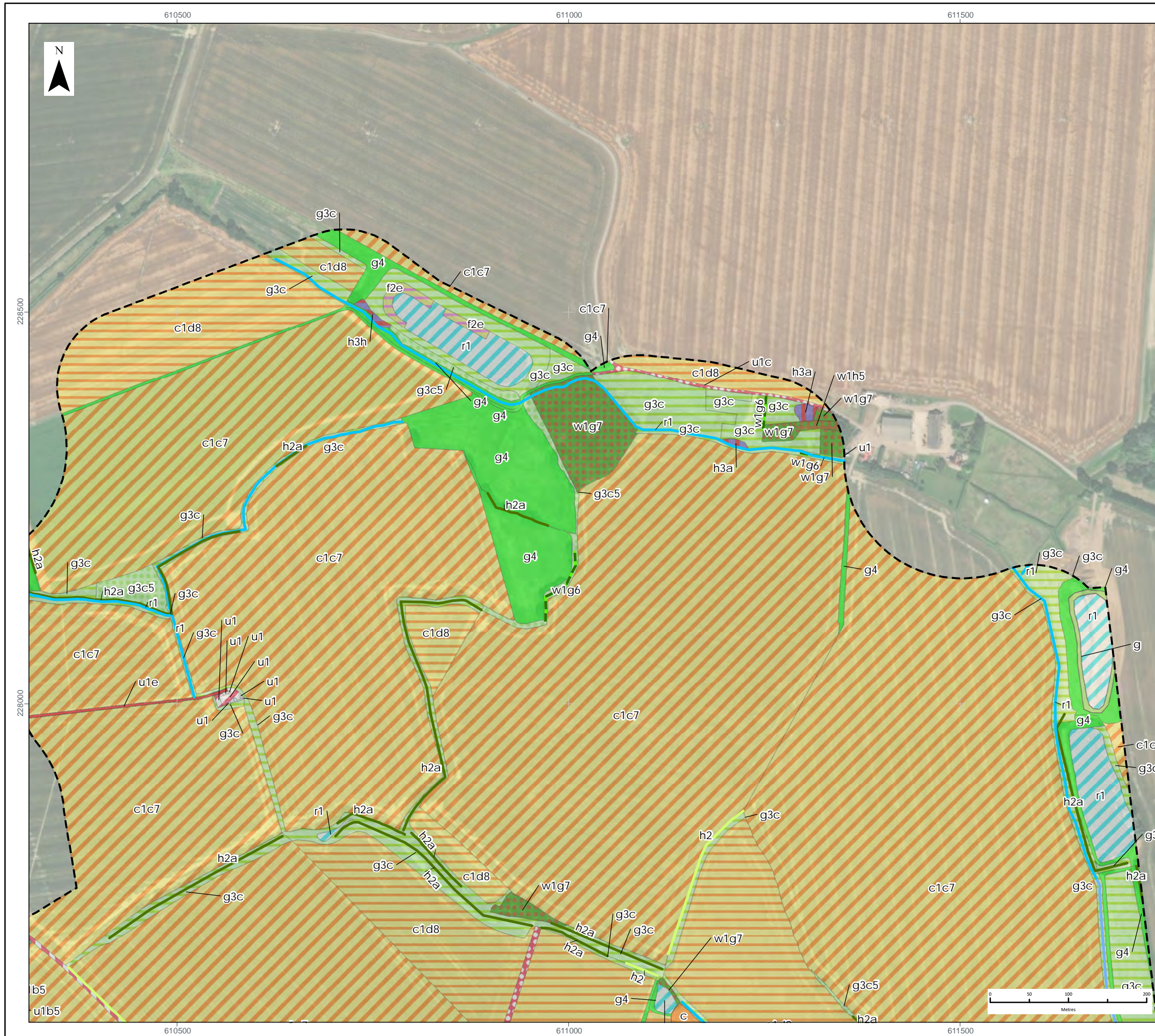
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LEGEND

UKHab Classification: Linear Features

- h2 - Hedgerow
- h2a - Hedgerow (Priority Habitat)
- r1 - Standing Open Water and Canals
- r2b - Other River and Stream
- w1g6 - Line of Trees

UKHab Classification: Area Features

- c - Cropland
- c1c7 - Other Cereal Crops
- c1d8 - Other Non-Cereal Crops
- f2e - Reedbeds
- g - Grassland
- g3c - Other Neutral Grassland
- g3c5 - Arrhenatherum Neutral Grassland
- g4 - Modified Grassland
- h3a - Blackthorn scrub
- h3h - Mixed scrub
- r1 - Standing Open Water and Canals
- u1 - Built-up Areas and Gardens
- u1b - Developed Land, Sealed Surface
- u1c - Artificial Unvegetated Unsealed Surface
- u1e - Built Linear Features
- w1 - Broadleaved, Mixed and Yew Woodland
- w1g7 - Other Broadleaved Woodland Types
- w1h5 - Other Woodland: Mixed; Mainly Broadleaved

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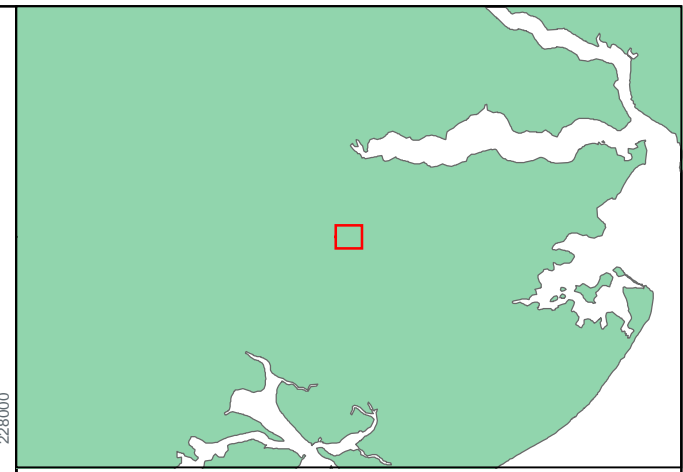
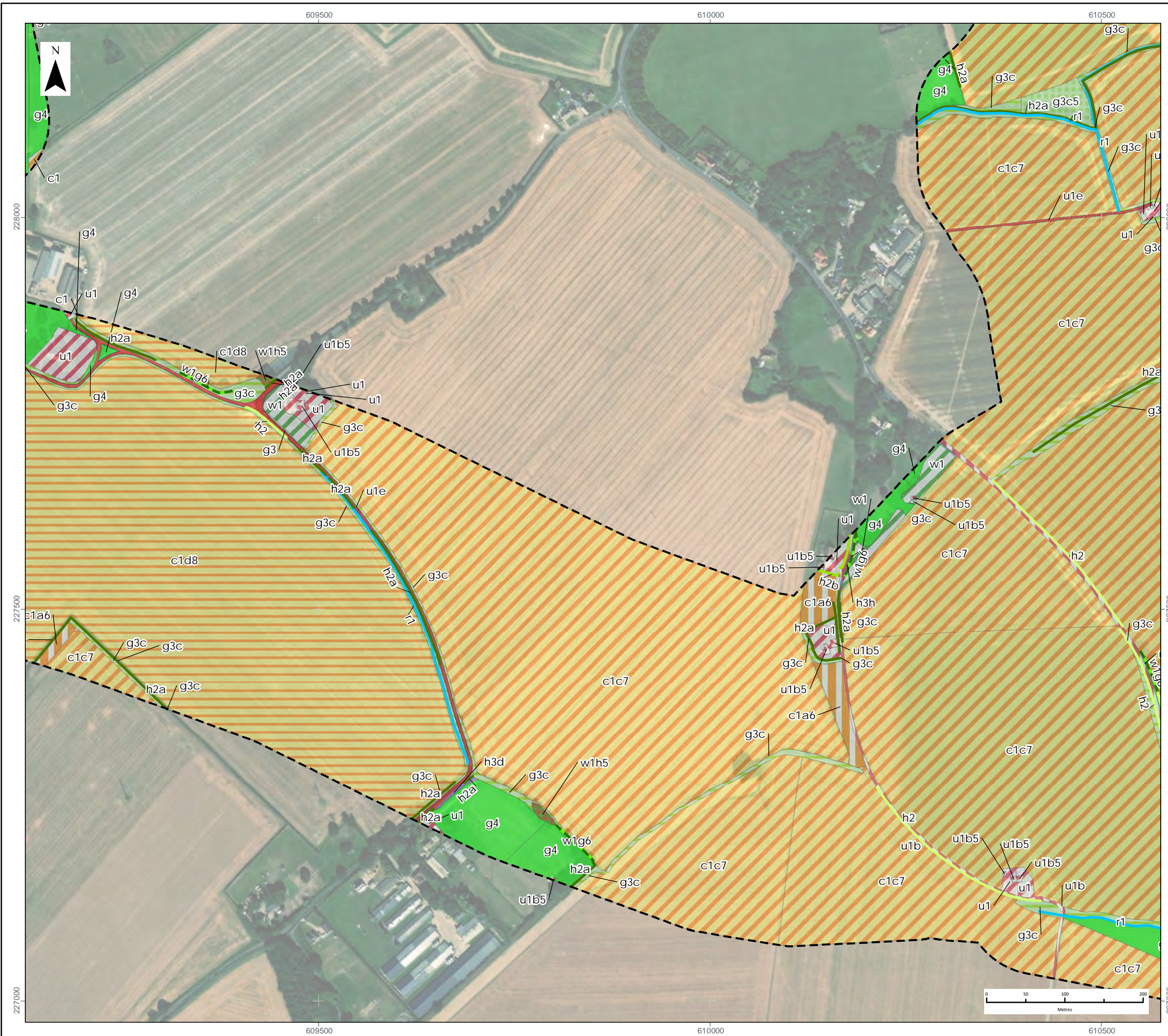
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LEGEND

Habitat Survey Area (2022)

UKHab Classification: Linear Features

- h2 - Hedgerow
- h2a - Hedgerow (Priority Habitat)
- h2b - Other Hedgerow
- r1 - Standing Open Water and Canals
- w1g6 - Line of Trees
- u1 - Built-up Areas and Gardens
- u1b - Developed Land, Sealed Surface
- u1b5 - Buildings
- u1e - Built Linear Features
- w1 - Broadleaved, Mixed and Yew Woodland
- w1h5 - Other Woodland; Mixed; Mainly Broadleaved

UKHab Classification: Area Features

- c1 - Arable and Horticulture
- c1a6 - Arable Margins Sown with Wild Flowers or a Pollen and Nectar Mix
- c1c7 - Other Cereal Crops
- c1d8 - Other Non-Cereal Crops
- g3 - Neutral Grassland
- g3c - Other Neutral Grassland
- g3c5 - Arrhenatherum Neutral Grassland
- g4 - Modified Grassland
- h3d - Bramble Scrub
- h3h - Mixed scrub

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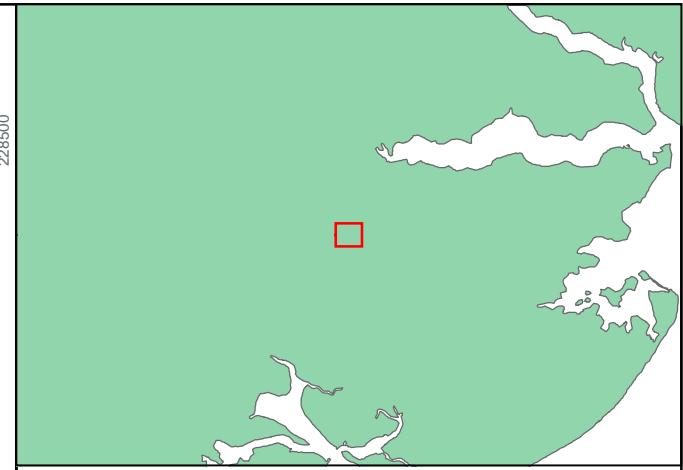
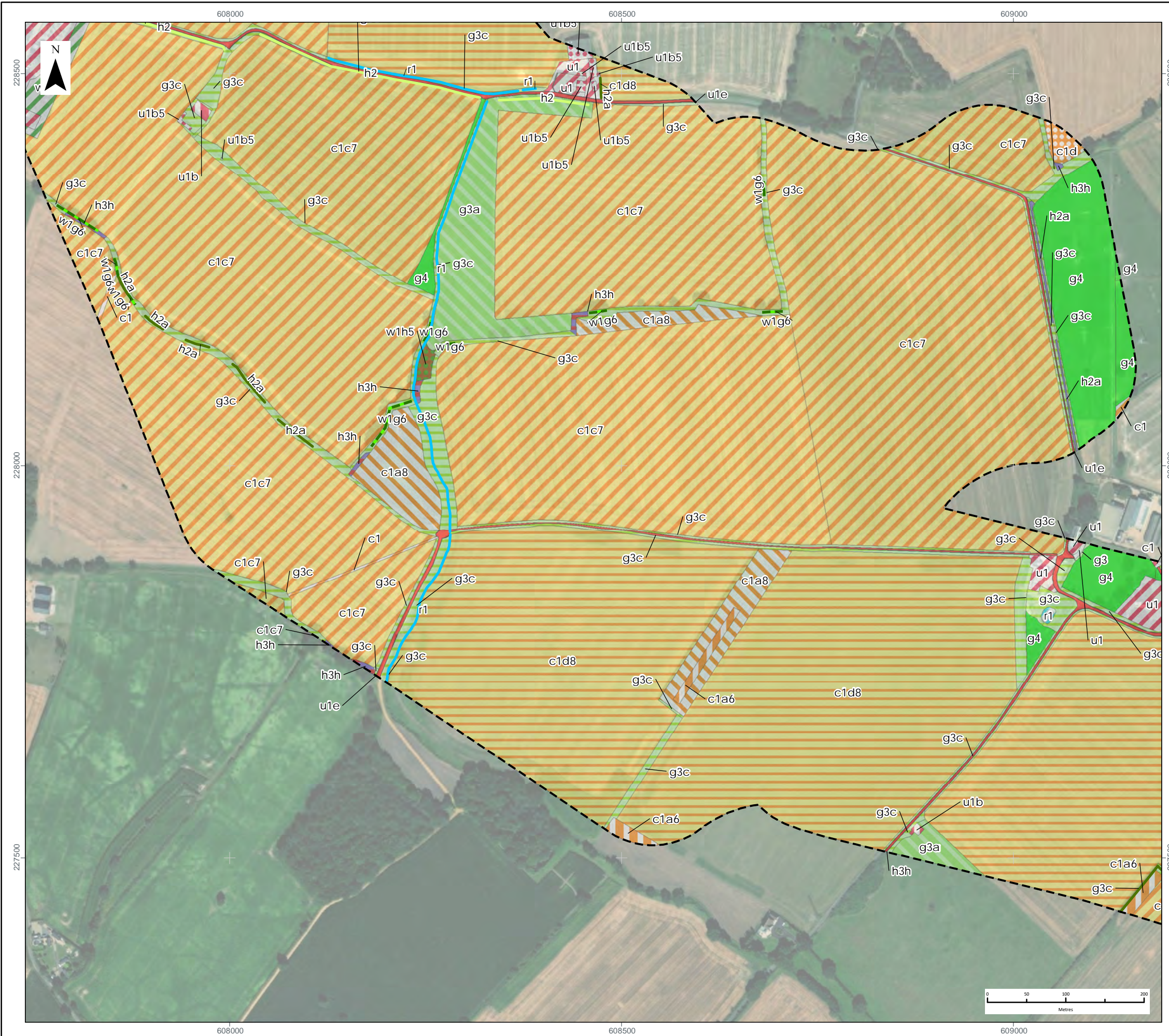
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LEGEND

Habitat Survey Area (2022)
 UKHab Classification: Linear Features

- h2 - Hedgerow
- h2a - Hedgerow (Priority Habitat)
- r1 - Standing Open Water and Canals
- w1g6 - Line of Trees

UKHab Classification: Area Features

- c1 - Arable and Horticulture
- c1a6 - Arable Margins Sown with Wild Flowers or a Pollen and Nectar Mix
- c1a8 - Game Bird Mix Strips and Corners
- c1c7 - Other Cereal Crops
- c1d - Non-Cereal Crops
- c1d8 - Other Non-Cereal Crops
- g3 - Neutral Grassland
- g3a - Lowland Meadows
- g3c - Other Neutral Grassland
- g4 - Modified Grassland
- h2a - Hedgerow (Priority Habitat)
- h3h - Mixed scrub
- r1 - Standing Open Water and Canals
- u1 - Built-up Areas and Gardens
- u1b - Developed Land, Sealed Surface
- u1b5 - Buildings
- u1e - Built Linear Features
- w1 - Broadleaved, Mixed and Yew Woodland
- w1h5 - Other Woodland; Mixed; Mainly Broadleaved

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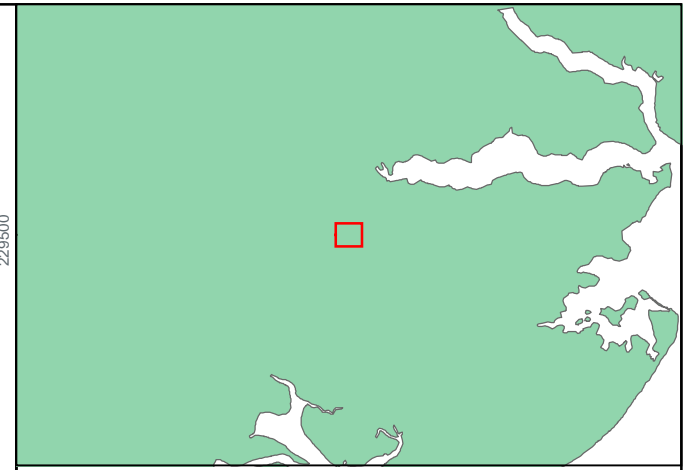
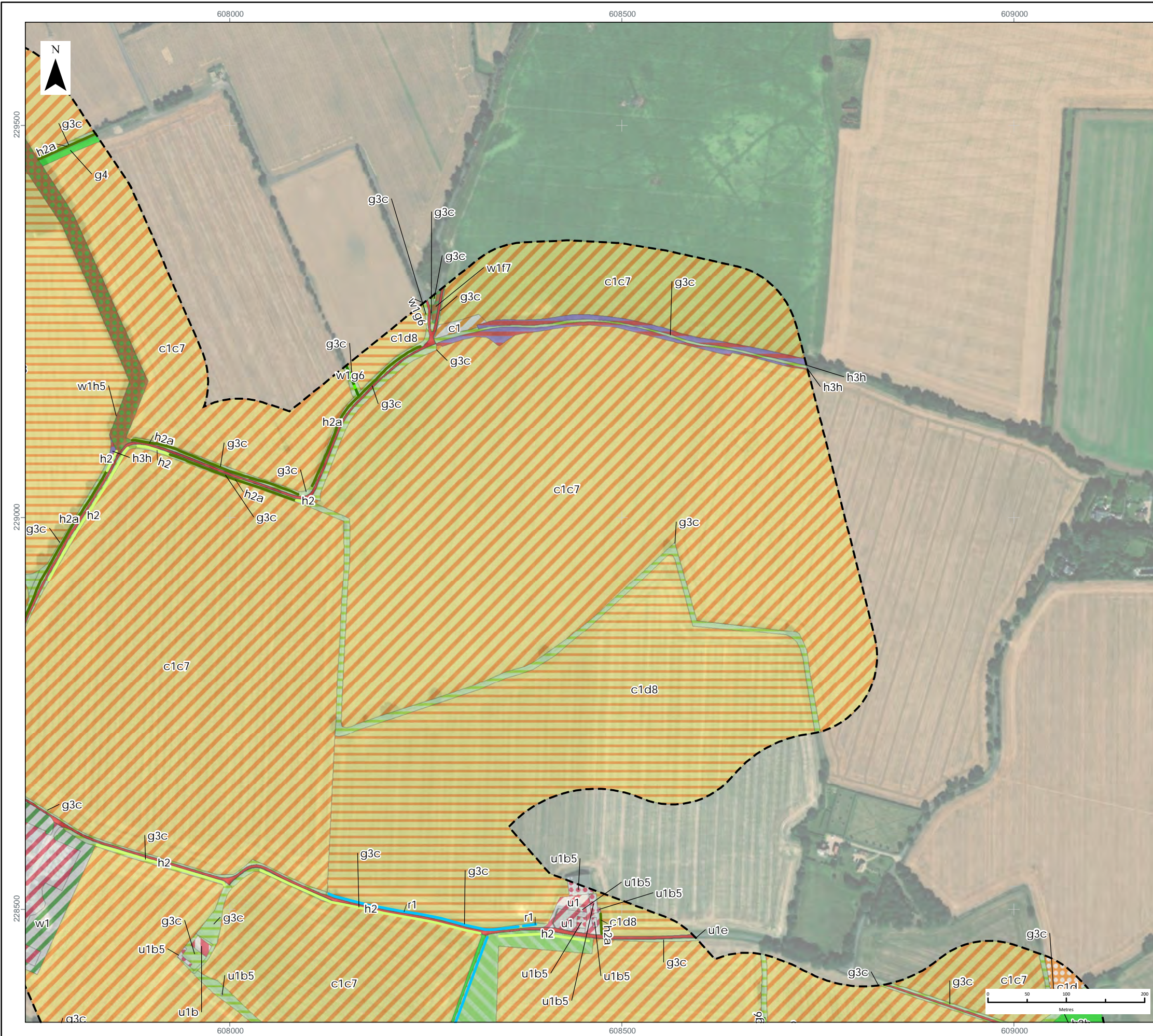
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LEGEND

Habitat Survey Area (2022)

UKHab Classification: Linear Features

- h2 - Hedgerow
- h2a - Hedgerow (Priority Habitat)
- r1 - Standing Open Water and Canals
- w1g6 - Line of Trees
- u1e - Built Linear Features
- w1 - Broadleaved, Mixed and Yew Woodland
- w1f7 - Other Lowland Mixed Deciduous Woodland
- w1h5 - Other Woodland; Mixed; Mainly Broadleaved

UKHab Classification: Area Features

- c1 - Arable and Horticulture
- c1c7 - Other Cereal Crops
- c1d - Non-Cereal Crops
- c1d8 - Other Non-Cereal Crops
- g3a - Lowland Meadows
- g3c - Other Neutral Grassland
- g4 - Modified Grassland
- h3h - Mixed scrub
- u1 - Built-up Areas and Gardens
- u1b - Developed Land, Sealed Surface
- u1b5 - Buildings

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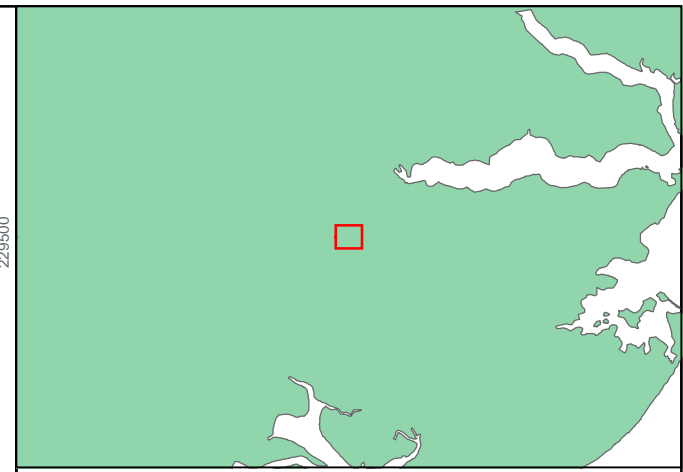
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LEGEND

Habitat Survey Area (2022)	u1 - Built-up Areas and Gardens
h2 - Hedgerow	u1b - Developed Land, Sealed Surface
h2a - Hedgerow (Priority Habitat)	u1b5 - Buildings
h2b - Other Hedgerow	u1e - Built Linear Features
r1 - Standing Open Water and Canals	w1 - Broadleaved, Mixed and Yew Woodland
w1g6 - Line of Trees	w1f7 - Other Lowland Mixed Deciduous Woodland
c1 - Arable and Horticulture	w1h5 - Other Woodland: Mixed; Mainly Broadleaved
c1a6 - Arable Margins Sown with Wild Flowers or a Pollen and Nectar Mix	w1h6 - Other Woodland: Mixed; Mainly Conifer
c1c7 - Other Cereal Crops	
c1d8 - Other Non-Cereal Crops	
g3 - Neutral Grassland	
g3c - Other Neutral Grassland	
g4 - Modified Grassland	
h3d - Bramble Scrub	
h3h - Mixed scrub	
r1 - Standing Open Water and Canals	

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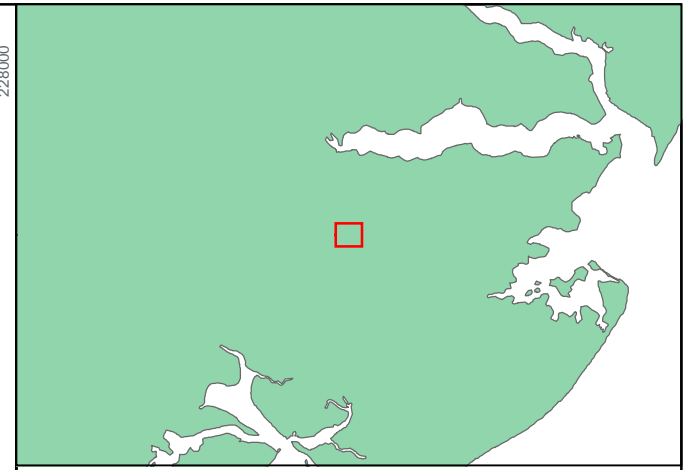
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FIGURE 2

Hedgerows Subject to Assessment



LEGEND

- Habitat Survey Area (2022)
- Hedgerow Subject to Hedgerow Assessment

Note:
No "Important Hedgerows" were identified during surveys.

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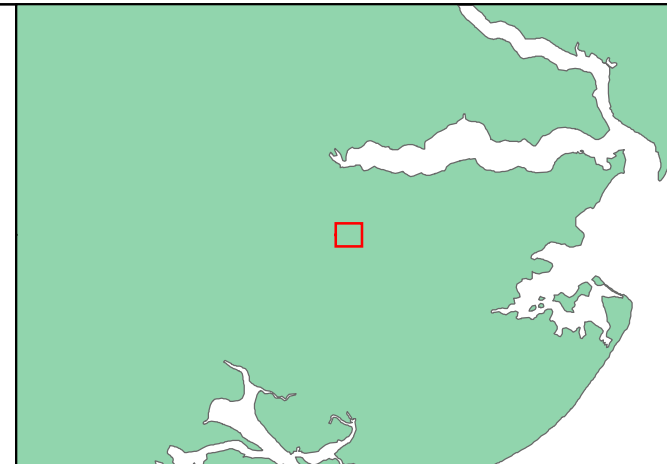
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Hedgerows Subject to Assessment

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LEGEND

- Habitat Survey Area (2022)
- Hedgerow Subject to Hedgerow Assessment

Note:
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Hedgerows Subject to Assessment

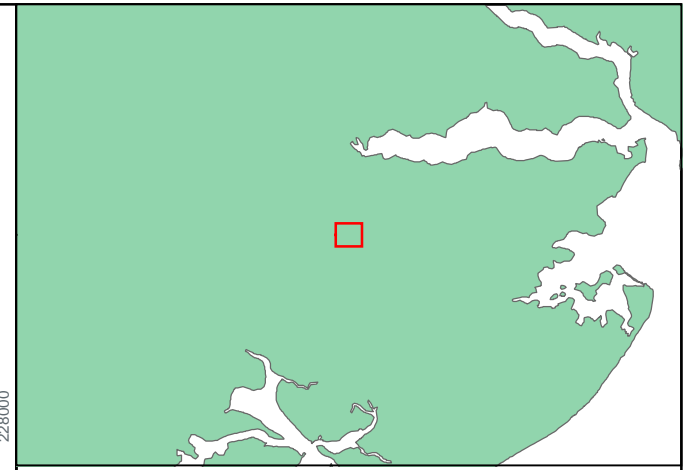
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SCALE: 1:5,000	PLOT SIZE: A3	DATUM: ODN	PROJECTION: British National Grid
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LEGEND

- Habitat Survey Area (2022)
- Hedgerow Subject to Hedgerow Assessment

Note:
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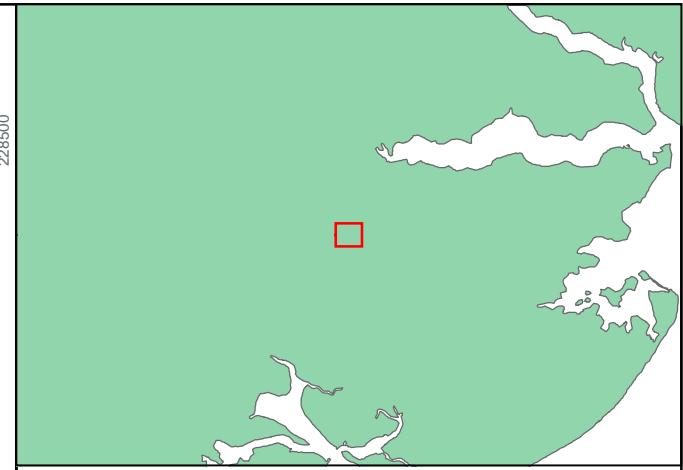
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SCALE: 1:5,000 PLOT SIZE: A3 DATUM: ODN PROJECTION: British National Grid





LEGEND

- Habitat Survey Area (2022)
- Hedgerow Subject to Hedgerow Assessment

Note:
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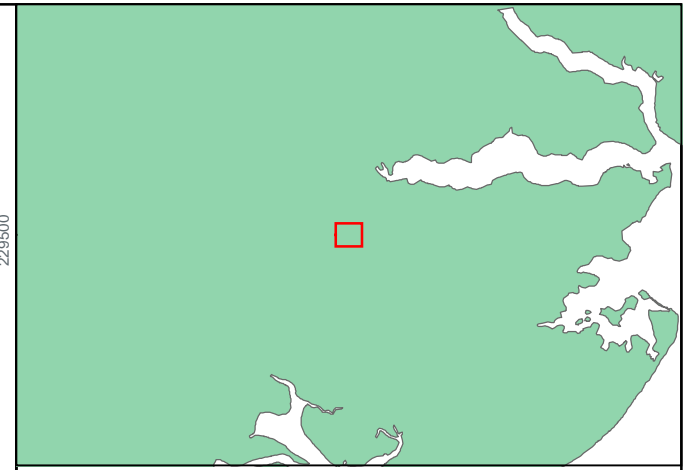
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Hedgerows Subject to Assessment

VER	DATE	REMARKS	Drawn	Checked
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SCALE: 1:5,000 PLOT SIZE: A3 DATUM: ODN PROJECTION: British National Grid





LEGEND

- Habitat Survey Area (2022)
- Hedgerow Subject to Hedgerow Assessment

Note:
No "Important Hedgerows" were identified during surveys.

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FIVE ESTUARIES OFFSHORE WINDFARM

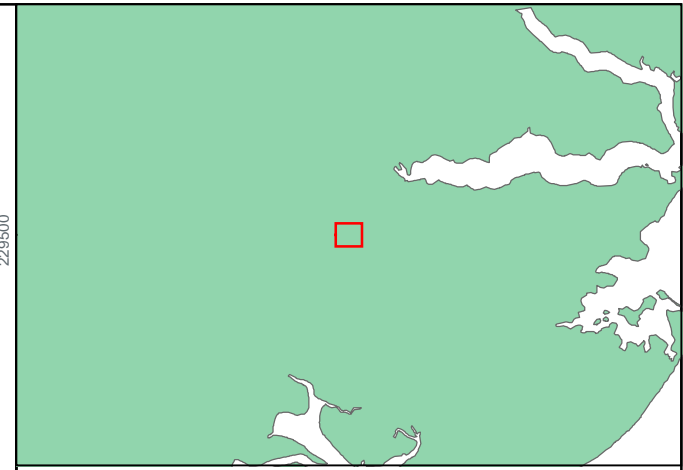
DRAWING TITLE:
Hedgerows Subject to Assessment

VER	DATE	REMARKS	Drawn	Checked
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SCALE: 1:5,000 PLOT SIZE: A3 DATUM: ODN PROJECTION: British National Grid





LEGEND

- Habitat Survey Area (2022)
- Hedgerow Subject to Hedgerow Assessment

Note:
No "Important Hedgerows" were identified during surveys.

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PROJECT TITLE:
FIVE ESTUARIES OFFSHORE WINDFARM

DRAWING TITLE:
Hedgerows Subject to Assessment

VER	DATE	REMARKS	Drawn	Checked
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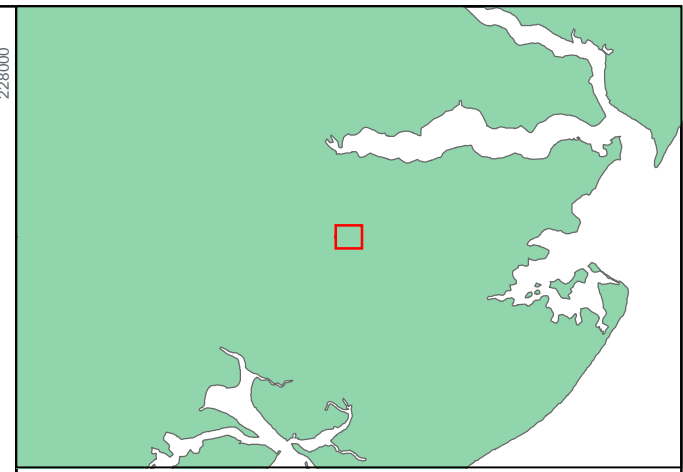
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SCALE: 1:5,000 PLOT SIZE: A3 DATUM: ODN PROJECTION: British National Grid



FIGURE 3

Important Habitats



- LEGEND**
- Habitat Survey Area (2022)
 - UKHab Classification: Linear Features
 - h2a - Hedgerow (Priority Habitat)
 - UKHab Classification: Area Features
 - f2e - Reedbeds
 - r1 - Standing Open Water and Canals
- Secondary Codes:
- | | |
|-------------------------------|------------------------------|
| 10 - Scattered Scrub | 117 - Dry |
| 11 - Scattered Trees | 140 - Anthills |
| 16 - Tall Herb | 148 - Flower Forage Abundant |
| 17 - Ruderal/Ephemeral | 160 - Sward Type Mosaic |
| 19 - Ponds (Priority Habitat) | 161 - Tall or Tussocky Sward |
| 56 - Young Trees - Planted | 190 - Hedgerow with Trees |
| 73 - Bare Ground | 1011 - Pasture or Meadow |

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PROJECT TITLE:
FIVE ESTUARIES OFFSHORE WINDFARM

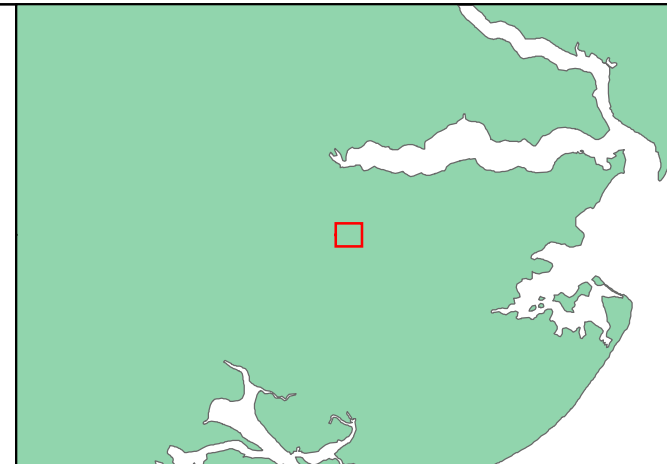
DRAWING TITLE:
Important Habitats

VER	DATE	REMARKS	Drawn	Checked
1	20/01/2023	For Issue	DB	JO

DRAWING NUMBER:
FIGURE 2
 Page 1 of 6

SCALE: 1:5,000 PLOT SIZE: A3 DATUM: ODN PROJECTION: British National Grid





LEGEND

- Habitat Survey Area (2022)
- UKHab Classification: Linear Features**
- h2a - Hedgerow (Priority Habitat)
- UKHab Classification: Area Features**
- f2e - Reedbeds
- r1 - Standing Open Water and Canals

- Secondary Codes:**
- | | |
|-------------------------------|------------------------------|
| 10 - Scattered Scrub | 117 - Dry |
| 11 - Scattered Trees | 140 - Anthills |
| 16 - Tall Herb | 148 - Flower Forage Abundant |
| 17 - Ruderal/Ephemeral | 160 - Sward Type Mosaic |
| 19 - Ponds (Priority Habitat) | 161 - Tall or Tussocky Sward |
| 56 - Young Trees - Planted | 190 - Hedgerow with Trees |
| 73 - Bare Ground | 1011 - Pasture or Meadow |

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FIVE ESTUARIES OFFSHORE WINDFARM

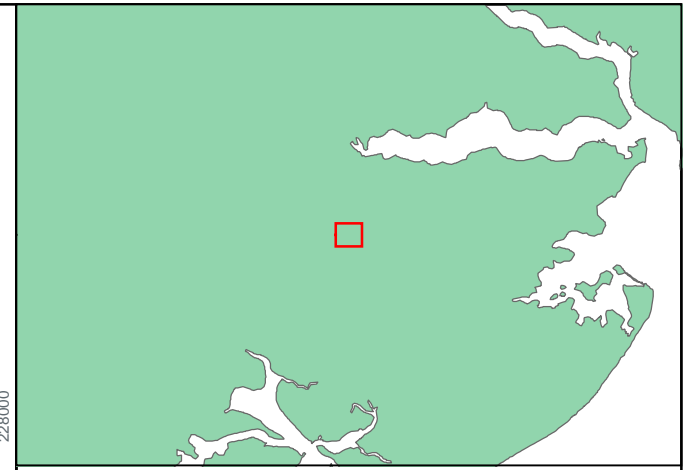
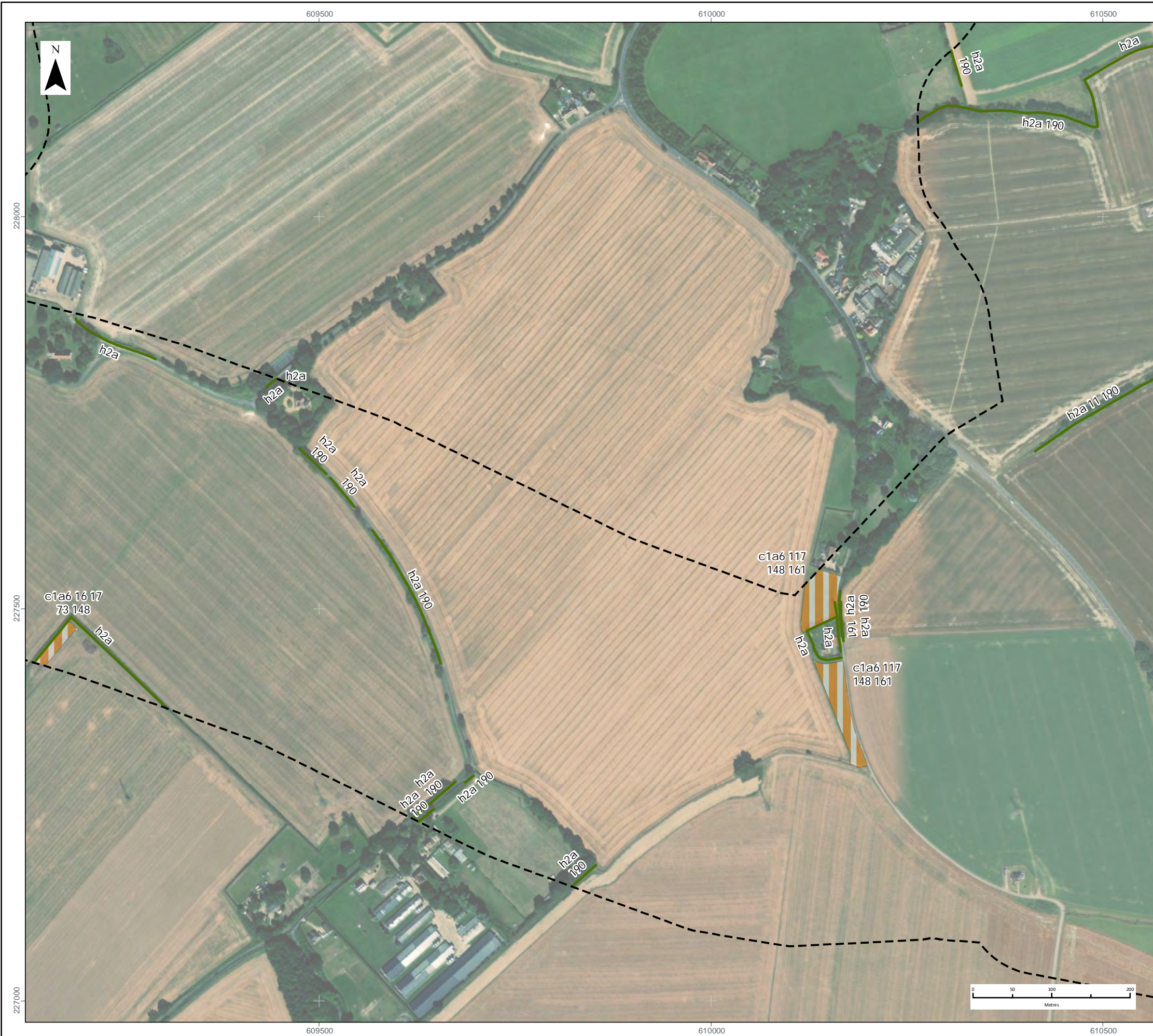
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1	20/01/2023	For Issue	DB	JO

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FIGURE 2
 Page 2 of 6

SCALE: 1:5,000	PLOT SIZE: A3	DATUM: ODN	PROJECTION: British National Grid
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LEGEND

Habitat Survey Area (2022)

UKHab Classification: Linear Features

h2a - Hedgerow (Priority Habitat)

UKHab Classification: Area Features

c1a6 - Arable Margins Sown with Wild Flowers or a Pollen and Nectar Mix

Secondary Codes:

10 - Scattered Scrub	117 - Dry
11 - Scattered Trees	140 - Anthills
16 - Tall Herb	148 - Flower Forage Abundant
17 - Ruderal/Ephemeral	160 - Sward Type Mosaic
19 - Ponds (Priority Habitat)	161 - Tall or Tussocky Sward
56 - Young Trees - Planted	190 - Hedgerow with Trees
73 - Bare Ground	1011 - Pasture or Meadow

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PROJECT TITLE:
FIVE ESTUARIES OFFSHORE WINDFARM

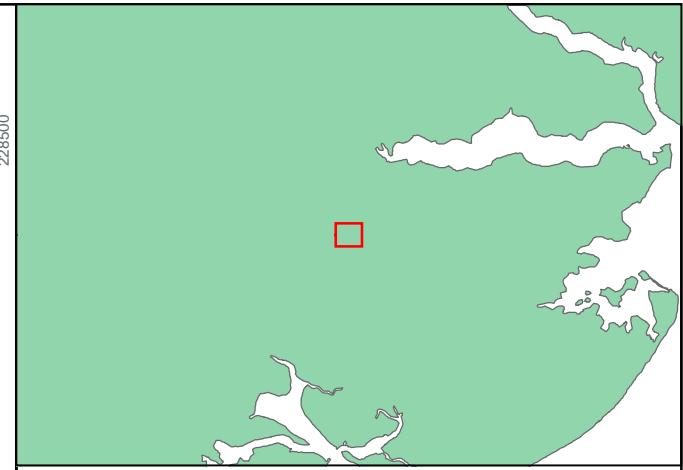
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Important Habitats

VER	DATE	REMARKS	Drawn	Checked
1	20/01/2023	For Issue	DB	JO

DRAWING NUMBER:
FIGURE 2
 Page 3 of 6

SCALE: 1:5,000 PLOT SIZE: A3 DATUM: ODN PROJECTION: British National Grid





LEGEND

Habitat Survey Area (2022)

UKHab Classification: Linear Features

- h2a - Hedgerow (Priority Habitat)

UKHab Classification: Area Features

- c1a6 - Arable Margins Sown with Wild Flowers or a Pollen and Nectar Mix
- c1a8 - Game Bird Mix Strips and Corners
- g3a - Lowland Meadows
- h2a - Hedgerow (Priority Habitat)

Secondary Codes:

10 - Scattered Scrub	117 - Dry
11 - Scattered Trees	140 - Anthills
16 - Tall Herb	148 - Flower Forage Abundant
17 - Ruderal/Ephemeral	160 - Sward Type Mosaic
19 - Ponds (Priority Habitat)	161 - Tall or Tussocky Sward
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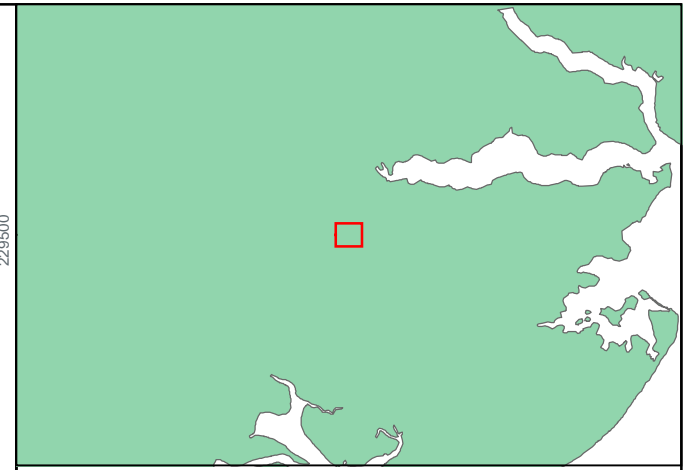
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Important Habitats

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DRAWING NUMBER:
FIGURE 2
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SCALE: 1:5,000 PLOT SIZE: A3 DATUM: ODN PROJECTION: British National Grid



LEGEND

Habitat Survey Area (2022)

UKHab Classification: Linear Features

h2a - Hedgerow (Priority Habitat)

UKHab Classification: Area Features

g3a - Lowland Meadows

Secondary Codes:

10 - Scattered Scrub	117 - Dry
11 - Scattered Trees	140 - Anthills
16 - Tall Herb	148 - Flower Forage Abundant
17 - Ruderal/Ephemeral	160 - Sward Type Mosaic
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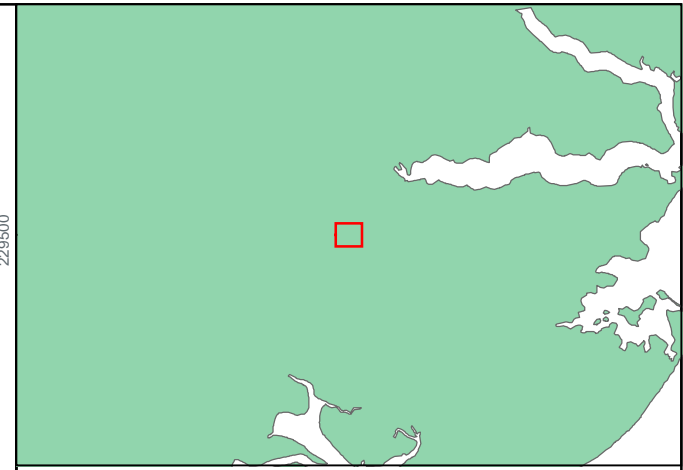
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FIGURE 2
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SCALE: 1:5,000 PLOT SIZE: A3 DATUM: ODN PROJECTION: British National Grid





LEGEND

Habitat Survey Area (2022)

UKHab Classification: Linear Features

h2a - Hedgerow (Priority Habitat)

UKHab Classification: Area Features

c1a6 - Arable Margins Sown with Wild Flowers or a Pollen and Nectar Mix

Secondary Codes:

10 - Scattered Scrub	117 - Dry
11 - Scattered Trees	140 - Anthills
16 - Tall Herb	148 - Flower Forage Abundant
17 - Ruderal/Ephemeral	160 - Sward Type Mosaic
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FIGURE 2
 Page 6 of 6

SCALE: 1:5,000 PLOT SIZE: A3 DATUM: ODN PROJECTION: British National Grid



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ECOLOGY
RESOURCES

Hazel Dormouse Survey Report

**Five Estuaries
Offshore Wind
Farm Ltd**

November 2022



Status	Name	Date
Draft	Jo Dent BSc (Hons) PGDip	08/11/2022
Rev 1	Georgina Davey MSc BSc (Hons) ACIEEM	06/12/2022
Rev 2	Gavin Mullan BA (Hons) MCIEEM	08/12/2022
Rev3	Georgina Davey MSc BSc (Hons) ACIEEM	05/01/2022

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EXECUTIVE SUMMARY

Ecology Resources Limited was commissioned by Royal HaskoningDHV on behalf of Five Estuaries Offshore Wind Farm Limited (VE OWFL), to undertake a hazel dormouse *Muscardinus avellanarius* habitat suitability assessment and, where suitable habitat is identified, complete a presence / likely absence survey within the Five Estuaries Offshore Wind Farm (VE) onshore project area plus a 100m buffer.

Twenty-two survey sites within the onshore project area (plus a 100m buffer) were identified as areas with suitable habitat to support hazel dormice. The full suite of seven dormouse survey visits were completed on 14 of these sites. Four sites were subject to six survey visits due to a delay in deploying survey equipment. One site had five survey visits and the remaining three sites had four visits, this was due to access restrictions at H136 and a revision of the onshore cable route in August 2022 which led to three survey sites being removed from the survey scope.

Hazel dormice were found to be present on 15 of the 22 survey sites.

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1. INTRODUCTION

Ecology Resources Limited was commissioned by Royal HaskoningDHV on behalf of Five Estuaries Offshore Wind Farm Limited (VE OWFL), to undertake a habitat suitability assessment and, where suitable habitat is identified, presence and absence survey for hazel dormouse *Muscardinus avellanarius* within the Five Estuaries Offshore Wind Farm (VE) onshore project area plus a 100m buffer.

1.1 Project Background

VE is a proposed extension to the operational Galloper Offshore Wind Farm (OWF) which consists of 56 wind turbine generators (WTGs). The 5 Estuaries will comprise up to 79 WTGs situated within two array areas to the east of the operational Galloper OWF. The array areas will be located approximately 30km off the coast of Suffolk, England.

Cables will connect the turbines to the offshore substation platforms and then export the power generated to shore. It is expected that there will be a number of inter-array cables, up to four export cables and up to two offshore substations platforms.

A landfall area has been identified between Holland-on Sea and Frinton-on-Sea on the Essex coast. The landfall point is yet to be determined but will be located within this area of coastline. A new VE onshore substation will be needed and will be constructed in an area to the north of the A120.

The VE cables will be installed underground between the landfall and the grid connection point north of the A120. A preferred corridor has not yet been determined with several corridors still under consideration at the time of writing. Potential substation land parcels and associated corridor options north of the A120 also remain under review at the time of writing.

A more detailed description of the project, several elements of which have yet to be finalised at this time, will be provided in the PEIR and ES in due course.

1.2 Legislation

The hazel dormouse is a fully protected species under both United Kingdom and European law. This is a brief summary of the legislation and is not to be regarded as a definitive legal opinion. When dealing with individual cases, the client is advised to consult the full texts of the relevant legislation and obtain further legal advice.

The hazel dormouse is fully protected under Schedule 5 of the Wildlife and Countryside Act (WCA) 1981 (as amended). Legal protection is also afforded by the Conservation of Habitats and Species (Amendment) (EU Exit) Regulations 2019, making the dormouse a European Protected Species. These two pieces of legislation operate in parallel, making it an offence to:

- deliberately capture, injure or kill a hazel dormouse;

- possess or control any live or dead specimen or anything derived from a hazel dormouse;
- deliberately disturb a hazel dormouse (in particular, disturbance which is likely to impair their ability to survive, to breed or reproduce, or to rear or nurture their young, to hibernate or migrate or to affect significantly the local distribution or abundance of the species to which they belong);
- damage or destroy a breeding site or resting place of a hazel dormouse; and
- possess, transport, advertise, sell or exchange a hazel dormouse (dead or alive) or any part of a hazel dormouse.

1.3 Priority species

Hazel dormice are listed as species of principal importance for the conservation of biodiversity in England, in accordance with Section 41 of the Natural Environment and Rural Communities (NERC) Act 2006. Under Section 40 of the NERC Act (2006) public bodies (including local planning authorities) have a duty to have regard for the conservation of species of principal importance when carrying out their functions, including determining planning applications.

2. METHODOLOGY

2.1 Habitat Suitability Assessment

An assessment of suitable habitat for hazel dormouse was made, based upon the previous Preliminary Ecological Appraisal report (SLR, 2022).

Habitats suitable for hazel dormice included woody habitats, including hedgerows and areas of species rich scrub and grassland that are connected to woodland areas which contain a high degree of species diversity within tree and shrub species.

A total of 19 areas of suitable habitat for dormice were recorded, primarily consisting of hazel-rich hedgerows connected to nearby woodland. Dormouse surveys were then undertaken on the 19 identified areas with suitable habitat. The location of these features are shown in Appendix A on Figure 1.1 to 1.3.

2.2 Presence / likely absence surveys

Survey Design

A dormouse nest-tube monitoring survey of all suitable hedgerows that may be impacted by construction works was undertaken in accordance with the current good practice guidelines set out in the Dormouse Conservation Handbook (Bright *et al.*, 2006). Sites outside the survey scope or not likely to be impacted by construction works were not subject to surveys.

Dormouse nest-tubes were installed approximately 15-20m apart within each suitable habitat area. The number of tubes installed at each survey site was determined by site geography (e.g length of hedgerow and hedgerow connections) and professional judgement. Nest tubes were numbered and

attached to the underside of branches using cable ties or wire. The opening of the tube pointed downwards, and at an angle no greater than 45 degrees.

For any identified suitable woodlands which may be impacted or have connectivity to potentially impacted hedgerows, dormouse nest box surveys were undertaken. Nest boxes were used within woodland as dormice more readily make nests within boxes than tubes, thereby increasing detectability (Chanin & Gubert, 2011). Nest boxes are not able to be used in hedgerows as they require a stable tree to attach to in a grid pattern. Nest boxes were attached with wire to suitable trees in a grid pattern approximately 20 metres apart (Bright *et al.*, 2006).

The GPS location of every nest tube and next box was recorded using Fulcrum digital mapping software, and notes and photographs were recorded to assist future surveyors to find the tubes.

The nest-tubes and boxes were checked once a month between May and November, following the 'Index of probability of finding dormice present in nest tubes' (Chanin & Woods, 2003) (see Table 3.1). The scoring system provides an overall index of effort by multiplying the sum of the months the tubes were checked by the number of tubes used. Likely absence should not be assumed on an overall score less than 20.

Table 1. Index of probability of finding dormice present in nest tubes in a single month (Bright *et al.*, 2006). Note this is based on a minimum of 50 nest tubes. A minimum score of 20 points must be achieved to reliably conclude likely absence of dormice.

Month	Index of probability (based on 50 nest tubes)
April	1
May	4
June	2
July	2
August	5
September	7
October	2
November	2

Field Survey

Each nest tube and box were carefully inspected, on inspection if evidence of activity of any small mammal (e.g., movement, nests, droppings) was found, the tube or box was taken down and opened within a large plastic bag (Bright *et al.*, 2006). This method allowed the surveyor to closely examine the contents of the box or tube and prevented any animals from escaping and the safe return of any animals to the tube or box before replacing.

Any dormice encountered were weighed and sexed with the age, class and breeding condition of each animal recorded. Any other small mammals such as wood mice *Apodemus sylvaticus*, shrew *Sorex sp.*, or field vole *Microtus agrestis* nests or individuals encountered were removed from the

tubes, unless a litter was present. Bird nests were not disturbed until chicks had fledged, at which point the nest was removed.

Table 2. Dormouse survey site set up details.

Site ID	No. tubes / boxes	Date of deployment
TN041	6	27.04.22
TN410	35	03.05.22
TN503	25	25.05.22
TN509	50	13.05.22
TN525	50	19.04.22
H075	26	27.04.22
H079	12	27.04.22
H085	12	27.04.22
H089	28	27.04.22
H136	15	28.04.22
H149	15	10.05.22
H154	20	28.04.22
H155	18	15.06.22
H156	10	15.06.22
H221	18	28.04.22
2047	15	24.05.22
2174	15	23.05.22
2196	9	25.05.22
2204	30	23.05.22
2250	10	25.05.22
2278	35	24.05.22
2345	10	25.05.22

2.3 Survey Personnel

All dormouse surveys were led by Kate Mann, (Natural England Level 1 dormouse survey licence number 2020-49128-CLS-CLS) an ecologist with over eight years' experience conducting dormouse surveys. In addition, assistant surveyors were all suitably experienced ecologists actively enrolled onto dormouse training courses and/or groups and have a professional interest in dormice. All

surveyors were either members of CIEEM, or followed the Code of Professional Conduct (CIEEM, 2022).

The surveys were carried out during the recommended survey season for dormice (Bright *et al*, 2006). They were undertaken between April and November (inclusive) in 2022.

2.4 Revised Onshore Cable Route August 2022

Throughout the life cycle of the project the cabling route has undergone several revisions and in August 2022, the onshore cable route was finalised, which led to the exclusion of dormouse survey sites 2047, 2250 and 2345, which were subsequently removed from scope.

However these sites had four, three and three surveys respectively. Dormouse survey tubes were collected from 2250 on 19th August, from 2345 on 31st August and from 2047 on 20th November 2022.

A small woven nest was recorded at survey site 2047 on 15th August 2022 and therefore this site was considered to have confirmed presence of hazel dormouse.

Survey sites 2250 and 2345 were removed from the scope of surveys before reaching the target of 20 points for surveys and therefore it cannot be concluded that dormice are likely absent from these sites.

2.5 Survey Limitations

Where possible the nest tubes and boxes were left in situ from April - November (inclusive), however survey sites H155 and H156 were identified at a later date and surveys were not formally instructed until May 2022. As such, these sites were subject to surveys between June – November (inclusive).

All sites were surveyed and accessed without limitations apart from two sites where access was the main limitation. Hedgerow H136 had repeated landowner access refusal until August 2022, this meant it did not align with the other survey areas. Due to this, the target survey point score of 20 was not reached, however dormice were confirmed as present on site and therefore access constraints are not considered to have impacted the results.

Survey site 2278 was spread over two land parcels with separate landowners, and access to one half of the site was refused in August, September and October 2022. This resulted in half of the site being subject to survey during these months, and the half of site with refused access remaining not surveyed until equipment collection in November. The target survey point score of 20 was reached and dormice were present on site (both halves recorded dormouse presence), therefore this access constraint is not considered to have impacted the results.

Surveys can only be used to determine likely absence of dormice from any given area. This is due to their elusive nature and natural population fluctuations, allowing colonisations of areas following the completion of surveys. An absence of dormice, or their field signs, found during a survey does not confirm with absolute certainty the absence of dormice.

For example, in suitable habitat where a lack of artificial nests are observed dormice may still be present. Dormice may be using natural nests, which are usually the size of a grapefruit and can be found low down in shrubs and bramble *Rubus fruticosus*, although these are more likely to be found in the Autumn months (Bright *et al*, 2006).

3. RESULTS

3.1 Habitat Suitability Assessment

The VE onshore project area plus a 100m buffer was assessed for their suitability to support dormice, this initial assessment was informed by the SLR Consulting Ltd. (2022) Five Estuaries Offshore Wind Farm Preliminary Ecological Appraisal, which was subsequently ground truthed in May 2022.

From this, 22 sites (four woodland stands and 18 hedgerows) were considered suitable for dormice and scoped in for further surveys. However, following the August 2022 revision of the proposed onshore cable route, three hedgerows were descope part-way through the suite of surveys. Details of the 22 sites subject to dormouse presence / likely absence surveys are outlined below in Table 3.

Table 3. Dormouse habitat suitability assessment results for sites scoped in for further survey.

Site ID	OS Grid Reference	Date	Notes
TN041	TM 20459 19619	23/06/2022	Dry ditch by a mixed species rich hedgerow that has zero gaps more than 5m wide. Food plants include field maple <i>Acer campestre</i> , bramble, elderberry <i>Sambucus nigra</i> , oak <i>Quercus robur</i> , ivy <i>Hedera helix</i> .
TN410	TM 19937 20422	24/06/2022	Broadleaved semi-natural woodland approx. 1.5 ha in area with excellent connectivity. Food plants include blackthorn <i>Prunus spinosa</i> , bramble, oak, wild cherry <i>Prunus avium</i> , ash <i>Fraxinus excelsior</i> , alder <i>Alnus glutinosus</i> .
TN503	TM 17800 23076	30/06/2022	Mixed semi-natural woodland approx. 0.7 ha in area and limited connectivity. Food plants include common hawthorn <i>Crataegus monogyna</i> , blackthorn, field maple, sycamore <i>Acer pseudoplatanus</i> , common honeysuckle <i>Lonicera periclymenum</i> , oak, bramble, dog-rose <i>Rosa canina</i> , elderberry, silver birch <i>Betula pendula</i> , field elm <i>Ulmus minor</i> , ash.
TN509	TM 16014 23982	28/06/2022	Mixed semi-natural woodland approx. 3.5 ha in area and excellent connectivity. Food plants include oak, field maple, common honeysuckle, elderberry, blackthorn, bramble, common hazel <i>Corylus avellana</i> , Scots pine <i>Pinus sylvestris</i> , common holly <i>Ilex aquifolium</i> , common hawthorn, wild cherry, Chestnut <i>Castanea sp.</i>
TN525	TM 15409 25034	12/06/2022	Mixed woodland plantation approx. 2 ha in area and excellent connectivity. Food plants include oak, ash, common hawthorn, elderberry, bramble, common

			hazel, blackthorn, sycamore, willow; <i>Salix</i> spp., alder, field maple.
H075	TM 20035 20004	24/06/2022	Species rich hedgerow with native trees. Zero gaps that are more than 5m wide. Food plants include oak, ash, bramble, common hawthorn.
H079	TM 20266 19690	23/06/2022	Defunct hedge with native trees. Zero gaps that are more than 5m wide. Food plants include bramble, oak, common hazel, elderberry.
H085	TM 20471 19750	23/06/2022	Species rich hedgerow approx. 3m high and 4m wide, with native trees. Zero gaps that are more than 5m wide. Food plants include oak, elderberry, bramble, common hawthorn.
H089	TM 20021 19977	24/06/2022	Species rich hedgerow with native trees. Zero gaps that are more than 5m wide. Food plants include bramble, oak, common hawthorn.
H136	TM 17287 23402	17/06/2022	Species rich hedgerow approx. 9m high and 5m wide, with native trees. Several gaps that are more than 5m wide. Some gaps contain bramble within tall ruderals. Excellent connectivity Food plants include blackthorn, common hazel, oak, ash, elm, bramble, dog-rose, common hawthorn, field maple, elderberry.
H149	TM 16577 23619	01/07/2022	Species poor hedgerow approx. 7m high and 10m wide, with native trees. Zero gaps that are more than 5m wide. Food plants include blackthorn, common hawthorn, common hazel, bramble, dog-rose, common dogwood <i>Cornus sanguinea</i> , field maple, <i>Prunus</i> spp., oak, common honeysuckle, common hornbeam <i>Carpinus betulus</i> , ash.
H154	TM 16184 23724	29/06/2022	Mixed species hedgerow approx. 3m high and 2m wide, with native trees. A couple gaps that are more than 5m wide and excellent connectivity. Food plants include common hawthorn, blackthorn, common hazel, oak, field maple, bramble.
H155	TM 16439 23935	28/06/2022	Mixed species hedgerow approx. 4m high and 3m wide, with native trees. One gap that is more than 5m wide and excellent connectivity. Food plants include elm, field maple, bramble, blackthorn, oak, <i>Rosa</i> sp., common honeysuckle, summer lilac <i>Buddleia Davidii</i> , common dogwood.

H156	TM 16283 23911	29/06/2022	Mixed species hedgerow approx. 3m high and 2m wide, with native trees. One gap that is more than 5m wide and excellent connectivity. Food plants include common hawthorn, blackthorn, common dogwood, bramble, common hazel, field maple.
H221	TM 16524 24157	28/06/2022	Species rich hedgerow approx. 8m high and 3m wide, with native trees. One gap that is more than 5m wide but connectivity is minor. Food plants include common honeysuckle, oak, English walnut <i>Juglans regia</i> , common hawthorn, blackthorn, common hazel, bramble, Rosa sp., field maple.
2047	TM 20517 19905	20/06/2022	Hedgerow 4m high and 5m wide. Food plants include blackthorn, common hazel, bramble, field maple, elm sp. <i>Ulmus spp.</i> , wild cherry.
2174	TM 19176 22724	18/06/2022	Species rich hedgerow approx. 5m high and 3m wide, with native trees. Food plants include oak, common hazel, blackthorn, field maple, bramble, ash, dog-rose.
2196	TM 17922 23037	18/06/2022	Species rich hedgerow approx. 5m high and 3m wide, with native trees. Good connectivity. Food plants include blackthorn, Prunus sp., bramble, common hazel, dog-rose, common honeysuckle.
2204	TM 19047 22902	18/06/2022	Species rich hedgerow approx. 5m high and 3m wide, with native trees. Food plants include dog-rose, common hazel, blackthorn, bramble, oak, elderberry.
2250	TM 19249 23389	18/06/2022	Hedgerow height 3m, width 4m. Food plants include blackthorn, blackthorn, dog-rose, elderberry, bramble.
2278	TM 17204 23416	18/06/2022	Species rich hedgerow approx. 5m high and 3m wide, with native trees. Excellent connectivity. Food plants include oak, hawthorn, bramble, blackthorn, field maple, common hazel, willow <i>Salix spp.</i>
2345	TM 14548 25784	13/06/2022	Young hedgerow, height 1m, width 1m. Food species include oak, bramble, dog-rose, common hazel, common hawthorn, field maple.

3.2 Field Survey

Results show that 15 of the 19 sites had confirmed evidence of hazel dormouse. These were survey sites: TN410, TN503, TN509, H075, H079, H085, H136, H149, H154, H155, H221, 2047, 2196, 2204 and 2278.

Survey sites TN041, TN525, H089, H156, 2174, 2250 and 2345 had no confirmed evidence of hazel dormice. Dormouse surveys at survey sites 2047, 2250 and 2345 began in May 2022 but were stopped when these sites were descoped after the August 2022 revision of the project onshore cable route.

All the survey meta data can be found in Appendix B and the photos to support the surveys can be found in Appendix C.

Table 4. Dormouse Survey Results.

TN041 -				
Survey visit	Date	Dormouse signs Y/N	Dormouse evidence	Notes
Deployment	27.04.22	N	N/A	N/A
1	11.05.22	N	N/A	N/A
2	17.06.22	N	N/A	N/A
3	25.07.22	N	N/A	N/A
4	15.08.22	N	N/A	N/A
5	26.09.22	N	N/A	N/A
6	28.10.22	N	N/A	N/A
7 + collection	30.11.22	N	N/A	Wood mouse nest.
TN410				
Survey visit	Date	Dormouse signs Y/N	Dormouse evidence	Notes
Deployment	03.05.22	N	N/A	N/A
1	27.05.22	N	N/A	N/A
2	17.06.22	N	N/A	N/A
3	28.07.22	N	N/A	N/A
4	31.08.22	N	N/A	N/A
5	26.09.22	Y	1x adult, 4x juveniles, 5x nests.	Box 2: 1x male juvenile dormouse with grey fur, not weighed. In nest. Box 7: 1x adult dormouse escaped nest on approach. Box 10: Dormouse nest, no animal present. Box 12: Dormouse nest with no entrance – likely maternity nest so left alone to avoid disturbance. Box 14: 3x juveniles in nest, not weighed. Grey fur present.
6	28.10.22	Y	1x adult, 9x male adult, 4x female adult, 6x juveniles, 12x nests.	Box 2: 1x adult escaped on approach. 2x male adults inside nest, 28g and 24g. Box 5: 1x male adult 22.5g, 1x juvenile (not weighed) in nest. Box 7: 1x male adult 23.5g, 1x adult female (this year's mature young) 26g with tick on face. In nest. Box 10: Dormouse nest, no animal present. Box 12: 1x female adult, 21.5g, 3x juveniles average weight 10g, in nest.

				<p>Box 13: 3x male adult, 25.5g, 20g with white tip and 18g.</p> <p>Box 14: Dormouse nest with wood mouse in.</p> <p>Box 16: 1x female juvenile 20g, 1x male juvenile 14g.</p> <p>Box 19: Dormouse nest, no animal present.</p> <p>Box 21: 1x female adult in nest, 19g.</p> <p>Box 23: 1x female adult in nest, 22.5g.</p> <p>Box 34: 2x male adult in nest, 20g.</p>
7 + collection	30.11.22	Y	17x nests.	<p>Box 2: Dormouse nest, no animal present.</p> <p>Box 4: Dormouse nest, no animal present.</p> <p>Box 5: Dormouse nest, no animal present.</p> <p>Box 8: Dormouse nest, no animal present.</p> <p>Box 10: Dormouse nest, no animal present.</p> <p>Box 11: Dormouse nest, no animal present.</p> <p>Box 12: Dormouse nest, no animal present.</p> <p>Box 13: Dormouse nest, no animal present.</p> <p>Box 14: Dormouse nest, no animal present.</p> <p>Box 16: Dormouse nest, no animal present.</p> <p>Box 17: Dormouse nest, no animal present.</p> <p>Box 18: Dormouse nest, no animal present.</p> <p>Box 19: Dormouse nest, no animal present.</p> <p>Box 21: Dormouse nest, no animal present.</p> <p>Box 22: Dormouse nest, no animal present.</p> <p>Box 23: Dormouse nest, no animal present.</p> <p>Box 34: Dormouse nest, no animal present.</p>
TN503				
Survey visit	Date	Dormouse signs Y/N	Dormouse evidence	Notes
Deployment	25.05.22	N	N/A	N/A
1	30.05.22	N	N/A	N/A
2	16.06.22	N	N/A	N/A
3	29.07.22	N	N/A	N/A
4	18.08.22	N	N/A	N/A
5	28.09.22	Y	1x juvenile.	Box 16: 1x juvenile in nest, not weighed.
6 + collection	28.11.22	Y	2x nests.	<p>Box 16: Old dormouse nest destroyed by other small mammal.</p> <p>Box 25: Dormouse nest, no animal present.</p>
TN509				
Survey visit	Date	Dormouse signs Y/N	Dormouse evidence	Notes
Deployment	13.05.22	N	N/A	N/A
1	26.05.22	N	N/A	N/A
2	14.06.22	N	N/A	N/A
3	29.07.22	N	N/A	N/A
4	19.08.22	N	N/A	N/A
5	30.09.22	Y	1x male adult, 1x nest.	Tube 40: 1x male adult, 15g, this year's young in breeding condition.
6	31.10.22	Y	1x nest.	Tube 40: Dormouse nest, no animal present.
7 + collection	29.11.22	Y	1x deceased dormouse, 3x nests.	<p>Box 3: Dormouse nest, no animal present.</p> <p>Tube 40: 1x deceased dormouse in nest, possible tumour to side of face.</p> <p>Tube 46: Dormouse nest, no animal present.</p>
TN525				

Survey visit	Date	Dormouse signs Y/N	Dormouse evidence	Notes
Deployment	19.04.22	N	N/A	N/A
1	10.05.22	N	N/A	5x bird nests recorded.
2	14.06.22	N	N/A	4x bird nests recorded.
3	03.07.22	N	N/A	N/A
4	31.08.22	N	N/A	N/A
5	12.09.22	N	N/A	N/A
6	31.10.22	N	N/A	N/A
7 + collection	29.11.22	N	N/A	4x other small mammal nests recorded.
H075				
Survey visit	Date	Dormouse signs Y/N	Dormouse evidence	Notes
Deployment	27.04.22	N	N/A	N/A
1	27.05.22	N	N/A	N/A
2	16.06.22	N	N/A	N/A
3	25.07.22	N	N/A	N/A
4	15.08.22	N	N/A	N/A
5	26.09.22	N	N/A	N/A
6	29.10.22	N	N/A	N/A
7 + collection	20.11.22	Y	1x nest	Tube 21: Dormouse nest, no animal present.
H079				
Survey visit	Date	Dormouse signs Y/N	Dormouse evidence	Notes
Deployment	27.04.22	N	N/A	N/A
1	11.05.22	N	N/A	N/A
2	17.06.22	N	N/A	N/A
3	25.07.22	N	N/A	N/A
4	15.08.22	N	N/A	N/A
5	26.09.22	Y	1x adult, 3x pinks, 1x nest	Tube 6: Adult escaped from nest before bunged - very vocal pinks within nest, minimum of 3 counted.
6	28.10.22	Y	1x nest	Tube 6: Dormouse nest. Not fully inspected due to pinks found previous month.
7 + collection	30.11.22	Y	4x nests	Tube 6: Dormouse nest, no animal present. Tube 7: Dormouse nest, no animal present. Tube 8: Dormouse nest with bracken, no animal present. Tube 11: Dormouse nest, no animal present.
H085				
Survey visit	Date	Dormouse signs Y/N	Dormouse evidence	Notes
Deployment	27.04.22	N	N/A	N/A
1	11.05.22	N	N/A	N/A
2	17.06.22	N	N/A	N/A
3	25.07.22	Y	1x female adult, 4x juvenile, 1x nest	Tube 18: Woven nest made from willow. Mother and 3 individuals escaped, and 4 th juvenile weighed 8.5g
4	15.08.22	Y	1x nest	Tube 18: Dormouse nest, no animal present.
5	26.09.22	Y	2x nests	Tube 18: Old dormouse nest Tube 20: Dormouse nest, empty
6	28.10.22	Y	2x nests	Tube 13: Dormouse nest, no animal present Tube 20: Escaped dormouse, nest present
7 + collection	30.11.22	Y	5x nests	Tube 13: Dormouse nest, no animal present.

				<p>Tube 18: Old dormouse nest. Tube 20: Dormouse nest, no animal present. Tube 21: Dormouse nest, no animal present. Tube 24: Dormouse nest partially destroyed by other small mammal, no animal present.</p>
H089				
Survey visit	Date	Dormouse signs Y/N	Dormouse evidence	Notes
Deployment	27.04.22	N/A	N/A	N/A
1	11.05.22	N/A	N/A	N/A
2	16.06.22	N/A	N/A	N/A
3	25.07.22	N/A	N/A	N/A
4	15.08.22	N/A	N/A	N/A
5	26.09.22	N/A	N/A	N/A
6	29.10.22	N/A	N/A	N/A
7 + collection	20.11.22	N/A	N/A	N/A
H136				
Survey visit	Date	Dormouse signs Y/N	Dormouse evidence	Notes
Deployment	28.04.22	N	N/A	N/A
1	16.08.22	Y	1x adult, 5x nests	<p>Tube 4: 1x adult escaped from nest. Tube 9: Dormouse nest, no animal present. Tube 11: Dormouse nest, no animal present. Tube 13: Dormouse nest, no animal present. Tube 14: Dormouse nest, no animal present.</p>
2	30.09.22	Y	2x female adult, 4x juveniles, 9x nests	<p>Tube 2: 1x adult female in nest, 18.5g. Tube 4: Dormouse nest, no animal present. Tube 7: 1x adult female and 4 juveniles in nest. Juveniles average 10g, mother not weighed. Tube 8: Dormouse nest, no animal present. Tube 9: Dormouse nest with wood mouse. Tube 10: Dormouse nest, shrew inside. Tube 11: Dormouse nest, no animal present. Tube 13: Dormouse nest, no animal present. Tube 14: Dormouse nest, no animal present.</p>
3	28.10.22	Y	9x nests. 4x juveniles	<p>Tube 1: 4x juveniles escaped nest on approach Tube 2: Dormouse nest, no animal present. Tube 4: Dormouse nest, no animal present. Tube 7: Dormouse nest, no animal, present. Tube 8: Dormouse nest, no animal present. Tube 9: Dormouse nest, no animal present. Tube 11: Dormouse nest, no animal present. Tube 13: Dormouse nest, no animal present. Tube 14: Dormouse nest, no animal present.</p>
4 + collection	29.11.22	Y	9x nests, 1x juvenile	<p>Tube 1: Old dormouse nest. Tube 2: Old dormouse nest. Tube 4: Old dormouse nest. Tube 7: Dormouse nest, no animal present. Tube 8: 1x juvenile, 14g, active in nest. Tube 9: Dormouse nest, no animal present. Tube 11: Old dormouse nest. Tube 13: Old dormouse nest. Tube 14: Old dormouse nest.</p>

H149				
Survey visit	Date	Dormouse signs Y/N	Dormouse evidence	Notes
Deployment	10.05.22	N	N/A	N/A
1	26.05.22	N	N/A	N/A
2	14.06.22	Y	2x male adult, 2x nests.	Tube 4: 1x male adult, 14g. Tube 5: 1x adult male, 16g. White tip on tail. Poorly structured nest. Tube 6: Beginning of dormouse nest.
3	15.07.22	Y	1x adult, 1x female juvenile, 3x nests.	Tube 2: 1x female juvenile dormouse, 14g, in poorly structured nest. Tube 6: Beginnings of dormouse nest. Tube 12: 1x adult dormouse escaped from nest on approach.
4	16.08.22	Y	5x nests.	Tube 2: Dormouse nest, no animal present. Tube 5: Old dormouse nest partially destroyed by other small mammal. Tube 6: Dormouse nest, no animal present. Tube 12: Dormouse nest, no animal present. Tube 14: Dormouse nest, no animal present.
5	27.09.22	Y	5x nests.	Tube 2: Old dormouse nest. Tube 6: Dormouse nest, no animal present. Tube 12: Dormouse nest, no animal present. Tube 13: Dormouse nest, no animal present. Tube 14: Dormouse nest, no animal present.
6	27.10.22	Y	1x juvenile, 5x nests.	Tube 2: 1x juvenile dormouse, 10g, showed signs of stress and subdued when placed back into nest. Tube 6: Dormouse nest, no animal present. Tube 12: Dormouse nest, no animal present. Tube 13: Old dormouse nest destroyed by other small mammal. Tube 14: Dormouse nest, no animal present.
7 + collection	30.11.22	Y	1x adult, 7x nests.	Tube 2: Old dormouse nest. Tube 6: Dormouse nest, no animal present. Tube 7: 1x adult dormouse escaped from nest on approach. Tube 11: Old dormouse nest destroyed by other small mammal. Tube 12: Dormouse nest, no animal present. Tube 13: Dormouse nest, no animal present. Tube 14: Old dormouse nest.
H154				
Survey visit	Date	Dormouse signs Y/N	Dormouse evidence	Notes
Deployment	28.04.22	N	N/A	N/A
1	10.05.22	N	N/A	N/A
2	14.06.22	N	N/A	N/A
3	15.07.22	N	N/A	N/A
4	16.08.22	N	N/A	N/A
5	27.09.22	Y	1x adult, 2x nests.	Tube 4: Dormouse nest, no animal present. Tube 20: 1x adult escaped from nest on approach.
6	27.10.22	Y	2x nests.	Tube 10: Beginning of dormouse nest. Tube 19: Dormouse nest, no animal present.

7 + collection	30.11.22	Y	3x nests.	Tube 17: Dormouse nest started but destroyed by other small mammal. Tube 18: Dormouse nest, no animal present. Tube 19: Dormouse nest with 4x wood mice.
H155.				
Survey visit	Date	Dormouse signs Y/N	Dormouse evidence	Notes
Deployment	15.06.22	N	N/A	N/A
1	30.06.22	N	N/A	N/A
2	15.07.22	N	N/A	N/A
3	16.08.22	N	N/A	N/A
4	27.09.22	Y	1x male adult, 5x nests.	Tube 2: Dormouse nest with other small mammal food cache. Tube 7: Beginning of dormouse nest. Tube 9: Dormouse nest, no animal present. Tube 12: 1x male adult in nest. Tube 14: Dormouse nest, spiders within and difficult to open tube.
5	27.10.22	Y	1x male adult, 4x juveniles, 9x nests.	Tube 2: Dormouse nest, no animal present. Tube 7: Dormouse nest, no animal present. Tube 9: 3x juveniles escaped from nest on approach. Tube 11: 1x adult male in nest, 23.5g with white tip on tail. Tube 12: Dormouse nest, no animal present. Tube 13: Dormouse nest, no animal present. Tube 14: Dormouse nest, no animal present. Tube 17: 1x juvenile, 10.5g in nest. Tube 18: Dormouse nest, no animal present. Tube 19: Dormouse nest, no animal present.
6 + collection	30.11.22	Y	1x adult, 8x nests.	Tube 2: Dormouse nest, no animal present. Tube 7: Dormouse nest, no animal present. Tube 9: Dormouse nest, no animal present. Tube 11: Dormouse nest, no animal present. Tube 12: Dormouse nest, no animal present. Tube 13: 1x adult dormouse escaped from nest. Tube 14: Dormouse nest, no animal present. Tube 18: Dormouse nest, no animal present.
H156				
Survey visit	Date	Dormouse signs Y/N	Dormouse evidence	Notes
Deployment	15.06.22	N	N/A	N/A
1	30.06.22	N	N/A	N/A
2	15.07.22	N	N/A	N/A
3	16.08.22	N	N/A	N/A
4	27.09.22	N	N/A	Other small mammal nest with food cache.
5	27.10.22	N	N/A	N/A
6 + collection	30.11.22	N	N/A	N/A
H221				
Survey visit	Date	Dormouse signs Y/N	Dormouse evidence	Notes
Deployment	28.04.22	N	N/A	N/A
1	26.05.22	N	N/A	N/A
2	15.06.22	N	N/A	N/A

3	28.07.22	Y	2x nests.	Tube 16: Dormouse nest, no animal present. Tube 18: Dormouse nest, no animal present.
4	16.08.22	Y	3x nests.	Tube 12: Dormouse nest, no animal present. Tube 16: Dormouse nest, no animal present. Tube 18: Dormouse nest, no animal present.
5	30.09.22	Y	4x nests.	Tube 5: Dormouse nest, no animal present. Tube 12: Dormouse nest, no animal present. Tube 16: Dormouse nest, no animal present. Tube 18: Dormouse nest, no animal present.
6	31.10.22	Y	4x nests.	Tube 5: Dormouse nest, no animal present. Tube 12: Dormouse nest, no animal present. Tube 16: Dormouse nest, no animal present. Tube 18: Dormouse nest, no animal present.
7 + collection	29.11.22	Y	7x nests.	Tube 2: Dormouse nest, no animal present. Tube 3: Dormouse nest, no animal present. Tube 4: Dormouse nest, no animal present. Tube 5: Dormouse nest, no animal present. Tube 12: Old dormouse nest. Tube 16: Old dormouse nest now with wood mice. Tube 18: Old dormouse nest.
2047				
Survey visit	Date	Dormouse signs Y/N	Dormouse evidence	Notes
Deployment	24.05.22	N	N/A	N/A
1	31.05.22	N	N/A	N/A
2	16.06.22	N	N/A	N/A
3	25.07.22	N	N/A	N/A
4	15.08.22	Y	1x nest	Tube 12: Structured nest, small in size - likely dormouse although no animal present to confirm.
5 + Collection	20.11.22	N	N/A	N/A
2174				
Survey visit	Date	Dormouse signs Y/N	Dormouse evidence	Notes
Deployment	23.05.22	N	N/A	N/A
1	31.05.22	N	N/A	N/A
2	13.06.22	N	N/A	N/A
3	28.07.22	N	N/A	N/A
4	19.08.22	N	N/A	N/A
5	12.09.22	N	N/A	N/A
6	27.10.22	N	N/A	N/A
7 + collection	20.11.22	N	N/A	8x other small mammal nests recorded.
2196				
Survey visit	Date	Dormouse signs Y/N	Dormouse evidence	Notes
Deployment	25.05.22	N	N/A	N/A
1	30.05.22	N	N/A	N/A
2	16.06.22	N	N/A	N/A
3	29.07.22	N	N/A	N/A
4	18.08.22	Y	2x nests	Tube 5: Dormouse nest, no animals present. Tube 7: Cannot conclusively say dormouse, green leaves and grass, not a great deal of

				structure, may have been dormouse trashed by other small mammal.
5	26.09.22	Y	4x nests	Tube 1: Dormouse nest partially trashed by wood mouse present at time of survey. Tube 5: Old dormouse nest with wood mouse and young. Tube 6: Old dormouse nest with wood mice. Tube 8: Dormouse nest very structured, stripped bark, and grasses- wood mouse present in nest.
6	29.10.22	Y	4x nests	Tube 1: Old dormouse nest. Tube 5: Old dormouse nest. Tube 7: Old dormouse nest. Tube 8: Old dormouse nest.
7 + collection	28.11.22	Y	4x nests	Tube 1: Old dormouse nest. Tube 5: Old dormouse nest. Tube 7: Old dormouse nest. Tube 8: Old dormouse nest.
2204				
Survey visit	Date	Dormouse signs Y/N	Dormouse evidence	Notes
Deployment	23.05.22	N	N/A	N/A
1	31.05.22	N	N/A	N/A
2	13.06.22	N	N/A	N/A
3	28.07.22	N	N/A	N/A
4	19.08.22	N	N/A	N/A
5	12.09.22	N	N/A	1x other small mammal nest, 1x other small mammal food cache.
6	27.10.22	Y	1x nest	Tube 29: Old dormouse nest with wood mouse present.
7 + collection	20.11.22	N	N/A	N/A
2250				
Survey visit	Date	Dormouse signs Y/N	Dormouse evidence	Notes
Deployment	25.05.22	N	N/A	N/A
1	31.05.22	N	N/A	N/A
2	13.06.22	N	N/A	N/A
3	28.07.22	N	N/A	N/A
4 + collection	19.08.22	N	N/A	N/A
2278				
Survey visit	Date	Dormouse signs Y/N	Dormouse evidence	Notes
Deployment	24.05.22	N	N/A	N/A
1	30.05.22	N	N/A	All 35 tubes surveyed.
2	13.06.22	N	N/A	All 35 tubes surveyed.
3	16.08.22	Y	1x adult female, 1x juvenile, 3x nests	Access permitted to only half the survey site – 16/35 tubes surveyed. Tube 21: Small woven dormouse nest, structured, no animals present. Tube 31: Adult female in woven structured nest, pregnant, 17g. Tube 33: Dormouse nest, juvenile dormouse escaped.

4	27.09.22	Y	3x female adult, 1x male adult, 9x juvenile, 4x nests	Access permitted to only half the survey site – 16/35 tubes surveyed. Tube 21: Grey eyes close x 2 not handled, mother absent. Tube 23: 5g young, female 20.5g. Tube 25: Structured dormouse nest male TS 18g female 12.5g. Tube 31: Min. of 3 pinks in nest, mother not weighed, handled to return to nest. Female small and still with some grey fur.
5	27.10.22	Y	1x male adult, 1x female adult, 6x nests	Access permitted to only half the survey site – 16/35 tubes surveyed. Tube 21: Dormouse nest, no animal present. Tube 23: 26.5g adult male, 22g adult female, nest. Tube 31: Previously maternity nest, no animals present. Tube 33: Dormouse nest, no animal present. Tube 34: Dormouse nest, no animal present. Tube 35: Dormouse nest, no animal present.
6 + collection	29.11.22	Y	14x nests	All 35 tubes surveyed and collected. Third survey for 19 tubes due to access restrictions. Tube 3: Old dormouse nest. Tube 5: Dormouse nest, no animal present. Tube 7: Dormouse nest, no animal present. Tube 9: Old dormouse nest. Tube 10: Dormouse nest, no animal present. Tube 12: Dormouse nest, no animal present. Tube 14: Dormouse nest, no animal present. Tube 16: Dormouse nest, no animal present. Tube 21: Dormouse nest with dead rat in tube. Tube 23: Old dormouse nest. Tube 25: Old dormouse nest. Tube 31: Old dormouse nest. Tube 34: Old dormouse nest. Tube 35: Old dormouse nest.
2345				
Survey visit	Date	Dormouse signs Y/N	Dormouse evidence	Notes
Deployment	25.05.22	N	N/A	N/A
1	30.05.22	N	N/A	N/A
2	17.06.22	N	N/A	N/A
3	13.07.22	N	N/A	N/A
4 + collection	31.08.22	N	N/A	N/A

4. Conclusion

Hazel dormice are mobile species, and although they are not known to disperse great distances, it is accepted that they will move across connected suitable habitat.

No evidence of hazel dormouse was recorded along hedgerow H156, however this hedgerow is connected at either end to H154 and H155 which both had confirmed dormouse presence. H156 is a younger hedgerow than H154 and H155, however the same plant food sources are present in all three hedgerows. It is therefore considered that H156 should be treated as having an *assumed presence* for hazel dormice, given their connectivity and the abundance of dormice found in H154 and H155.

Similarly, no evidence of hazel dormouse was recorded in H089, however this survey site relates to the southern side of a hedgerow, of which the northern side (H075) did have confirmed evidence of dormouse. It is advised that H075 and H085 should be considered together to conclude presence of hazel dormouse in this hedgerow.

Finally, no evidence of hazel dormouse was recorded at TN041 however this survey site is connected at the northern end to both H079 and H085, both of which had confirmed presence of dormouse. This network of hedgerows is additionally connected to survey site 2047, which also had confirmed presence of dormouse. It is considered that TN041 should be treated as having *assumed presence* for hazel dormice, given the very few survey tubes established on this section of hedgerow and it's connection to the hedgerow network of H079, H085 and 2047.

5. REFERENCES

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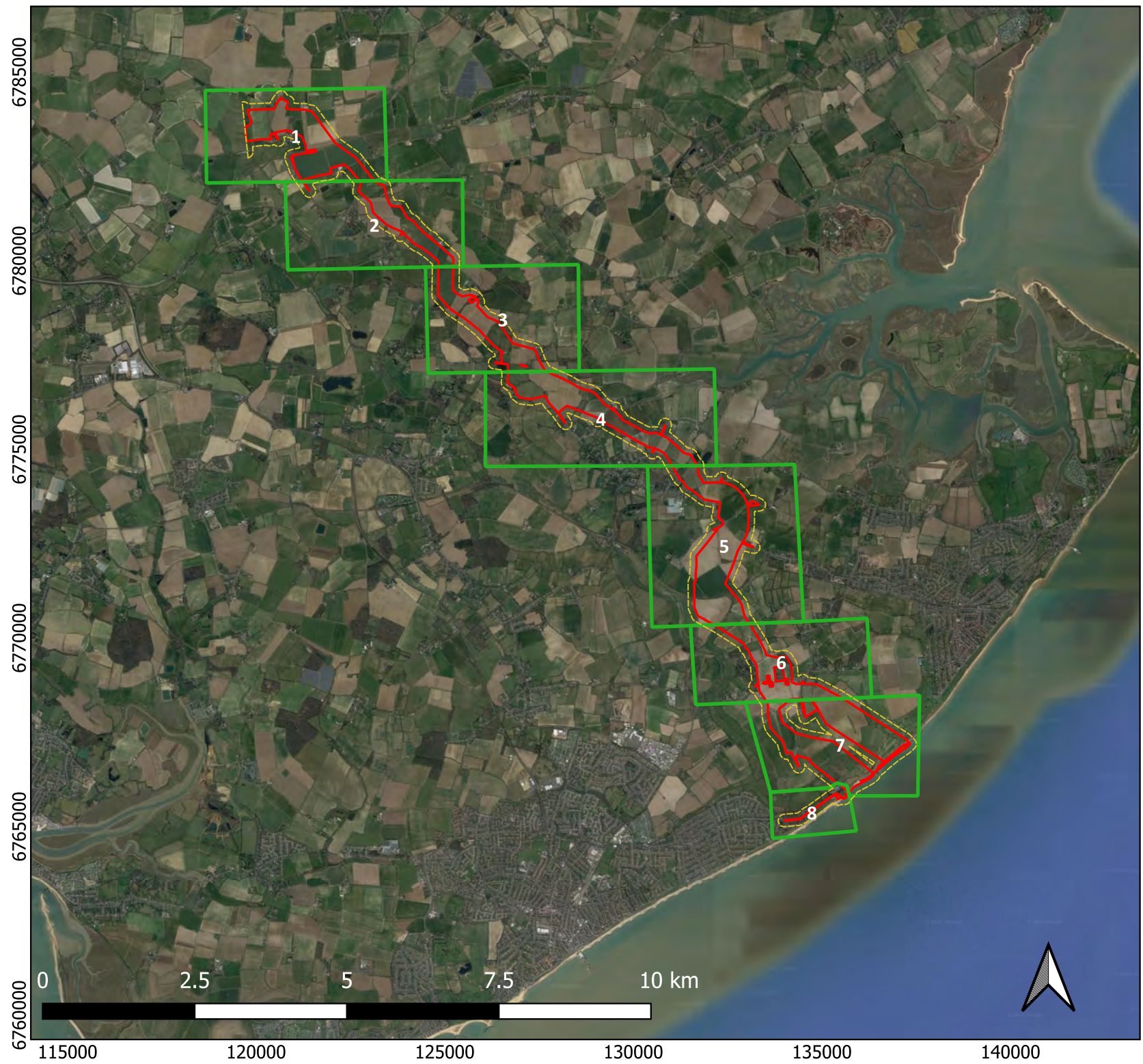
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

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APPENDIX A: Survey Location Site Plan



LEGEND

-  100m Buffer Zone
-  BNG Map Sections
-  Site Boundary

Site map including map sections 1-8 (Figure 2.)
FIGURE 1

PROJECT TITLE:
 Five Estuaries Off Shore Wind Farm Ltd.

CLIENT:
 Royal HaskoningDHV

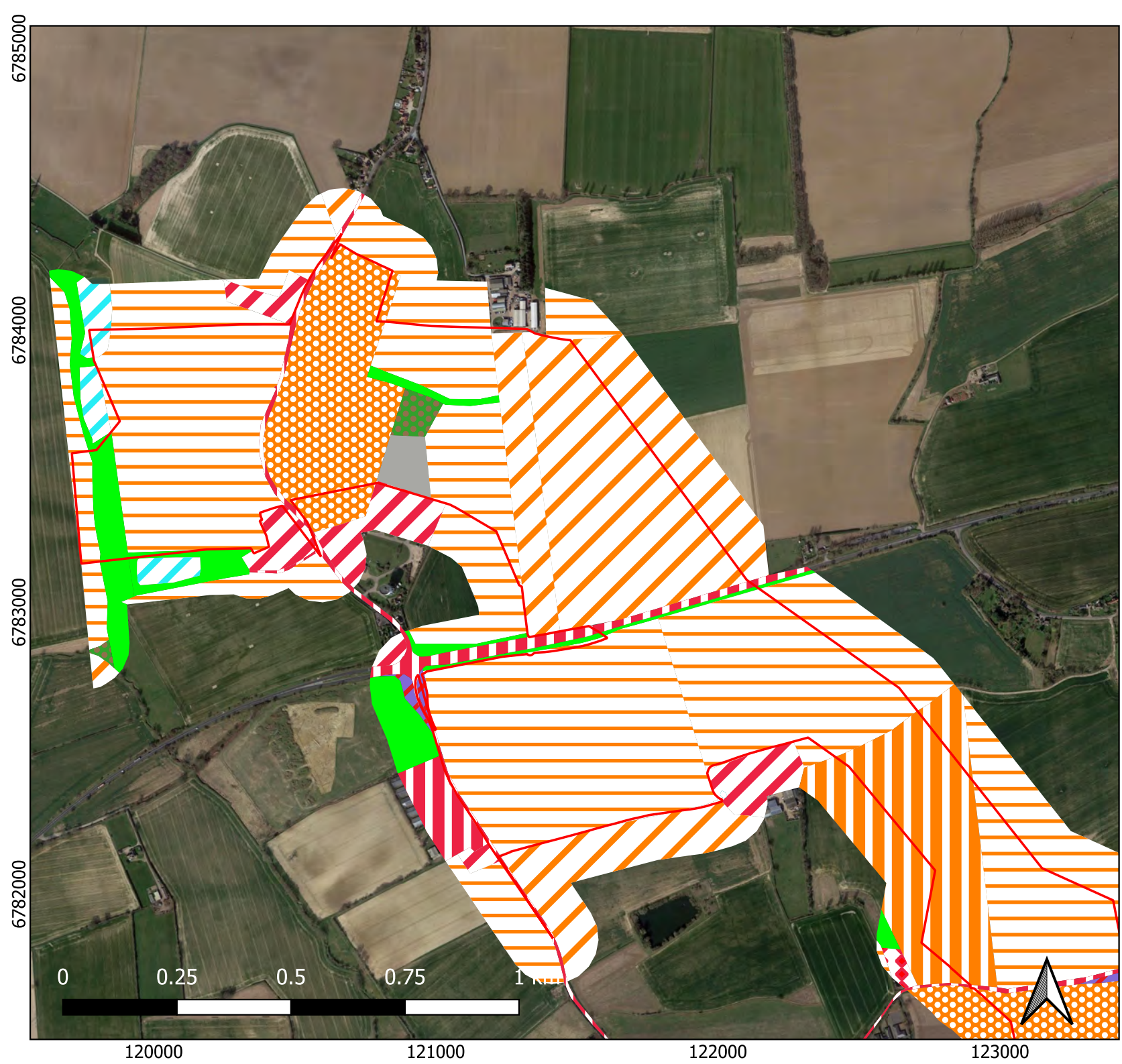
DATE:
 25.10.2022

PRODUCED BY:
 B. Turner

REF: 22043



ECOLOGY
 RESOURCES



LEGEND

-  c1 - arable and horticulture
-  c1b - temporary grass and clover leys
-  c1c - cereal crops
-  c1d - non-cereal crops
-  g4 - modified grassland
-  h3h - Mixed scrub
-  r1 - standing open water and canals
-  r1a - eutrophic standing waters
-  s - sparsely vegetated land
-  u1 - built-up areas and gardens
-  u1b - developed land, sealed surface
-  w1g - other woodland-broadleaved

Habitat map of section 1
FIGURE 2a

PROJECT TITLE:
Five Estuaries Off Shore Wind Farm Ltd.

CLIENT:
Royal HaskoningDHV

DATE:
25.10.2022

PRODUCED BY:
B. Turner

REF: 22043





LEGEND

- c1 - arable and horticulture
- c1b - temporary grass and clover leys
- c1c - cereal crops
- c1d - non-cereal crops
- g4 - modified grassland
- h3h - Mixed scrub
- r1a - eutrophic standing waters
- u1 - built-up areas and gardens
- u1b - developed land, sealed surface
- u1d - suburban mosaic
- w1g - other woodland-broadleaved

Habitat map of section 2
FIGURE 2b

PROJECT TITLE:
 Five Estuaries Off Shore Wind Farm Ltd.

CLIENT:
 Royal HaskoningDHV















DATE:
 25.10.2022

PRODUCED BY:
 B. Turner

REF: 22043





- LEGEND**
-  c1 - arable and horticulture
 - c1c - cereal crops
 -  c1d - non-cereal crops
 -  f2d - aquatic marginal vegetation
 -  g4 - modified grassland
 -  h3h - Mixed scrub
 -  r1a - eutrophic standing waters
 -  u1 - built-up areas and gardens
 -  u1b - developed land. sealed surface
 -  u1b6 - other developed land
 -  w1g - other woodland-broadleaved
 -  w1h - other woodland mixed
 -  w1h5 - Mixed broadleaved woodland
 -  w1h6 - Mixed conifer woodland
 -  w2c - other coniferous woodland

Habitat map of section 3
FIGURE 2c

PROJECT TITLE:
Five Estuaries Off Shore Wind Farm Ltd.

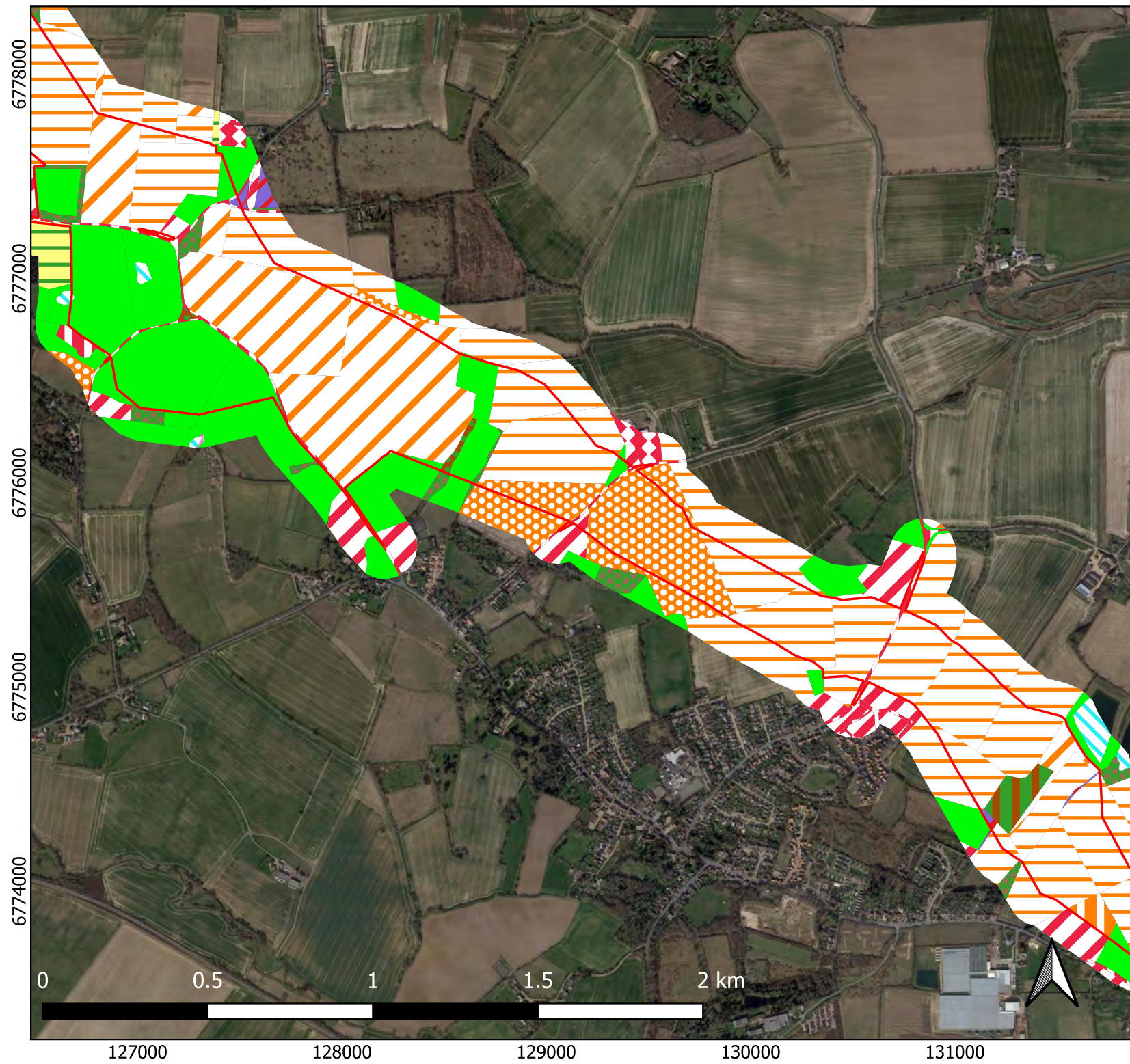
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DATE:
25.10.2022














PRODUCED BY:
B. Turner

REF: 22043





LEGEND

-  c1 - arable and horticulture
- c1c - cereal crops
-  c1d - non-cereal crops
-  f2d - aquatic marginal vegetation
-  g4 - modified grassland
-  h3d - Bramble scrub
-  r1a - eutrophic standing waters
-  u1 - built-up areas and gardens
-  u1b - developed land, sealed surface
-  u1b6 - other developed land
-  w1g - other woodland-broadleaved
-  w1h - other woodland mixed
-  w1h5 - Mixed broadleaved woodland
-  w1h6 - Mixed conifer woodland

Habitat map of section 4
FIGURE 2d

PROJECT TITLE:

Five Estuaries Off Shore Wind Farm Ltd.

CLIENT:

Royal HaskoningDHV

DATE:

25.10.2022

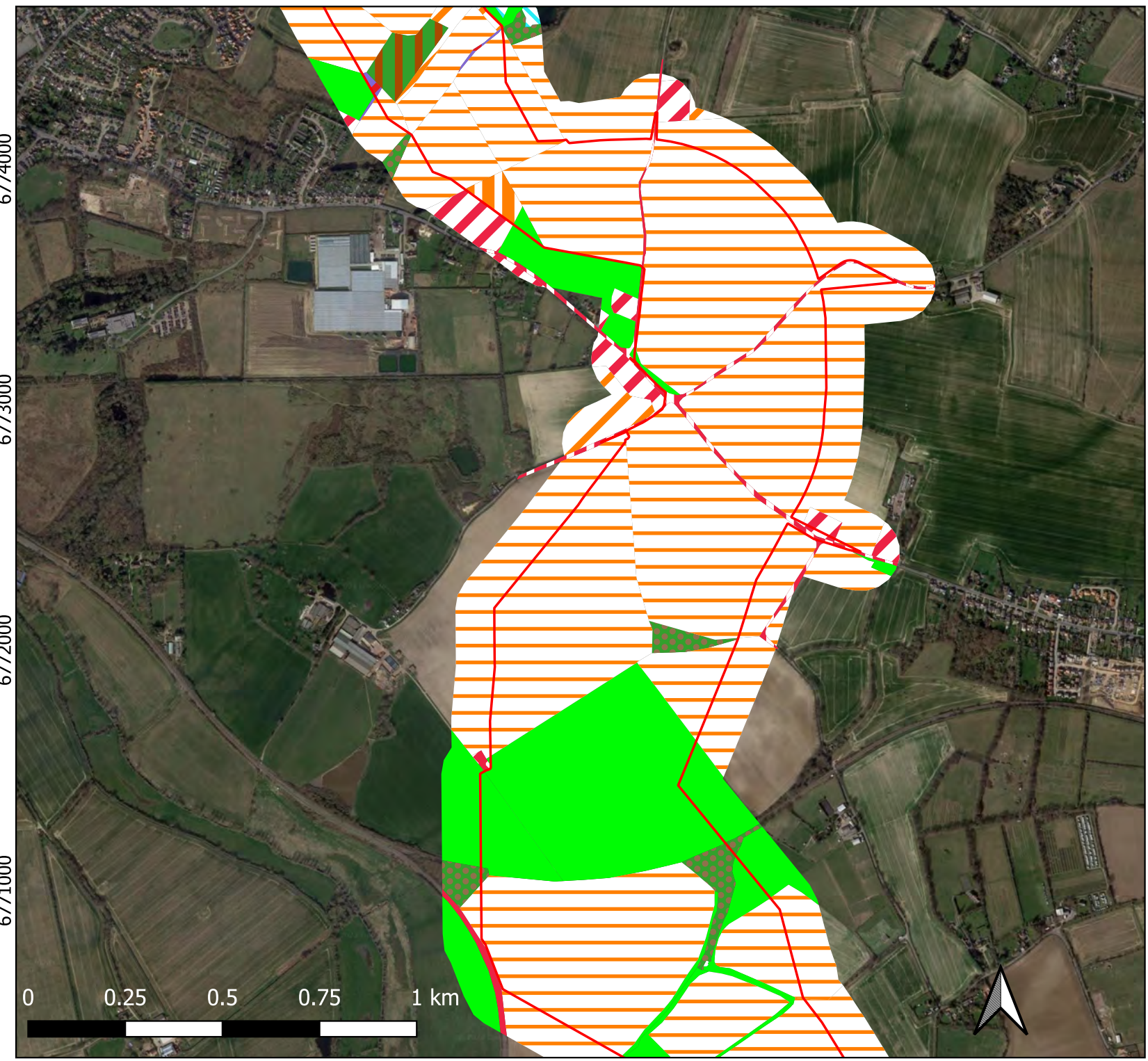
PRODUCED BY:

B. Turner














REF: 22043



ECOLOGY
 RESOURCES



LEGEND

-  c1 - arable and horticulture
-  c1b - temporary grass and clover leys
-  c1c - cereal crops
-  g4 - modified grassland
-  h3 - dense scrub
-  h3d - Bramble scrub
-  r1a - eutrophic standing waters
-  u1 - built-up areas and gardens
-  u1b - developed land, sealed surface
-  u1b6 - other developed land
-  u1e - built linear features
-  w1g - other woodland-broadleaved
-  w1h5 - Mixed broadleaved woodland

Habitat map of section 5
FIGURE 2e

PROJECT TITLE:
Five Estuaries Off Shore Wind Farm Ltd.

CLIENT:
Royal HaskoningDHV

DATE:
25.10.2022

PRODUCED BY:
B. Turner

REF: 22043





LEGEND

- c1 - arable and horticulture
- c1a8 - Game bird mix strips and corners
- c1c - cereal crops
- c1d - non-cereal crops
- g1c - bracken
- g3 - neutral grassland
- g4 - modified grassland
- h3d - Bramble scrub
- h3h - Mixed scrub
- u1 - built-up areas and gardens
- u1b - developed land, sealed surface
- u1c - artificial unvegetated unsealed surface
- w1g - other woodland-broadleaved
- w1g7 - Other broadleaved woodland
- w1h - other woodland mixed
- w1h5 - Mixed broadleaved woodland

Habitat map of section 6
FIGURE 2f

PROJECT TITLE:
Five Estuaries Off Shore Wind Farm Ltd.

CLIENT:
Royal HaskoningDHV

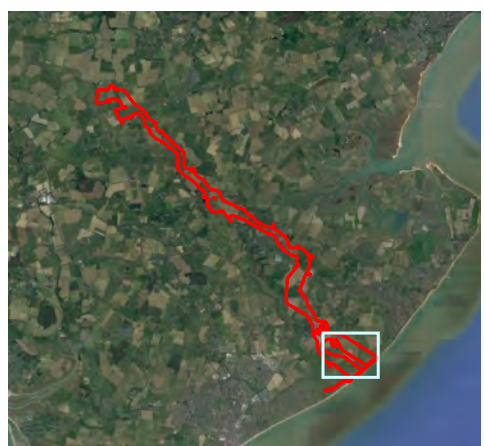
DATE:
25.10.2022

PRODUCED BY:
B. Turner

REF: 22043



ECOLOGY
RESOURCES



- LEGEND**
- Out of scope (Holland Haven Marsh SSSI)
 - c1a8 - Game bird mix strips and corners
 - c1c - cereal crops
 - c1d - non-cereal crops
 - g4 - modified grassland
 - h3d - Bramble scrub
 - h3h - Mixed scrub
 - r1a - eutrophic standing waters
 - s1 - inland rock
 - t1 - littoral rock
 - u1 - built-up areas and gardens
 - u1b - developed land, sealed surface
 - u1c - artificial unvegetated unsealed surface

Habitat map of section 7
FIGURE 2g

PROJECT TITLE:
 Five Estuaries Off Shore Wind Farm Ltd.

CLIENT:
 Royal HaskoningDHV

DATE:
 25.10.2022

PRODUCED BY:
 B. Turner

REF: 22043



ECOLOGY
 RESOURCES



LEGEND

-  Out of scope (Holland Haven Marsh SSSI)
-  g4 - modified grassland
-  h3h - Mixed scrub
-  r2 - rivers and streams
-  t1 - littoral rock
-  t2 - littoral sediment
-  t2h - Beach
-  u1 - built-up areas and gardens
-  u1b - developed land, sealed surface
-  u1b5 - buildings
-  u1b6 - other developed land
-  u1c - artificial unvegetated unsealed surface
-  u1d - suburban mosaic
-  w1h - other woodland mixed

Habitat map of section 8
FIGURE 2h

PROJECT TITLE:
Five Estuaries Off Shore Wind Farm Ltd.

CLIENT:
Royal HaskoningDHV

DATE:
25.10.2022

PRODUCED BY:
B. Turner

REF: 22043



APPENDIX B: Survey Metadata

H075			
Survey number	Date	Weather conditions	Dormice / evidence found
1	27.05.22	16°C. Sunny, dry with light breeze	N
2	16.06.22	21°C. Sunny, dry with light breeze	N
3	25.07.22	24°C. Sunny intervals, strong breeze, 70% cloud cover	N
4	15.08.22	27°C. Light breeze, cloud cover 70%	N
5	26.09.22	13°C. Changeable, light rain, strong breeze	N
6	29.10.22	18°C. Sunny intervals, moderate breeze, cloud cover 80%	N
7	20.11.22	10°C. Rain before survey, moderate breeze	Y

H079			
Survey number	Date	Weather conditions	Dormice / evidence found
1	11.05.22	16°C. Sunny/ dry/ light breeze	N
2	17.06.22	25°C. Sunny/ dry/ light breeze	N
3	25.07.22	24°C. Strong breeze/ humid/ cloud cover 60%	N
4	15.08.22	28°C. no breeze/ cloud cover 90 %	N
5	26.09.22	13°C. Changeable/ light on off rain/ cloud cover 90%	Y
6	28.10.22	20°C. Moderate breeze/ cloud cover 20%	Y
7	30.11.22	8°C. Cold, light breeze.	Y

H085			
Survey number	Date	Weather conditions	Dormice / evidence found
1	11.05.22	16°C. Sunny/ dry/ light breeze	N
2	17.06.22	25°C. Sunny/ dry/ light breeze	N
3	25.07.22	24°C. strong breeze/ cloud cover 60%	Y
4	15.08.22	25°C. light breeze/ cloud cover 60%	Y
5	26.09.22	13°C. Changeable/ very windy with light rain on and off	Y
6	28.10.22	20°C. Sunny/ moderate breeze/ cloud cover 20%	Y
7	30.11.22	9°C. Cold, light breeze.	Y

H089			
Survey number	Date	Weather conditions	Dormice/evidence found
1	11.05.22	16°C. Sunny, dry, light breeze	N
2	16.06.22	21°C. Sunny, dry, light breeze	N
3	25.07.22	24°C. Sunny intervals, strong breeze, cloud cover 70%	N
4	15.08.22	27°C. light breeze, cloud cover 70 %	N
5	26.09.22	14°C. Changeable, light rain, very windy, cloud cover 60%	N
6	29.10.22	18°C. Overcast, moderate breeze, cloud cover 80%	N
7	20.11.22	10°C. Rain prior to survey, moderate breeze.	N

H136			
Survey number	Date	Weather conditions	Dormice/evidence found
1	16.08.22	22°C. Light rain/ moderate breeze/ cloud cover 80%	Y
2	30.09.22	12°C. Sunny spells/ light breeze/ cloud cover 80%	Y
3	28.10.22	18°C. Sunny/ light breeze/ rain overnight	Y
4	29.11.22	10°C. Light cold breeze, cloud cover 50%.	Y

H149			
Survey number	Date	Weather conditions	Dormice/evidence found
1	10.05.22	15°C. Sunny/ dry/ light breeze	N
2	14.06.22	20°C. Sunny/ dry/ light breeze	Y
3	15.07.22	23°C. Sunny/ light breeze/ cloud cover 5%	Y
4	16.08.22	23°C. light breeze/ dry/ cloud cover 90%,	Y
5	27.09.22	11°C. Cold wind/ cloud cover 80%	Y
6	27.10.22	18°C. Sunny/ light breeze/ cloud cover 5%	Y
7	30.11.22	10°C. Light breeze, cloud cover 70%	Y

H154			
Survey number	Date	Weather conditions	Dormice/evidence found
1	10.05.22	13°C. Sunny/ dry/ light breeze	N
2	14.06.22	22°C. Sunny/ dry/ light breeze	N
3	15.07.22	23°C. Sunny/ light breeze/ cloud cover 20%,	N
4	16.08.22	24°C. Light breeze/ cloud cover 70%	N
5	27.09.22	11°C. Cloud cover 70%	Y
6	27.10.22	15°C. Moderate breeze/ light rain before survey	Y
7	30.11.22	11°C. Light breeze, cloud cover 80%	Y

H155			
Survey number	Date	Weather conditions	Dormice/evidence found
1	30.06.22	19°C. Sunny spells/ dry/ slight breeze/ cloud cover 60%	N
2	15.07.22	23°C. Sunny/ slight breeze/ cloud cover 20%,	N
3	16.08.22	24°C. Light breeze/ cloud cover 70%	N
4	27.09.22	11°C. Strong wind/ cloud cover 60%	Y
5	27.10.22	15°C. Sunny/ moderate breeze/ light rain before survey/ cloud cover 30%	Y
6	30.11.22	11°C. Light breeze, cloud cover 80%	Y

H156			
Survey number	Date	Weather conditions	Dormice/evidence found
1	30.06.22	19°C. Sunny spells/dry/ slight breeze/ cloud cover 80%	N
2	15.07.22	23°C. Sunny/ slight breeze/ cloud cover 20%,	N
3	16.08.22	24°C. Light breeze/ cloud cover 70%	N
4	27.09.22	13°C. Light breeze/ cloud cover 40%	N

5	27.10.22	15°C. Sunny/ moderate breeze/ light rain before survey/ cloud cover 30%	N
6	30.11.22	10°C. Light breeze, cloud cover 80%	N

H221			
Survey number	Date	Weather conditions	Dormice/evidence found
1	26.05.22	16°C. Sunny/ dry with light breeze	N
2	15.06.22	23°C. Very sunny with no cloud cover/ dry with light breeze	N
3	28.07.22	22°C. Moderate breeze/ 60% cloud cover	Y
4	16.08.22	25°C. Light rain/ light breeze/ cloud cover 90%	Y
5	30.09.22	9°C. Cool breeze/ very misty	Y
6	31.10.22	17°C. Moderate breeze/ light rain before survey/ cloud cover 80%	Y
7	29.11.22	8°C. Light breeze, cloud cover 70%	Y

TN041			
Survey number	Date	Weather conditions	Dormice/evidence found
1	11.05.22	16°C. Sunny/ dry/ light breeze	N
2	17.06.22	25°C. Sunny/ dry/ light breeze	N
3	25.07.22	24°C. Sunny/ strong breeze	N
4	15.08.22	25°C. Light breeze/ cloud cover 70%	N
5	26.09.22	13°C. Changeable/ on off light rain/ cloud cover 90%	N
6	28.10.22	20°C. Sunny/ moderate breeze/ cloud cover 20%	N
7	30.11.22	8°C. Cold light breeze, cloud cover 90%, light rain	N

TN410			
Survey number	Date	Weather conditions	Dormice/evidence found
1	27.05.22	15°C. Sunny/ dry/ light breeze	N
2	17.06.22	25°C. Sunny/ dry/ light breeze	N
3	28.07.22	22°C. Moderate breeze/ cloud cover 50%	N
4	31.08.22	21°C. Sunny/ dry/moderate breeze	N
5	26.09.22	13°C. Changeable/ moderate wind/ cloud starting at 70%	Y
6	28.10.22	18°C. Light breeze/ overnight rain/ cloud cover 0%	Y
7	30.11.22	9°C. Light breeze, cloud cover 80%	Y

TN503			
Survey number	Date	Weather conditions	Dormice/evidence found
1	30.05.22	15°C. Sunny/ dry/ light breeze	N
2	16.06.22	21°C. Sunny/ dry/ light breeze	N
3	29.07.22	24°C. Moderate breeze/ cloud cover 80%	N
4	18.08.22	23°C. Moderate breeze/ cloud cover 40%	N
5	28.09.22	14°C. Sunny/ dry/ moderate breeze/ cloud cover 50%	Y
6	28.11.22	10°C. Sunny, light breeze, cloud cover 5%	Y

TN509			
Survey number	Date	Weather conditions	Dormice/evidence found
1	26.05.22	15°C. Sunny/ dry/ light breeze	N
2	14.06.22	19°C. Sunny/ dry/ light breeze	N
3	29.07.22	24°C. Moderate breeze/ cloud cover 80%	N
4	19.08.22	26°C. Light breeze/ cloud cover 40%	N
5	30.09.22	9°C. Misty/ light breeze	Y
6	31.10.22	16°C. Light breeze/ cloud cover 70%	Y
7	29.11.22	9°C. Light breeze, cloud cover 80%	Y

TN525			
Survey number	Date	Weather conditions	Dormice/evidence found
1	10.05.22	15°C. Sunny/ dry/ light breeze	N
2	14.06.22	21°C. Sunny/ dry/ light breeze	N
3	13.07.22	25°C. Sunny/ cloud cover 20%	N
4	31.08.22	23°C. Sunny/ dry/ moderate breeze	N
5	13.09.22	17°C. Sunny spells/ moderate breeze/ cloud cover 40%	N
6	31.10.22	16°C. Light rain/ moderate breeze/ cloud cover 90%	N
7	29.11.22	8°C. Light breeze, misty. Cloud cover 80%	N

2047			
Survey number	Date	Weather conditions	Dormice/evidence found
1	31.05.22	15°C. Sunny/ dry/ moderate breeze	N
2	16.06.22	21°C. Sunny/ dry/ moderate breeze	N
3	25.07.22	24°C. Strong breeze/ cloud cover 60%, sunny intervals	N
4	15.08.22	25°C. Light breeze/ cloud cover 80%,	Y
5	20.11.22	10°C. Rain on and off during survey, moderate breeze. Cloud cover 100%	N

2174			
Survey number	Date	Weather conditions	Dormice/evidence found
1	31.05.22	15°C. Sunny/ dry/ moderate breeze	N
2	13.06.22	19°C. Sunny/ dry/ light breeze/ cloud cover 30%	N
3	28.07.22	23°C. Moderate breeze/ cloud cover 60%	N
4	19.08.22	26°C. Light breeze/ cloud cover 80%,	N
5	12.09.22	22°C. Dry/slight breeze/ cloud cover 80%	N
6	27.10.22	15°C. Moderate breeze, cloud cover 60%	N
7	20.11.22	10°C. Rain on and off during survey, moderate breeze. Cloud cover 100%	N

2196			
Survey number	Date	Weather conditions	Dormice/evidence found
1	30.05.22	15°C. Sunny/ dry/ moderate breeze	N
2	16.06.22	21°C. Sunny/ dry/ moderate breeze	N
3	29.07.22	24°C. Moderate breeze/ cloud cover 80%	N
4	18.08.22	24°C. Moderate breeze/ humid/ Cloud cover 60%	Y
5	28.09.22	14°C. Sunny/ moderate breeze/ dry	Y
6	29.10.22	18°C. Sunny intervals/ moderate breeze/ cloud cover 90%	Y
7	28.11.22	10 °C. Sunny, light breeze. Cloud cover 5%	Y

2204			
Survey number	Date	Weather conditions	Dormice/evidence found
1	31.05.22	15°C. Sunny/ dry/ moderate breeze	N
2	13.06.22	19°C. Sunny/ dry/ moderate breeze/ cloud cover 30%	N
3	28.07.22	23°C. Moderate breeze/ cloud cover 60%	N
4	19.08.22	23°C. Moderate breeze/ cloud cover 90%	N
5	12.09.22	22°C. Dry/ slight breeze/ cloud cover 80%,	N
6	27.10.22	15°C. Overcast/ light breeze/ cloud cover 90%,	Y
7	20.11.22	9°C. Rain on and off during survey. Moderate breeze, cloud cover 100%.	N




2250			
Survey number	Date	Weather conditions	Dormice/evidence found
1	31.05.22	15°C. Sunny/ dry/ moderate breeze	N
2	13.06.22	19°C. Sunny/ dry/ moderate breeze/ cloud cover 30%	N
3	28.07.22	23°C. Light rain/ no breeze/ cloud cover 90%	N
4	19.08.22	23°C. Moderate breeze/ cloud cover 90%	N





2278			
Survey number	Date	Weather conditions	Dormice/evidence found
1	30.05.22	15°C. Sunny/ dry/ moderate breeze	N
2	13.06.22	22°C. Sunny/ dry/ moderate breeze/ cloud cover 20%	N
3	16.08.22	23°C. Light rain/ no breeze/ cloud cover 90%	Y
4	27.09.22	13°C. Sunny/ moderate wind	Y
5	27.10.22	19°C. Sunny/ light breeze/ cloud cover 20%/ earlier rain	Y
6	29.11.22	10°C. Light breeze, cloud cover 50%	Y

2345			
Survey number	Date	Weather conditions	Dormice/evidence found
1	30.05.22	15°C. Sunny/ dry/ moderate breeze	N
2	17.06.22	25°C. Sunny/ dry/ moderate breeze	N
3	13.07.22	25°C. Sunny, cloud cover 20%	N
4	31.08.22	24°C. Dry, moderate breeze, sunny	N





APPENDIX C: Survey Photographs

Survey Site	Month	Photo(s)	Description
H085	July		1 female adult, 4 juveniles, nest
	October		Nest, no animal present
H136	August		4x nests with no animal present. Single adult escaped on approach to one nest.

Survey Site	Month	Photo(s)	Description
			
	September		<p>1x female adult, 1x nest with no animal present.</p>
H149	July		<p>1x other small mammal nest. 1x nest with female juvenile dormouse. 1x nest with escaped adult.</p>

Survey Site	Month	Photo(s)	Description
			
	August		2x dormouse nests with no animal present.
	September		Dormouse nest with no animal present.
H154	September		Dormouse nest with no animal present.

Survey Site	Month	Photo(s)	Description
	October		Dormouse nest with no animal present.
H155	September		1x dormouse nest with other small mammal food cache. 1x beginning of nest with stripped bark. 1x dormouse nest with no animal present. 1x male adult.
	October		1x nest with 3 escaped juveniles. 1x male adult. 1x dormouse nest with no animal present. 1x juvenile.

Survey Site	Month	Photo(s)	Description
			
H221	July		Dormouse nest with no animal present.
	August		Dormouse nest with no animal present.
	September		3x dormouse nests with no animals present.

Survey Site	Month	Photo(s)	Description
			
TN410	October		2x juveniles in box with nest.



ECOLOGY
RESOURCES

**eDNA
Great Crested
Newt
Results Report**

**5 Estuaries
Offshore Wind
Farm Ltd**

August 2022

The logo for FIVE ESTUARIES features the word 'FIVE' in a sans-serif font. The 'I' is grey, 'V' is purple, and 'E' is pink. To the right of 'FIVE' are three wavy lines representing water, colored blue, green, and yellow from top to bottom.

FIVE
ESTUARIES
OFFSHORE WIND FARM

FIVE ESTUARIES
OFFSHORE WIND FARM
PRELIMINARY ENVIRONMENTAL
INFORMATION REPORT

VOLUME 5, ANNEX 4.5: GREAT CRESTED
NEWT SURVEY REPORT: SOUTH OF A120

Document Reference 004685545-01
Revision A
Date August 2022



Status	Name	Date
Draft	Francesca Austin BSc (Hons) QCIEEM	20/10/2022
Rev 1	Gavin Mullan BA (Hons) MCIEEM	01/11/22
Rev 2	Gavin Mullan BA (Hons) MCIEEM	17/11/22
Rev 3		

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EXECUTIVE SUMMARY

Ecology Resources Limited was commissioned by Royal HaskoningDHV on behalf of Five Estuaries Offshore Wind Farm Limited (VE OWFL), to undertake Habitat Suitability Index (HSI) and environmental DNA (eDNA) sampling of suitable standing water bodies within the onshore project area plus a 250m buffer.

In total 106 standing water bodies were identified as having potential for great crested newts, two of which were found incidentally during site walkovers.

A total of 84 HSI assessments and eDNA sampling surveys were completed within the great crested newt survey area, 15 of which returned positive eDNA results confirming great crested newt presence. The outstanding 22 standing water bodies were not subject to HSI assessments and eDNA sampling due to land access restrictions or were unsuitable at time of survey.

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1. INTRODUCTION

Ecology Resources Limited was commissioned by Royal HaskoningDHV on behalf of Five Estuaries Offshore Wind Farm Limited (VE OWFL), to undertake Habitat Suitability Index (HSI) and environmental DNA (eDNA) sampling for great crested newt *Triturus cristatus* for suitable standing water bodies within the onshore project area plus a 250m buffer (herein ‘the great crested newt survey area’).

1.1 Project Background

Five Estuaries Offshore Windfarm (VE) is a proposed extension to the operational Galloper Offshore Wind Farm (OWF) which consists of 56 wind turbine generators (WTGs). The VE will comprise up to 79 WTGs situated within two array areas to the east of the operational Galloper OWF. The array areas will be located approximately 30km off the coast of Suffolk, England.

Cables will connect the turbines to the offshore substation platforms and then export the power generated to shore. It is expected that there will be a number of inter-array cables, up to four export cables and up to two offshore substations platforms.

A landfall area has been identified between Holland-on Sea and Frinton-on-Sea on the Essex coast. The landfall point is yet to be determined but will be located within this area of coastline. A new VE onshore substation will be needed and will be constructed in an area to the north of the A120.

The VE cables will be installed underground between the landfall and the grid connection point north of the A120. A preferred corridor has not yet been determined with several corridors still under consideration at the time of writing. Potential substation land parcels and associated corridor options north of the A120 also remain under review at the time of writing.

A more detailed description of the project, several elements of which have yet to be finalised at this time, will be provided in the PEIR and ES in due course.

1.2 Legislation

Great crested newts are a European protected species, as such they are afforded a high level of protection. The animals and their eggs, breeding sites and resting places are protected by law. Great crested newts are fully protected under the following UK legislation / international agreements: Bern Convention 1979: Appendix II Strictly Protected Fauna Species, Wildlife & Countryside Act (as amended) 1981, The Conservation of Habitats and Species Regulations 2017 (The Conservation of Habitats and Species Regulations 2017 transposes into UK law the EU Habitats Directive Council Directive 92/43/EEC) and Countryside Rights of Way Act 2000 (CRoW 2000). Protection under these laws makes it an offence to: -

- intentionally kill, injure, or capture, or take great crested newts;
- deliberately take or destroy eggs of great crested newts;
- possess or control alive or dead great crested newt or any part or thing derived from them;
- intentionally or recklessly damage, destroy, or obstruct access to, any structure or place which great crested newts use for breeding, shelter or protection;
- intentionally or recklessly disturb great crested newts while occupying a structure or place which it uses for that purpose;

- sell, offer, or expose for sale, or possess or transport for the purpose of sale, any live or dead great crested newt or any part or thing derived from them. It is also an offence to publish or cause to be published any advertisement likely to be understood as conveying that great crested newts, or parts or derived things of them are bought, sold, or are intended to be. This applies to all stages in their life cycle;
- keep, or transport, or exchange great crested newts or any part or thing derived from them.

2.0 METHODOLOGY

The great crested newt eDNA survey was completed in accordance with Natural England’s *Technical advice note for field and laboratory sampling of great crested newt (Triturus cristatus) environmental DNA* (Biggs *et al.*, 2014). Each of the suitable standing water bodies were visited once and eDNA samples taken following the field protocol set out in Biggs *et al.* (2014).

In total 106 standing water bodies were identified as having potential for great crested newts, two of which were found incidentally during site walkovers (i.e. not part of the original scope). A total of 84 HSI assessments and eDNA sampling surveys were completed within the great crested newt survey area.

2.1 Habitat Suitability Index

The HSI for great crested newts is a measure of habitat suitability, where water bodies are assessed and scored on the following ten key variables which are known to influence breeding populations of great crested newts, in accordance with standard methods (ARG UK, 2010):

- geographic location;
- water body area;
- water body permanence;
- water quality;
- water body shading;
- presence/impact of waterfowl;
- fish stocks;
- number of water bodies within 1km;
- terrestrial habitat around the water body; and
- macrophyte cover of the water body

Table 1: Water body suitability category based on HSI score

HSI Score	Water body Suitability
<0.5	Poor
0.5 – 0.59	Below Average
0.6 – 0.69	Average
0.7- 0.79	Good

2.2 eDNA Water Sampling

The eDNA sampling collection was by led Johnnie Johnson, Great Crested Newt Level 1 licence holder (2018-33728-CLS-CLS). Each of the suitable water bodies was visited once, and all standing water bodies

were subject to eDNA analysis irrespective of their HSI score. The samples were taken following the field protocol set out in Biggs *et al.* (2014). Visits were made between late-April and mid-June, during the newt breeding season (survey dates provided in Table 3, page 13.)



Samples underwent laboratory analysis by Surescreen Scientifics Ltd. and followed the laboratory protocol detailed in Biggs *et al.* (2014). The laboratory testing provided a 'presence / likely absence' result for each standing water body. The laboratory testing provided a 'presence / likely absence' result for each standing water body, shown in Appendix B.


2.3 Survey Limitations



Most of the standing water bodies were surveyed and assessed without any limitations to survey, however 22 standing water bodies were not surveyed due to restricted land access (10 water bodies) and water body unsuitability i.e., dry at time of survey (12 water bodies). Table 2 page 7 identifies the reasons water bodies were not surveyed.



Table 2: Standing Water Bodies not Surveyed

Ponds labelled with PO preceding the number are also ponds within the North Falls survey area

Water body ID	Photograph	Constraint	Land Parcel Number and Grid Reference
1	Photograph unavailable	No pond present, does not exist	LPN 144; TM2113217604
12		No pond was present, waterbody was dry	LPN 93; TM2019219517
14		No pond was present, waterbody was dry	LPN 93; TM2020519520
21	Photograph unavailable	No access granted	LPN 46; TM1924321450
31	Photograph unavailable	No access granted	LPN 351; TM1764823088
32	Photograph unavailable	No access granted	LPN 352; TM1764723156

Water body ID	Photograph	Constraint	Land Parcel Number and Grid Reference
PO32	Photograph unavailable	No pond was present, waterbody was dry	LPN 163; TM2170117823
33	Photograph unavailable	No access granted	LPN 356; TM1769323220
PO33		No pond was present, waterbody was dry	LPN 176; TM2234318446
35	Photograph unavailable	No access granted	LPN 337; TM1726223355
58	Photograph unavailable	No access granted	LPN 1273; TM15481 4343
59	Photograph unavailable	No access granted	LPN 1273; TM1535224415

Water body ID	Photograph	Constraint	Land Parcel Number and Grid Reference
67		No pond was present, waterbody was dry	LPN 1112; TM1433026076
68	Photograph unavailable	No access granted	LPN 1013; TM1368026232
PO70		Waterbody is dry	LPN 93; TM2023219499

Water body ID	Photograph	Constraint	Land Parcel Number and Grid Reference
71	Photograph unavailable	Waterbody is dry	LPN 985; TM1339326865
PO77		No pond was present, waterbody was dry	LPN 90; TM1991920198
PO80	Photograph unavailable	No access granted	No LPN; TM1939521824
PO110		No pond was present, waterbody was dry	LPN 235; TM1565224379

Water body ID	Photograph	Constraint	Land Parcel Number and Grid Reference
IP0192		Temporary pond but the waterbody was dry	No LPN; TM1528326070
PO196	Photograph unavailable	No access granted	No LPN; TM1859723220


3.0 RESULTS



In total 106 standing water bodies were identified within the great crested newt survey area, two of which were identified as incidental finds during site walkovers, a location plan of all the water bodies that were assessed within the great crested newt survey can be found in Appendix A; Figures 1a – 1e. The ponds identified in table 1 that were dry at the time of survey are excluded from these figures.



Twenty two of the standing water bodies were either unsuitable or inaccessible for reasons identified in Table 2: Water Bodies not surveyed, Page 7. As a result, these were not subject to HSI assessments and eDNA sampling.



A total of 84 HSI assessments and eDNA sampling surveys were completed across the great crested newt survey area, 15 of which returned positive eDNA results confirming great crested newt presence. Those water bodies which returned positive eDNA results are detailed in Table 3. The full eDNA results for all waterbodies are shown in Appendix B.

Table 3: Water body HSI and Positive eDNA Results



Water body ID	Photograph	Grid Reference	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
6		TM 20262 19255	0.5	Below Average	Positive	eDNA – 16/05/2022



Water body ID	Photograph	Grid Reference	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
8		TM 20193 19342	0.58	Below Average	Positive	HSI - 20/09/2021 eDNA - 16/05/2022
10		TM 20121 19408	0.57	Below Average	Positive	HSI - 20/09/2021 eDNA - 16/05/2022


Water body ID	Photograph	Grid Reference	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
11		TM 20182 19480	0.49	Poor	Positive	HSI – 08/06/2022 eDNA – 08/06/2022
13		TM 20202 19503	0.53	Below Average	Positive	HSI - 20/09/2021 eDNA - 16/05/2022
24	*Image not available for this pond*	TM 19080 22018	0.7	Good	Positive	HSI – 09/06/2022 eDNA – 09/06/2022

Water body ID	Photograph	Grid Reference	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
25		TM 19728 22526	0.72	Good	Positive	HSI – 13/10/22 eDNA - 10/05/2022
50		TM 16475 23393	0.81	Excellent	Positive	HSI – 23/09/2022 eDNA - 12/05/2022

Water body ID	Photograph	Grid Reference	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
53		TM 16161 23542	0.63	Average	Positive	HSI – 12/40/21 eDNA - 11/05/2022
54		TM 16098 23859	0.61	Average	Positive	HSI – 11/05/2022 eDNA - 11/05/2022

Water body ID	Photograph	Grid Reference	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
57		TM 15739 24129	0.66	Average	Positive	HSI - 01/06/2022 eDNA - 01/06/2022
65		TM 15438 25475	0.65	Average	Positive	HSI – 10/05/2022 eDNA – 10/05/2022

Water body ID	Photograph	Grid Reference	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
72		TM 12979 26811	0.72	Good	Positive	HSI – 01/06/2022 eDNA – 01/06/2022
PO102		TM 16082 23551	0.80	Excellent	Positive	HSI – 24/09/2021 eDNA - 11/05/2022

Water body ID	Photograph	Grid Reference	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
PO192		TM 15211 26033	0.78	Good	Positive	HSI – 11/05/2022 eDNA – 11/05/2022

4.0 CONCLUSION

In total 106 standing water bodies were identified as having potential for great crested newts, two of which were found incidentally during site walkovers.

A total of 84 HSI assessments and eDNA sampling surveys were completed across the great crested newt survey area, 15 of which returned positive eDNA results confirming great crested newt presence. The outstanding 22 standing water bodies were not subject to HSI assessments and eDNA sampling due to land access restrictions (10 waterbodies) or were unsuitable (12 waterbodies) at time of survey.

5.0 REFERENCES

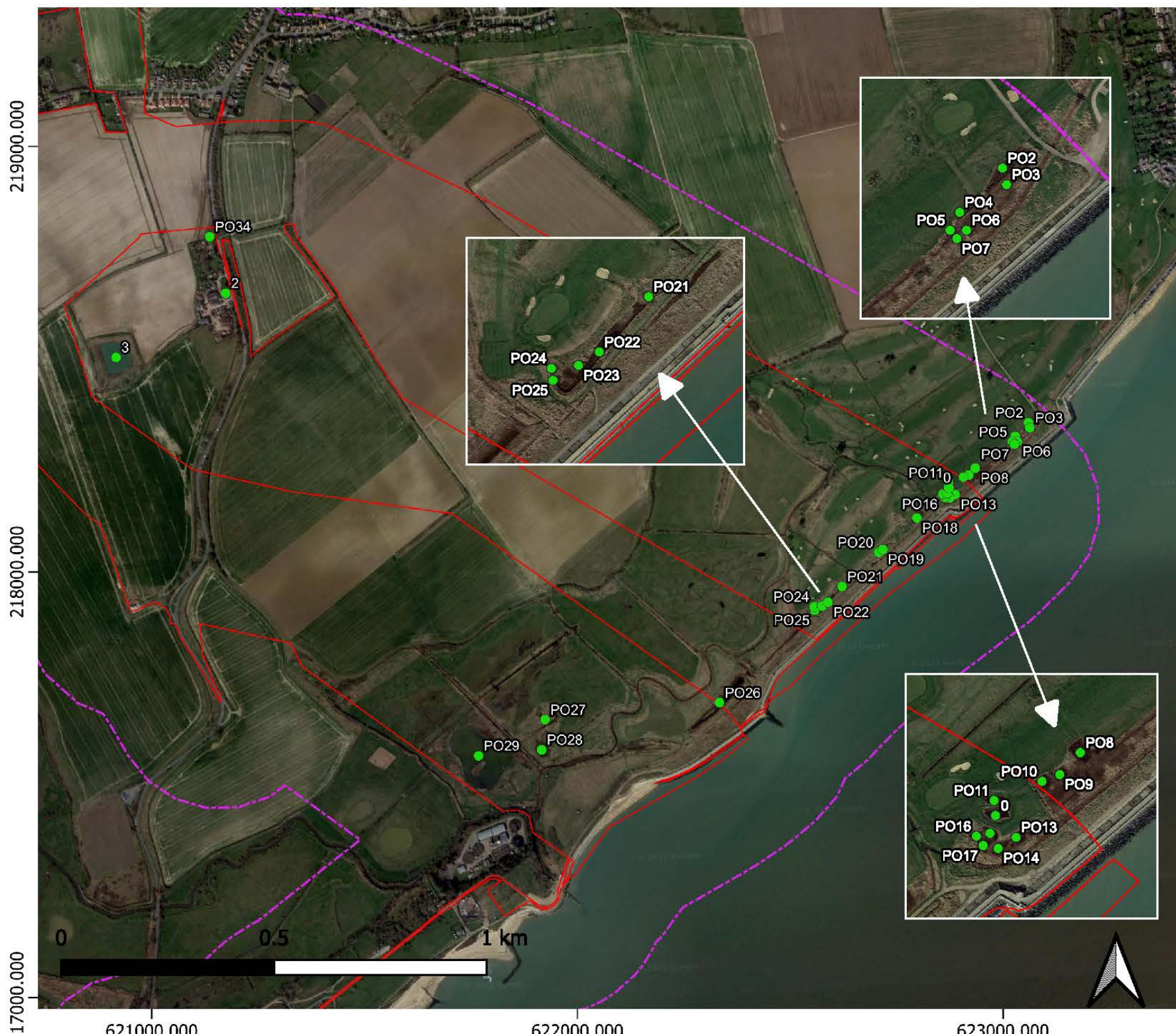
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APPENDIX A: Standing Water Body Location Plan



LEGEND

- GCN Absent
- GCN Present
- No Access Granted
- eDNA Not Complete
- Five Estuaries 250m Buffer
- Five Estuaries Onshore

**GREAT CRESTED NEWT WATERBODIES
FIGURE 1a**

PROJECT TITLE:
5 Estuaries Offshore Wind Farm

CLIENT:
Royal HaskoningDHV

DATE:
27/10/2022

PRODUCED BY:
F. Austin

REF: 22043



ECOLOGY
RESOURCES



LEGEND

- GCN Absent
- GCN Present
- No Access Granted
- eDNA Not Complete
- Five Estuaries 250m Buffer
- Five Estuaries Onshore

**GREAT CRESTED NEWT WATERBODIES
FIGURE 1b**

PROJECT TITLE:
5 Estuaries Offshore Wind Farm

CLIENT:
Royal HaskoningDHV

DATE:
27/10/2022

PRODUCED BY:
F. Austin

REF: 22043



ECOLOGY
RESOURCES



LEGEND

- GCN Absent
- GCN Present
- No Access Granted
- eDNA Not Complete
- Five Estuaries 250m Buffer
- Five Estuaries Onshore

**GREAT CRESTED NEWT WATERBODIES
FIGURE 1c**

PROJECT TITLE:
5 Estuaries Offshore Wind Farm

CLIENT:
Royal HaskoningDHV

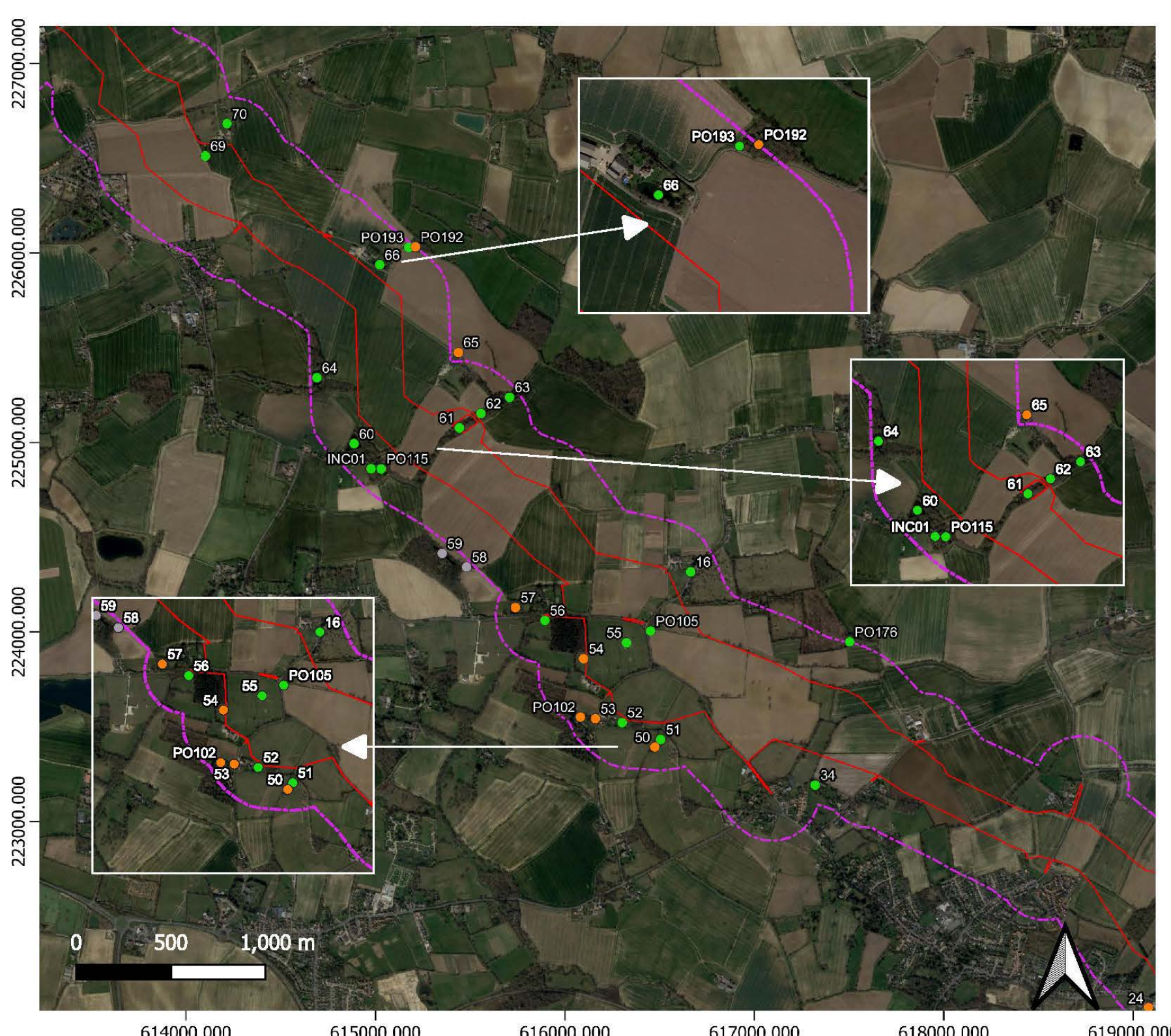
DATE:
27/10/2022

PRODUCED BY:
F. Austin

REF: 22043



ECOLOGY
RESOURCES



LEGEND

- GCN Absent
- GCN Present
- No Access Granted
- eDNA Not Complete
- Five Estuaries 250m Buffer
- Five Estuaries Onshore

**GREAT CRESTED NEWT WATERBODIES
FIGURE 1d**

PROJECT TITLE:
5 Estuaries Offshore Wind Farm

CLIENT:
Royal HaskoningDHV

DATE:
27/10/2022

PRODUCED BY:
F. Austin

REF: 22043



ECOLOGY
RESOURCES



LEGEND

- GCN Absent
- GCN Present
- No Access Granted
- eDNA Not Complete
- Five Estuaries 250m Buffer
- Five Estuaries Onshore

**GREAT CRESTED NEWT WATERBODIES
FIGURE 1e**

PROJECT TITLE:
5 Estuaries Offshore Wind Farm

CLIENT:
Royal HaskoningDHV

DATE:
27/10/2022



PRODUCED BY:
F. Austin



REF: 22043





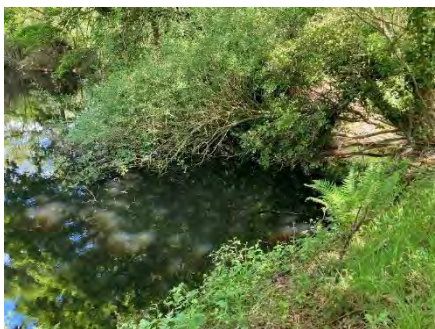

**ECOLOGY
RESOURCES**



APPENDIX B: All Standing Water Body Results



Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
0		TM 22873 18198	1	0.1	1	0.67	1	1	0.67	0.65	0.33	0.9	0.62	Average	Negative	17/05/2022
2		TM 21174 18655	1	0.2	0.9	1	1	0.01	0.01	0.9	1	0.6	0.31	Poor	Negative	06/06/2022



Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
P02		TM 23060 18351	1	0.91 54	1	0.67	1	1	0.67	0.65	0.67	0.8	0.82	Excellent	Negative	17/05/2022
3	No photograph available	TM 20916 18504	1	0.87 7	0.9	0.67	1	0.67	0.67	0.72	0.67	0.4	0.73	Good	Negative	16/05/2022
P03		TM 23063 18339	1	0.91 54	1	0.67	1	1	0.67	0.65	0.67	0.8	0.82	Excellent	Negative	17/05/2022



Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
4		TM 20451 18437	1	0.80 01	0.9	0.67	1	0.67	0.67	0.69	0.67	0.35	0.71	Good	Negative	16/05/2022
P04	No photograph available	TM 23029 18319	1	0.91 54	1	0.67	1	1	0.67	0.65	0.67	0.8	0.82	Excellent	Negative	17/05/2022
5		TM 20329 19152	1	0.91 54	1	0.67	1	1	0.67	0.9	0.67	0.9	0.86	Excellent	Negative	16/05/2022
P05	No photograph available	TM 23022 18306	1	0.91 54	1	0.67	1	1	0.67	0.9	0.67	0.9	0.86	Excellent	Negative	17/05/2022


Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
6		TM 20262 19255	1	0.00	0.90	0.33	0.70	0.01	0.67	1	1.00	0.35	0.50	Below Average	Positive	16/05/22
P06	No photograph available	TM 23034 18306	1	0.91 54	1	0.67	1	1	0.67	0.9	0.67	0.9	0.86	Excellent	Negative	17/05/2022
7		TM 20225 19307	1	1.00	0.10	0.33	1.00	0.67	1.00	1	1.00	0.40	0.62	Average	Negative	16/05/22
P07	No photograph available	TM 23027 18300	1	0.91 54	1	0.67	1	1	0.67	0.9	0.67	0.9	0.86	Excellent	Negative	17/05/2022



Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
8		TM 20193 19342	1	0.21	0.90	0.33	0.50	0.67	0.67	1	1.00	0.30	0.58	Below Average	Positive	16/05/22
P08	No photograph available	TM 22935 18244	1	0.91 54	1	0.67	1	1	0.67	0.9	0.67	0.9	0.86	Excellent	Negative	17/05/2022
9		TM 20149 19368	1	0.4	1	1	0.7	1	1	0.95	1	0.9	0.86	Excellent	Negative	16/05/2022 17/05/2022



Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
P09	No photograph available	TM 22920 18228	1	0.91 54	1	0.67	1	1	0.67	0.9	0.67	0.9	0.86	Excellent	Negative	17/05/2022
10		TM 20121 19407	1	0.50	0.10	0.33	1.00	0.67	1.00	1	1.00	0.35	0.57	Below Average	Positive	16/05/22
P010		TM 22907 18223	1	0.91 54	1	0.67	1	1	0.67	0.90	0.67	0.90	0.86	Excellent	Negative	17/05/2022


Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
11		TM 20182 19480	1	0.1	0.1	0.33	1	1	1	0.65	0.33	1	0.49	Poor	Positive	08/06/2022
P011		TM 22872 18209	1	0.2	0.5	0.67	1	1	0.67	0.9	0.67	0.9	0.68	Average	Negative	17/05/2022

Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
13		TM 20202 19503	1	0.21	0.10	0.33	0.60	0.67	0.67	1	1.00	0.90	0.53	Below Average	Positive	16/05/2022
P013	No photograph available	TM 22869 18185	1	1	0.5	0.67	1	1	0.67	0.7	0.67	0.9	0.79	Excellent	Negative	17/05/22
14		TM 20205 19520	1	0.5	0.1	0.01	1	1	1	1	0.01	0.3	0.2	Poor	N/A	07/06/2022
P014	No photograph available	TM 61544 22507	1	1	0.5	0.67	1	1	0.67	0.7	0.67	0.9	1	Excellent	Negative	17/05/22




Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
15		TM 20228 19525	1	0.5	0.1	0.01	1	1	1	1	0.1	0.3	0.2	Poor	N/A	07/06/2022
P015	No photograph available	TM 62286 21818	1	1	0.5	0.67	1	1	0.67	0.7	0.67	0.9	1	Excellent	Negative	17/05/22
16	No photograph available	TM 16664 24317	1	0.60	0.90	0.33	1.00	0.67	0.67	0.69	0.67	0.30	0.64	Average	Negative	27/09/21 06/05/22
P016	No photograph available	TM 22859 18183	1	1	0.5	0.67	1	1	0.67	0.7	0.67	0.9	0.79	Excellent	Negative	17/05/2022
17	No photograph available	TM 20629 19731	1	0.80 01	0.9	0.67	1	0.67	0.67	1	0.67	0.5	0.76	Good	Negative	14/06/2022
P017	No photograph available	TM 22864 18176	1	1	0.5	0.67	1	1	0.67	0.7	0.67	0.9	0.79	Excellent	Negative	17/05/2022



Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
P018		TM 22798 18127	1	0.7	0.5	0.67	1	1	0.67	0.7	0.67	0.9	0.76	Good	Negative	17/05/2022
P019		TM 22718 18053	1	1	0.5	0.67	1	1	0.67	0.7	0.67	0.9	0.79	Excellent	Negative	17/05/2022
20	No photograph available	TM 20198 20524	1	0.2	0.9	1	1	0.01	0.01	0.95	0.67	0.6	0.3	Poor	Negative	14/06/2022




Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
P020		TM 22708 18046	1	1	0.5	0.67	1	1	0.67	0.7	0.67	1	0.79	Excellent	Negative	17/05/2022
P021		TM 22622 17966	1	0.2	1	0.67	1	1	0.67	0.7	0.67	1	0.72	Good	Negative	17/05/2022


Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
22		TM 20380 21316	1	0.5	0.9	0.67	1	10.6 7	0.67	0.66	0.67	0.4	0.68	Average	Negative	06/05/2022
P022		TM 22589 17929	1	0.3	0.5	0.67	1	0.67	0.67	0.78	0.67	0.9	0.68	Average	Negative	17/05/2022



Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
23		TM 19500 21879	1	0.3	0.5	0.67	1	1	1	0.9	0.67	0.5	0.7	Good	Negative	10/05/2022
P023		TM 22575 17920	1	0.3	0.5	0.67	1	0.67	0.67	0.8	0.67	0.9	0.68	Average	Negative	17/05/2022
24	No photograph available	TM 19080 22018	1	0.3	1	0.67	1	1	0.67	0.8	0.67	0.45	0.7	Good	Positive	09/06/2022

Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
P024		TM 22557 17918	1	0.3	0.5	0.67	1	0.67	0.67	0.78	0.67	0.9	0.68	Average	Negative	17/05/2022
25		TM 19728 22526	1	0.80	0.90	0.33	0.60	0.67	0.67	0.85	0.67	1.00	0.72	Good	Positive	10/05/22
P025		TM 22558 17910	1	03	0.5	0.67	1	0.67	0.67	0.78	0.67	0.9	0.68	Average	Negative	17/05/2022



Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
26	No photograph available	TM 19281 22613	1	0.9	0.9	0.67	1	1	0.67	0.84	0.67	0.4	0.77	Good	Negative	01/06/2022
P026	No photograph available	TM 22334 17693	1	0.80 01	1	0.67	1	0.67	0.67	1	0.67	0.9	0.82	Excellent	Negative	18/05/2022
27		TM 19415 22677	1	0.00	0.90	0.67	1.00	0.67	0.67	0.9	1.00	0.35	0.80	Excellent	Negative	10/05/2022
P027		TM 21924 17653	1	0.1	0.1	0.33	1	0.67	1	0.7	1	0.95	0.52	Below Average	Negative	20/05/2022



Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
28		TM 19273 22709	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Negative	10/05/2022
P028		TM 21916 17582	1	0.6	1	0.67	1	1	0.67	0.7	0.67	0.7	0.5	Good	Negative	20/05/2022
29		TM 19189 22953	1	0.90	0.90	0.33	1.00	0.67	0.67	0.85	0.67	0.30	0.68	Average	Negative	10/05/22



Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
P029		TM 21768 17568	1	0	1	0.67	1	0.67	0.67	0.95	0.67	0.3	0.72	Good	Negative	18/05/2022
30	*Image not available for this pond*	TM 19408 23333	1	0.3	1	0.67	1	1	1	0.68	0.67	0.35	0.7	Good	Negative	01/06/2022



Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
34		TM 17321 23192	1	0.3	0.9	1	1	0.01	0.01	0.9	0.67	0.7	0.32	Poor	Negative	08/06/2022
P034	No photograph available	TM 21136 18788	1	0.2	1	0.67	0.6	0.67	0.67	0.65	0.67	0.3	0.58	Below Average	Negative	16/05/2022
50		TM 16475 23393	1	1.00	0.90	0.67	0.60	0.67	0.67	0.98	1.00	0.80	0.81	Excellent	Positive	12/05/2022



Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
51		TM 16505 23432	1	0.98	0.90	0.67	1.00	0.01	0.67	0.96	1.00	0.50	0.53	Below Average	Negative	12/05/2022
52		TM 16302 23522	1	1.10	0.90	0.33	1.00	0.67	0.33	0.93	1.00	0.45	0.70	Good	Negative	11/05/2022
53		TM 16161 23542	1	0.21	0.90	0.67	0.60	0.67	0.67	0.9	1.00	0.30	0.63	Average	Positive	11/05/2022



Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
54		TM 16098 23859	1	0.3	0.5	0.33	1	0.67	1	0.92	0.67	0.35	0.61	Average	Positive	11/05/2022
55		TM 16325 23943	1	0.83	0.90	0.33	1.00	0.67	1.00	0.93	1.00	0.30	0.73	Good	Negative	12/10/21 16/06/22
56		TM 15894 24061	1	0.1	0.5	0.67	1	1	1	0.55	1	1	0.67	Average	Negative	26/04/2022


Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
57		TM 15739 24129	1	0.1	1	0.67	1	1	1	0.7	1	0.35	0.66	Average	Positive	01/06/2022
60		TM 14887 24994	1	0	0.9	0.67	1	0.67	0.67	0.75	0.67	0.4	0.72	Good	Negative	26/04/2022



Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
61		TM 15443 25077	1	0.95 39	0.5	0.33	0.6	1	1	0.65	0.67	0.35	0.65	Average	Negative	10/05/2022
62		TM 15557 25153	1	0.88 47	1	0.33	0.6	0.67	0.67	0.7	1	0.4	0.68	Average	Negative	11/05/2022 12/07/2022



Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
63		TM 15708 25239	1	0	0.9	1	1	0.67	0.67	0.65	1	0.3	0.75	Good	Negative	26/04/2022
64		TM 14691 25342	1	0.00	0.90	0.67	1.00	0.67	0.01	0.85	1.00	0.30	0.54	Below Average	Negative	26/04/2022



Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
65		TM 15438 25475	1	0.2	0.5	0.33	1	1	1	0.65	0.67	0.9	0.65	Average	Positive	10/05/2022
66		TM 15022 25938	1	N/A	0.90	0.67	1.00	0.67	0.33	0.85	0.33	0.50	0.70	Good	Negative	26/04/2022



Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
69		TM 14102 26512	1	0.92	0.50	0.33	0.20	0.67	0.67	0.9	0.33	0.50	0.54	Below Average	Negative	26/04/2022
70		TM 14216 26683	1	0.2	0.9	0.67	1	0.67	1	0.69	0.67	0.35	0.64	Average	Negative	12/05/2022



Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
P070		TM 20232 19499	1	0.05	0.1	0.01	1	1	1	1	0.01	0.3	0.2	Poor	Negative	07/06/2022
72		TM 12979 26811	1	0.80 01	0.9	0.67	1	0.67	0.67	1	0.67	0.65	0.72	Good	Positive	01/06/2022
73	No photograph available	TM 13093 27091	1	0.60	0.90	0.67	1.00	0.67	0.67	0.58	1.00	0.35	0.71	Good	Negative	28/04/2022

Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
74	No photograph available	TM 12555 28104	1	0.31	0.90	0.33	1.00	0.67	0.67	0.58	0.33	0.40	0.56	Below Average	Negative	28/04/2022
P083		TM 19706 22392	1	0.80 01	0.9	0.67	1	0.67	0.67	0.68	0.67	0.35	0.69	Average	Negative	10/05/2022
P0102		TM 16082 23551	1	0.98	0.90	0.67	1.00	0.67	0.67	0.96	1.00	0.40	0.80	Excellent	Positive	11/05/2022

Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
P0105		TM 16452 24005	1	0.31	0.90	0.33	0.20	0.67	0.67	0.76	1.00	0.35	0.54	Average	Negative	27/09/21 26/04/22
P0115	No photograph available	TM 15030 24861	1	0.2	0.5	0.33	1	0.67	1	0.65	1	0.95	0.65	Average	Excellent	26/04/2022
P0142		TM 11851 27529	1	0.80 01	0.9	0.67	1	0.67	0.67	0.68	0.67	0.6	0.75	Good	Negative	11/05/2022

Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
P0176		TM 17503 23948	1	0.50	0.90	0.33	1.00	0.67	0.67	0.96	1.00	0.90	0.75	Good	Negative	07/06/22
P0183		TM 11680 27882	1	0.80 01	0.9	0.67	1	0.67	0.67	0.68	0.67	0.35	0.71	Good	Negative	11/05/2022

Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
P0192		TM 15211 26033	1	0.8	1	0.67	1	0.67	0.67	0.89	1	0.4	0.78	Good	Positive	11/05/2022
IP0192		TM 15283 26070	1	0.1	0.1	0.67	1	1	1	0.6	0.67	1	0.55	Below Average	N/A	11/05/2022

Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
P0193		TM 15175 26030	1	0.1	0.1	0.33	1	0.67	1	0.88	1	0.7	0.51	Below Average	Negative	12/05/2022
P0195		TM 11643 28069	1	0.81 54	1	0.67	1	0.67	0.67	0.7	0.67	0.35	0.72	Good	Negative	11/05/2022
INC01	No photograph available	TM 14977 24862	1	0.3	1	0.67	1	0.67	0.33	0.75	1	0.6	0.68	Average	Negative	26/04/2022

APPENDIX C: Surescreen Scientifics – eDNA Results

Folio No: E13559
 Report No: 1
 Purchase Order: 173EM1204/22042
 Client: ECOLOGY RESOURCES
 Contact: Elliot Mack

TECHNICAL REPORT

ANALYSIS OF ENVIRONMENTAL DNA IN POND WATER FOR THE DETECTION OF GREAT CRESTED NEWTS (*TRITURUS CRISTATUS*)

SUMMARY

When great crested newts (GCN), *Triturus cristatus*, inhabit a pond, they continuously release small amounts of their DNA into the environment. By collecting and analysing water samples, we can detect these small traces of environmental DNA (eDNA) to confirm GCN habitation or establish GCN absence.

RESULTS

Date sample received at Laboratory: 13/05/2022
Date Reported: 23/05/2022
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
1737	PO83	TM 19706 22392	Pass	Pass	Pass	Negative	0
1738	PO84	TM 19728 22526	Pass	Pass	Pass	Positive	1
1739	PO86	TM 19273 22709	Pass	Pass	Pass	Negative	0
1740	PO85	TM 19415 22677	Pass	Pass	Pass	Negative	0
1741	PO87	TM 19189 22953	Pass	Pass	Pass	Negative	0
1742	PO88	TM 19326 23209	Pass	Pass	Pass	Negative	0
1744	PO143	TM 11123 27625	Pass	Pass	Pass	Negative	0



1747	PO124	TM 14102 26511	Pass	Pass	Pass	Negative	0
1749	PO129	TM 13255 28265	Pass	Pass	Pass	Negative	0
1751	PO128	TM 12555 28100	Pass	Pass	Pass	Negative	0
1752	PO120	TM 15022 25938	Pass	Pass	Pass	Negative	0
1753	PO118	TM 14691 25342	Pass	Pass	Pass	Negative	0
1754	PO115	TM 15030 24861	Pass	Pass	Pass	Negative	0
1756	PO105	TM 16452 24005	Pass	Pass	Pass	Negative	0
1757	POIncol	614980 224854	Pass	Pass	Pass	Negative	0
1758	PO116	TM 14887 24994	Pass	Pass	Pass	Negative	0
1759	PO107	TM 15894 24061	Pass	Pass	Pass	Negative	0
1760	PO112	TM 15709 25239	Pass	Pass	Pass	Negative	0
1857	PO106	TM 16664 24317	Pass	Pass	Pass	Negative	0
1859	PO132	TM 13265 29325	Pass	Pass	Pass	Negative	0
1860	PO138	TM 10198 30183	Pass	Pass	Pass	Negative	0
1861	PO131	TM 13205 29298	Pass	Pass	Pass	Negative	0
1862	PO130	TM 13137 29295	Pass	Pass	Pass	Negative	0
1863	PO127	TM 13093 27091	Pass	Pass	Pass	Negative	0

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

Reported by: Esther Strafford

Approved by: Gabriela Danickova



METHODOLOGY

The samples detailed above have been analysed for the presence of GCN eDNA following the protocol stated in DEFRA WC1067 'Analytical and methodological development for improved surveillance of the Great Crested Newt, Appendix 5.' (Biggs et al. 2014). Each of the 6 sub-sample tubes are first centrifuged and pooled together into a single sample which then undergoes DNA extraction. The extracted sample is then analysed using real time PCR (qPCR), which uses species-specific molecular markers to amplify GCN DNA within a sample. These markers are unique to GCN DNA, meaning that there should be no detection of closely related species.

If GCN DNA is present, the DNA is amplified up to a detectable level, resulting in positive species detection. If GCN DNA is not present then amplification does not occur, and a negative result is recorded.

Analysis of eDNA requires scrupulous attention to detail to prevent risk of contamination. True positive controls, negative controls and spiked synthetic DNA are included in every analysis and these have to be correct before any result is declared and reported. Stages of the DNA analysis are also conducted in different buildings at our premises for added security.

SureScreen Scientifics Ltd is ISO9001 accredited and participate in Natural England's proficiency testing scheme for GCN eDNA testing. We also carry out regular inter-laboratory checks on accuracy of results as part of our quality control procedures.

INTERPRETATION OF RESULTS

- SIC:** **Sample Integrity Check** [Pass/Fail]
When samples are received in the laboratory, they are inspected for any tube leakage, suitability of sample (not too much mud or weed etc.) and absence of any factors that could potentially lead to inconclusive results.
- DC:** **Degradation Check** [Pass/Fail]
Analysis of the spiked DNA marker to see if there has been degradation of the kit or sample between the date it was made to the date of analysis. Degradation of the spiked DNA marker may lead indicate a risk of false negative results.
- IC:** **Inhibition Check** [Pass/Fail]
The presence of inhibitors within a sample are assessed using a DNA marker. If inhibition is detected, samples are purified and re-analysed. Inhibitors cannot always be removed, if the inhibition check fails, the sample should be re-collected.
- Result:** **Presence of GCN eDNA** [Positive/Negative/Inconclusive]
Positive: GCN DNA was identified within the sample, indicative of GCN presence within the sampling location at the time the sample was taken or within the recent past at the sampling location.
Positive Replicates: Number of positive qPCR replicates out of a series of 12. If one or more of these are found to be positive the pond is declared positive for GCN presence. It may be assumed that small fractions of positive analyses suggest low level presence, but this cannot currently be used for population studies. In accordance with Natural England protocol, even a score of 1/12 is declared positive. 0/12 indicates negative GCN presence.
Negative: GCN eDNA was not detected or is below the threshold detection level and the test result should be considered as evidence of GCN absence, however, does not exclude the potential for GCN presence below the limit of detection.



Folio No: E13850
 Report No: 1
 Purchase Order: 173EM1204/22042
 Client: ECOLOGY RESOURCES
 Contact: Elliot Mack

TECHNICAL REPORT

ANALYSIS OF ENVIRONMENTAL DNA IN POND WATER FOR THE DETECTION OF GREAT CRESTED NEWTS (TRITURUS CRISTATUS)

SUMMARY

When great crested newts (GCN), *Triturus cristatus*, inhabit a pond, they continuously release small amounts of their DNA into the environment. By collecting and analysing water samples, we can detect these small traces of environmental DNA (eDNA) to confirm GCN habitation or establish GCN absence.

RESULTS

Date sample received at Laboratory: 27/05/2022
Date Reported: 09/06/2022
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
1745	PO98	TM 16505 23432	Pass	Pass	Pass	Negative	0
1746	PO141	TM 10858 28469	Pass	Pass	Pass	Negative	0
1761	PO101	TM 16161 23542	Pass	Pass	Pass	Positive	4
1762	PO103	TM 16098 23862	Pass	Pass	Pass	Positive	3
1764	PO102	TM 16082 23551	Pass	Pass	Pass	Positive	10
1765	PO142	TM 11851 27529	Pass	Pass	Pass	Negative	0
1766	PO183	TM 11680 27882	Pass	Pass	Pass	Negative	0



1767	PO195	TM 11643 28069	Pass	Pass	Pass	Negative	0
1768	PO100	TM 16303 23523	Pass	Pass	Pass	Negative	0
1769	PO114	TM 15442 25077	Pass	Pass	Pass	Negative	0
1770	PO113	TM 15557 25153	Pass	Pass	Pass	Negative	0
1772	PO99	TM 16475 23393	Pass	Pass	Pass	Positive	12
1774	PO193	TM 15175 26030	Pass	Pass	Pass	Negative	0
1775	PO124	TM 14102 26511	Pass	Pass	Pass	Negative	0
1781	PO117	TM 15438 25475	Pass	Pass	Pass	Positive	1
1782	PO81	TM 19500 21879	Pass	Pass	Pass	Negative	0
1812	PO18	TM 22798 18127	Pass	Pass	Pass	Negative	0
1816	PO03	TM 23063 18339	Pass	Pass	Pass	Negative	0
1817	PO11	TM 22872 18209	Pass	Pass	Pass	Negative	0
1819	PO02	TM 23060 18351	Pass	Pass	Pass	Negative	0
1822	PO01	TM 23238 18578	Pass	Pass	Pass	Negative	0
1823	PO13-17	TM 22869 18185	Pass	Pass	Pass	Negative	0
1824	PO12	TM 22873 18193	Pass	Pass	Pass	Negative	0
1858	PO125	TM 14217 26683	Pass	Pass	Pass	Negative	0

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

Reported by: Esther Strafford

Approved by: Chris Troth



METHODOLOGY

The samples detailed above have been analysed for the presence of GCN eDNA following the protocol stated in DEFRA WC1067 'Analytical and methodological development for improved surveillance of the Great Crested Newt, Appendix 5.' (Biggs et al. 2014). Each of the 6 sub-sample tubes are first centrifuged and pooled together into a single sample which then undergoes DNA extraction. The extracted sample is then analysed using real time PCR (qPCR), which uses species-specific molecular markers to amplify GCN DNA within a sample. These markers are unique to GCN DNA, meaning that there should be no detection of closely related species.

If GCN DNA is present, the DNA is amplified up to a detectable level, resulting in positive species detection. If GCN DNA is not present then amplification does not occur, and a negative result is recorded.

Analysis of eDNA requires scrupulous attention to detail to prevent risk of contamination. True positive controls, negative controls and spiked synthetic DNA are included in every analysis and these have to be correct before any result is declared and reported. Stages of the DNA analysis are also conducted in different buildings at our premises for added security.

SureScreen Scientifics Ltd is ISO9001 accredited and participate in Natural England's proficiency testing scheme for GCN eDNA testing. We also carry out regular inter-laboratory checks on accuracy of results as part of our quality control procedures.

INTERPRETATION OF RESULTS

- SIC:** **Sample Integrity Check** [Pass/Fail]
When samples are received in the laboratory, they are inspected for any tube leakage, suitability of sample (not too much mud or weed etc.) and absence of any factors that could potentially lead to inconclusive results.
- DC:** **Degradation Check** [Pass/Fail]
Analysis of the spiked DNA marker to see if there has been degradation of the kit or sample between the date it was made to the date of analysis. Degradation of the spiked DNA marker may lead indicate a risk of false negative results.
- IC:** **Inhibition Check** [Pass/Fail]
The presence of inhibitors within a sample are assessed using a DNA marker. If inhibition is detected, samples are purified and re-analysed. Inhibitors cannot always be removed, if the inhibition check fails, the sample should be re-collected.
- Result:** **Presence of GCN eDNA** [Positive/Negative/Inconclusive]
Positive: GCN DNA was identified within the sample, indicative of GCN presence within the sampling location at the time the sample was taken or within the recent past at the sampling location.
Positive Replicates: Number of positive qPCR replicates out of a series of 12. If one or more of these are found to be positive the pond is declared positive for GCN presence. It may be assumed that small fractions of positive analyses suggest low level presence, but this cannot currently be used for population studies. In accordance with Natural England protocol, even a score of 1/12 is declared positive. 0/12 indicates negative GCN presence.
Negative: GCN eDNA was not detected or is below the threshold detection level and the test result should be considered as evidence of GCN absence, however, does not exclude the potential for GCN presence below the limit of detection.



Folio No: E13885
 Report No: 1
 Purchase Order: 173EM1204/22042
 Client: ECOLOGY RESOURCES
 Contact: Elliot Mack

TECHNICAL REPORT

ANALYSIS OF ENVIRONMENTAL DNA IN POND WATER FOR THE DETECTION OF GREAT CRESTED NEWTS (TRITURUS CRISTATUS)

SUMMARY

When great crested newts (GCN), *Triturus cristatus*, inhabit a pond, they continuously release small amounts of their DNA into the environment. By collecting and analysing water samples, we can detect these small traces of environmental DNA (eDNA) to confirm GCN habitation or establish GCN absence.

RESULTS

Date sample received at Laboratory: 30/05/2022
Date Reported: 09/06/2022
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
1743	PO147	TM 09673 27216	Pass	Pass	Pass	Negative	0
1771	PO192	TM 15211 26033	Pass	Pass	Pass	Positive	1
1777	PO27	TM 21924 17653	Pass	Pass	Pass	Negative	0
1783	PO28	TM 21916 17582	Pass	Pass	Pass	Negative	0
1801	PO26	TM 22334 17693	Pass	Pass	Pass	Negative	0
1806	PO29	TM 21768 17568	Pass	Pass	Pass	Negative	0
1809	PO25	TM 22558 17910	Pass	Pass	Pass	Negative	0



1810	PO19	TM 22718 18053	Pass	Pass	Pass	Negative	0
1811	PO20	TM 22708 18046	Pass	Pass	Pass	Negative	0
1813	PO22	TM 22589 17929	Pass	Pass	Pass	Negative	0
1814	PO24	TM 22557 17918	Pass	Pass	Pass	Negative	0
1815	PO23	TM 22575 17920	Pass	Pass	Pass	Negative	0
1818	PO21	TM 22622 17966	Pass	Pass	Pass	Negative	0
1820	PO37	TM 20452 18437	Pass	Pass	Pass	Negative	0
1821	PO64	TM 20225 19307	Pass	Pass	Pass	Negative	0
1825	PO69	TM 20202 19503	Pass	Pass	Pass	Positive	12
1826	PO59	TM 20329 19152	Pass	Pass	Pass	Negative	0
1827	PO67	TM 20121 19408	Pass	Pass	Pass	Positive	9
1828	PO65	TM 20193 19342	Pass	Pass	Pass	Positive	2
1829	PO36	TM 20916 18505	Pass	Pass	Pass	Negative	0
1830	PO34	TM 21136 18788	Pass	Pass	Pass	Negative	0
1831	PO60	TM 20262 19254	Pass	Pass	Pass	Positive	7
1832	PO66	TM 20149 19368	Pass	Pass	Pass	Negative	0

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

Reported by: Esther Strafford

Approved by: Chris Troth

METHODOLOGY



The samples detailed above have been analysed for the presence of GCN eDNA following the protocol stated in DEFRA WC1067 'Analytical and methodological development for improved surveillance of the Great Crested Newt, Appendix 5.' (Biggs et al. 2014). Each of the 6 sub-sample tubes are first centrifuged and pooled together into a single sample which then undergoes DNA extraction. The extracted sample is then analysed using real time PCR (qPCR), which uses species-specific molecular markers to amplify GCN DNA within a sample. These markers are unique to GCN DNA, meaning that there should be no detection of closely related species.

If GCN DNA is present, the DNA is amplified up to a detectable level, resulting in positive species detection. If GCN DNA is not present then amplification does not occur, and a negative result is recorded.

Analysis of eDNA requires scrupulous attention to detail to prevent risk of contamination. True positive controls, negative controls and spiked synthetic DNA are included in every analysis and these have to be correct before any result is declared and reported. Stages of the DNA analysis are also conducted in different buildings at our premises for added security.

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INTERPRETATION OF RESULTS

- SIC:** **Sample Integrity Check** [Pass/Fail]
When samples are received in the laboratory, they are inspected for any tube leakage, suitability of sample (not too much mud or weed etc.) and absence of any factors that could potentially lead to inconclusive results.
- DC:** **Degradation Check** [Pass/Fail]
Analysis of the spiked DNA marker to see if there has been degradation of the kit or sample between the date it was made to the date of analysis. Degradation of the spiked DNA marker may lead indicate a risk of false negative results.
- IC:** **Inhibition Check** [Pass/Fail]
The presence of inhibitors within a sample are assessed using a DNA marker. If inhibition is detected, samples are purified and re-analysed. Inhibitors cannot always be removed, if the inhibition check fails, the sample should be re-collected.
- Result:** **Presence of GCN eDNA** [Positive/Negative/Inconclusive]
Positive: GCN DNA was identified within the sample, indicative of GCN presence within the sampling location at the time the sample was taken or within the recent past at the sampling location.
Positive Replicates: Number of positive qPCR replicates out of a series of 12. If one or more of these are found to be positive the pond is declared positive for GCN presence. It may be assumed that small fractions of positive analyses suggest low level presence, but this cannot currently be used for population studies. In accordance with Natural England protocol, even a score of 1/12 is declared positive. 0/12 indicates negative GCN presence.
Negative: GCN eDNA was not detected or is below the threshold detection level and the test result should be considered as evidence of GCN absence, however, does not exclude the potential for GCN presence below the limit of detection.



Folio No: E14177
 Report No: 1
 Purchase Order: 173EM1204/22042
 Client: ECOLOGY RESOURCES
 Contact: Elliot Mack

TECHNICAL REPORT

ANALYSIS OF ENVIRONMENTAL DNA IN POND WATER FOR THE DETECTION OF GREAT CRESTED NEWTS (TRITURUS CRISTATUS)

SUMMARY

When great crested newts (GCN), *Triturus cristatus*, inhabit a pond, they continuously release small amounts of their DNA into the environment. By collecting and analysing water samples, we can detect these small traces of environmental DNA (eDNA) to confirm GCN habitation or establish GCN absence.

RESULTS

Date sample received at Laboratory: 15/06/2022
Date Reported: 23/06/2022
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
1763	PO 068	TM 20182 19480	Pass	Pass	Pass	Positive	1
1773	PO 176	TM 17503 23948	Pass	Pass	Pass	Negative	0
1776	ID391-LP49 5E	TM 192 226	Pass	Pass	Pass	Negative	0
1780	ID222-LP239 5 Estuaries	TM 157 241	Pass	Pass	Pass	Positive	12
1794	PO82	TM 19080 22018	Pass	Pass	Pass	Positive	4
1796	PO 134	TM 12275 29994	Pass	Pass	Pass	Negative	0



1797	PO135	TM 11336 29737	Pass	Pass	Pass	Negative	0
1798	PO178	TM 11446 29778	Pass	Pass	Pass	Negative	0
1799	PO94	TM 17317 23195	Pass	Pass	Pass	Negative	0
1800	PO35	TM 21174 18656	Pass	Pass	Pass	Negative	0
1803	PO79	TM 20381 21316	Pass	Pass	Pass	Negative	0
1807	10260 - LP996 5E	TM 130 268	Pass	Pass	Pass	Positive	1
1808	ID145 - LP75 5 Estuaries	TM 193 233	Pass	Pass	Pass	Negative	0

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

Reported by: Esther Strafford

Approved by: Chelsea Warner

METHODOLOGY

The samples detailed above have been analysed for the presence of GCN eDNA following the protocol stated in DEFRA WC1067 'Analytical and methodological development for improved surveillance of the Great Crested Newt, Appendix 5.' (Biggs et al. 2014). Each of the 6 sub-sample tubes are first centrifuged and pooled together into a single sample which then undergoes DNA extraction. The extracted sample is then analysed using real time PCR (qPCR), which uses species-specific molecular markers to amplify GCN DNA within a sample. These markers are unique to GCN DNA, meaning that there should be no detection of closely related species.

If GCN DNA is present, the DNA is amplified up to a detectable level, resulting in positive species detection. If GCN DNA is not present then amplification does not occur, and a negative result is recorded.

Analysis of eDNA requires scrupulous attention to detail to prevent risk of contamination. True positive controls, negative controls and spiked synthetic DNA are included in every analysis and these have to be correct before any result is declared and reported. Stages of the DNA analysis are also conducted in different buildings at our premises for added security.

SureScreen Scientifics Ltd is ISO9001 accredited and participate in Natural England's proficiency testing scheme for GCN eDNA testing. We also carry out regular inter-laboratory checks on accuracy of results as part of our quality control procedures.

INTERPRETATION OF RESULTS

SIC: **Sample Integrity Check** [Pass/Fail]



When samples are received in the laboratory, they are inspected for any tube leakage, suitability of sample (not too much mud or weed etc.) and absence of any factors that could potentially lead to inconclusive results.

DC: Degradation Check [Pass/Fail]

Analysis of the spiked DNA marker to see if there has been degradation of the kit or sample between the date it was made to the date of analysis. Degradation of the spiked DNA marker may lead indicate a risk of false negative results.

IC: Inhibition Check [Pass/Fail]

The presence of inhibitors within a sample are assessed using a DNA marker. If inhibition is detected, samples are purified and re-analysed. Inhibitors cannot always be removed, if the inhibition check fails, the sample should be re-collected.

Result: Presence of GCN eDNA [Positive/Negative/Inconclusive]

Positive: GCN DNA was identified within the sample, indicative of GCN presence within the sampling location at the time the sample was taken or within the recent past at the sampling location.

Positive Replicates: Number of positive qPCR replicates out of a series of 12. If one or more of these are found to be positive the pond is declared positive for GCN presence. It may be assumed that small fractions of positive analyses suggest low level presence, but this cannot currently be used for population studies. In accordance with Natural England protocol, even a score of 1/12 is declared positive. 0/12 indicates negative GCN presence.

Negative: GCN eDNA was not detected or is below the threshold detection level and the test result should be considered as evidence of GCN absence, however, does not exclude the potential for GCN presence below the limit of detection.



Folio No: E14893
Report No: 1
Purchase Order: 173EM/204/22042
Client: ECOLOGY RESOURCES
Contact: Elliot Mack

TECHNICAL REPORT

ANALYSIS OF ENVIRONMENTAL DNA IN POND WATER FOR THE DETECTION OF GREAT CRESTED NEWTS (*TRITURUS CRISTATUS*)

SUMMARY

When great crested newts (GCN), *Triturus cristatus*, inhabit a pond, they continuously release small amounts of their DNA into the environment. By collecting and analysing water samples, we can detect these small traces of environmental DNA (eDNA) to confirm GCN habitation or establish GCN absence.

RESULTS

Date sample received at Laboratory: 05/07/2022
Date Reported: 19/07/2022
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
1787	PO 174	TM 22864 18176	Pass	Pass	Pass	Negative	0
1788	PO 104	TM 16325 23943	Pass	Pass	Pass	Negative	0
1791	PO 76	TM 20614 20151	Pass	Pass	Pass	Negative	0

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

Reported by: Chelsea Warner

Approved by: Chelsea Warner



METHODOLOGY

The samples detailed above have been analysed for the presence of GCN eDNA following the protocol stated in DEFRA WC1067 'Analytical and methodological development for improved surveillance of the Great Crested Newt, Appendix 5.' (Biggs et al. 2014). Each of the 6 sub-sample tubes are first centrifuged and pooled together into a single sample which then undergoes DNA extraction. The extracted sample is then analysed using real time PCR (qPCR), which uses species-specific molecular markers to amplify GCN DNA within a sample. These markers are unique to GCN DNA, meaning that there should be no detection of closely related species.

If GCN DNA is present, the DNA is amplified up to a detectable level, resulting in positive species detection. If GCN DNA is not present then amplification does not occur, and a negative result is recorded.

Analysis of eDNA requires scrupulous attention to detail to prevent risk of contamination. True positive controls, negative controls and spiked synthetic DNA are included in every analysis and these have to be correct before any result is declared and reported. Stages of the DNA analysis are also conducted in different buildings at our premises for added security.

SureScreen Scientifics Ltd is ISO9001 accredited and participate in Natural England's proficiency testing scheme for GCN eDNA testing. We also carry out regular inter-laboratory checks on accuracy of results as part of our quality control procedures.

INTERPRETATION OF RESULTS

- SIC:** **Sample Integrity Check** [Pass/Fail]
When samples are received in the laboratory, they are inspected for any tube leakage, suitability of sample (not too much mud or weed etc.) and absence of any factors that could potentially lead to inconclusive results.
- DC:** **Degradation Check** [Pass/Fail]
Analysis of the spiked DNA marker to see if there has been degradation of the kit or sample between the date it was made to the date of analysis. Degradation of the spiked DNA marker may lead indicate a risk of false negative results.
- IC:** **Inhibition Check** [Pass/Fail]
The presence of inhibitors within a sample are assessed using a DNA marker. If inhibition is detected, samples are purified and re-analysed. Inhibitors cannot always be removed, if the inhibition check fails, the sample should be re-collected.
- Result:** **Presence of GCN eDNA** [Positive/Negative/Inconclusive]
Positive: GCN DNA was identified within the sample, indicative of GCN presence within the sampling location at the time the sample was taken or within the recent past at the sampling location.
Positive Replicates: Number of positive qPCR replicates out of a series of 12. If one or more of these are found to be positive the pond is declared positive for GCN presence. It may be assumed that small fractions of positive analyses suggest low level presence, but this cannot currently be used for population studies. In accordance with Natural England protocol, even a score of 1/12 is declared positive. 0/12 indicates negative GCN presence.
Negative: GCN eDNA was not detected or is below the threshold detection level and the test result should be considered as evidence of GCN absence, however, does not exclude the potential for GCN presence below the limit of detection.





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Registered in England and Wales
company number 12292474





ECOLOGY
RESOURCES

**eDNA
Great Crested
Newt
Results Report**

**5 Estuaries
Offshore Wind
Farm Ltd**

August 2022



Status	Name	Date
Draft	Francesca Austin BSc (Hons) QCIEEM	20/10/2022
Rev 1	Gavin Mullan BA (Hons) MCIEEM	01/11/22
Rev 2	Gavin Mullan BA (Hons) MCIEEM	17/11/22
Rev 3		

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EXECUTIVE SUMMARY

Ecology Resources Limited was commissioned by Royal HaskoningDHV on behalf of Five Estuaries Offshore Wind Farm Limited (VE OWFL), to undertake Habitat Suitability Index (HSI) and environmental DNA (eDNA) sampling of suitable standing water bodies within the onshore project area plus a 250m buffer.

In total 106 standing water bodies were identified as having potential for great crested newts, two of which were found incidentally during site walkovers.

A total of 84 HSI assessments and eDNA sampling surveys were completed within the great crested newt survey area, 15 of which returned positive eDNA results confirming great crested newt presence. The outstanding 22 standing water bodies were not subject to HSI assessments and eDNA sampling due to land access restrictions or were unsuitable at time of survey.

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1. INTRODUCTION

Ecology Resources Limited was commissioned by Royal HaskoningDHV on behalf of Five Estuaries Offshore Wind Farm Limited (VE OWFL), to undertake Habitat Suitability Index (HSI) and environmental DNA (eDNA) sampling for great crested newt *Triturus cristatus* for suitable standing water bodies within the onshore project area plus a 250m buffer (herein ‘the great crested newt survey area’).

1.1 Project Background

Five Estuaries Offshore Windfarm (VE) is a proposed extension to the operational Galloper Offshore Wind Farm (OWF) which consists of 56 wind turbine generators (WTGs). The VE will comprise up to 79 WTGs situated within two array areas to the east of the operational Galloper OWF. The array areas will be located approximately 30km off the coast of Suffolk, England.

Cables will connect the turbines to the offshore substation platforms and then export the power generated to shore. It is expected that there will be a number of inter-array cables, up to four export cables and up to two offshore substations platforms.

A landfall area has been identified between Holland-on Sea and Frinton-on-Sea on the Essex coast. The landfall point is yet to be determined but will be located within this area of coastline. A new VE onshore substation will be needed and will be constructed in an area to the north of the A120.

The VE cables will be installed underground between the landfall and the grid connection point north of the A120. A preferred corridor has not yet been determined with several corridors still under consideration at the time of writing. Potential substation land parcels and associated corridor options north of the A120 also remain under review at the time of writing.

A more detailed description of the project, several elements of which have yet to be finalised at this time, will be provided in the PEIR and ES in due course.

1.2 Legislation

Great crested newts are a European protected species, as such they are afforded a high level of protection. The animals and their eggs, breeding sites and resting places are protected by law. Great crested newts are fully protected under the following UK legislation / international agreements: Bern Convention 1979: Appendix II Strictly Protected Fauna Species, Wildlife & Countryside Act (as amended) 1981, The Conservation of Habitats and Species Regulations 2017 (The Conservation of Habitats and Species Regulations 2017 transposes into UK law the EU Habitats Directive Council Directive 92/43/EEC) and Countryside Rights of Way Act 2000 (CRoW 2000). Protection under these laws makes it an offence to: -

- intentionally kill, injure, or capture, or take great crested newts;
- deliberately take or destroy eggs of great crested newts;
- possess or control alive or dead great crested newt or any part or thing derived from them;
- intentionally or recklessly damage, destroy, or obstruct access to, any structure or place which great crested newts use for breeding, shelter or protection;
- intentionally or recklessly disturb great crested newts while occupying a structure or place which it uses for that purpose;

- sell, offer, or expose for sale, or possess or transport for the purpose of sale, any live or dead great crested newt or any part or thing derived from them. It is also an offence to publish or cause to be published any advertisement likely to be understood as conveying that great crested newts, or parts or derived things of them are bought, sold, or are intended to be. This applies to all stages in their life cycle;
- keep, or transport, or exchange great crested newts or any part or thing derived from them.

2.0 METHODOLOGY

The great crested newt eDNA survey was completed in accordance with Natural England’s *Technical advice note for field and laboratory sampling of great crested newt (Triturus cristatus) environmental DNA* (Biggs *et al.*, 2014). Each of the suitable standing water bodies were visited once and eDNA samples taken following the field protocol set out in Biggs *et al.* (2014).

In total 106 standing water bodies were identified as having potential for great crested newts, two of which were found incidentally during site walkovers (i.e. not part of the original scope). A total of 84 HSI assessments and eDNA sampling surveys were completed within the great crested newt survey area.

2.1 Habitat Suitability Index

The HSI for great crested newts is a measure of habitat suitability, where water bodies are assessed and scored on the following ten key variables which are known to influence breeding populations of great crested newts, in accordance with standard methods (ARG UK, 2010):

- geographic location;
- water body area;
- water body permanence;
- water quality;
- water body shading;
- presence/impact of waterfowl;
- fish stocks;
- number of water bodies within 1km;
- terrestrial habitat around the water body; and
- macrophyte cover of the water body

Table 1: Water body suitability category based on HSI score

HSI Score	Water body Suitability
<0.5	Poor
0.5 – 0.59	Below Average
0.6 – 0.69	Average
0.7- 0.79	Good

2.2 eDNA Water Sampling

The eDNA sampling collection was by led Johnnie Johnson, Great Crested Newt Level 1 licence holder (2018-33728-CLS-CLS). Each of the suitable water bodies was visited once, and all standing water bodies

were subject to eDNA analysis irrespective of their HSI score. The samples were taken following the field protocol set out in Biggs *et al.* (2014). Visits were made between late-April and mid-June, during the newt breeding season (survey dates provided in Table 3, page 13.)



Samples underwent laboratory analysis by Surescreen Scientifics Ltd. and followed the laboratory protocol detailed in Biggs *et al.* (2014). The laboratory testing provided a 'presence / likely absence' result for each standing water body. The laboratory testing provided a 'presence / likely absence' result for each standing water body, shown in Appendix B.


2.3 Survey Limitations



Most of the standing water bodies were surveyed and assessed without any limitations to survey, however 22 standing water bodies were not surveyed due to restricted land access (10 water bodies) and water body unsuitability i.e., dry at time of survey (12 water bodies). Table 2 page 7 identifies the reasons water bodies were not surveyed.



Table 2: Standing Water Bodies not Surveyed

Ponds labelled with PO preceding the number are also ponds within the North Falls survey area

Water body ID	Photograph	Constraint	Land Parcel Number and Grid Reference
1	Photograph unavailable	No pond present, does not exist	LPN 144; TM2113217604
12		No pond was present, waterbody was dry	LPN 93; TM2019219517
14		No pond was present, waterbody was dry	LPN 93; TM2020519520
21	Photograph unavailable	No access granted	LPN 46; TM1924321450
31	Photograph unavailable	No access granted	LPN 351; TM1764823088
32	Photograph unavailable	No access granted	LPN 352; TM1764723156

Water body ID	Photograph	Constraint	Land Parcel Number and Grid Reference
PO32	Photograph unavailable	No pond was present, waterbody was dry	LPN 163; TM2170117823
33	Photograph unavailable	No access granted	LPN 356; TM1769323220
PO33		No pond was present, waterbody was dry	LPN 176; TM2234318446
35	Photograph unavailable	No access granted	LPN 337; TM1726223355
58	Photograph unavailable	No access granted	LPN 1273; TM15481 4343
59	Photograph unavailable	No access granted	LPN 1273; TM1535224415

Water body ID	Photograph	Constraint	Land Parcel Number and Grid Reference
67		No pond was present, waterbody was dry	LPN 1112; TM1433026076
68	Photograph unavailable	No access granted	LPN 1013; TM1368026232
PO70		Waterbody is dry	LPN 93; TM2023219499

Water body ID	Photograph	Constraint	Land Parcel Number and Grid Reference
71	Photograph unavailable	Waterbody is dry	LPN 985; TM1339326865
PO77		No pond was present, waterbody was dry	LPN 90; TM1991920198
PO80	Photograph unavailable	No access granted	No LPN; TM1939521824
PO110		No pond was present, waterbody was dry	LPN 235; TM1565224379

Water body ID	Photograph	Constraint	Land Parcel Number and Grid Reference
IP0192		Temporary pond but the waterbody was dry	No LPN; TM1528326070
PO196	Photograph unavailable	No access granted	No LPN; TM1859723220


3.0 RESULTS



In total 106 standing water bodies were identified within the great crested newt survey area, two of which were identified as incidental finds during site walkovers, a location plan of all the water bodies that were assessed within the great crested newt survey can be found in Appendix A; Figures 1a – 1e. The ponds identified in table 1 that were dry at the time of survey are excluded from these figures.



Twenty two of the standing water bodies were either unsuitable or inaccessible for reasons identified in Table 2: Water Bodies not surveyed, Page 7. As a result, these were not subject to HSI assessments and eDNA sampling.



A total of 84 HSI assessments and eDNA sampling surveys were completed across the great crested newt survey area, 15 of which returned positive eDNA results confirming great crested newt presence. Those water bodies which returned positive eDNA results are detailed in Table 3. The full eDNA results for all waterbodies are shown in Appendix B.

Table 3: Water body HSI and Positive eDNA Results



Water body ID	Photograph	Grid Reference	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
6		TM 20262 19255	0.5	Below Average	Positive	eDNA – 16/05/2022



Water body ID	Photograph	Grid Reference	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
8		TM 20193 19342	0.58	Below Average	Positive	HSI - 20/09/2021 eDNA - 16/05/2022
10		TM 20121 19408	0.57	Below Average	Positive	HSI - 20/09/2021 eDNA - 16/05/2022


Water body ID	Photograph	Grid Reference	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
11		TM 20182 19480	0.49	Poor	Positive	HSI – 08/06/2022 eDNA – 08/06/2022
13		TM 20202 19503	0.53	Below Average	Positive	HSI - 20/09/2021 eDNA - 16/05/2022
24	*Image not available for this pond*	TM 19080 22018	0.7	Good	Positive	HSI – 09/06/2022 eDNA – 09/06/2022

Water body ID	Photograph	Grid Reference	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
25		TM 19728 22526	0.72	Good	Positive	HSI – 13/10/22 eDNA - 10/05/2022
50		TM 16475 23393	0.81	Excellent	Positive	HSI – 23/09/2022 eDNA - 12/05/2022

Water body ID	Photograph	Grid Reference	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
53		TM 16161 23542	0.63	Average	Positive	HSI – 12/40/21 eDNA - 11/05/2022
54		TM 16098 23859	0.61	Average	Positive	HSI – 11/05/2022 eDNA - 11/05/2022

Water body ID	Photograph	Grid Reference	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
57		TM 15739 24129	0.66	Average	Positive	HSI - 01/06/2022 eDNA - 01/06/2022
65		TM 15438 25475	0.65	Average	Positive	HSI – 10/05/2022 eDNA – 10/05/2022

Water body ID	Photograph	Grid Reference	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
72		TM 12979 26811	0.72	Good	Positive	HSI – 01/06/2022 eDNA – 01/06/2022
PO102		TM 16082 23551	0.80	Excellent	Positive	HSI – 24/09/2021 eDNA - 11/05/2022

Water body ID	Photograph	Grid Reference	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
PO192		TM 15211 26033	0.78	Good	Positive	HSI – 11/05/2022 eDNA – 11/05/2022

4.0 CONCLUSION

In total 106 standing water bodies were identified as having potential for great crested newts, two of which were found incidentally during site walkovers.

A total of 84 HSI assessments and eDNA sampling surveys were completed across the great crested newt survey area, 15 of which returned positive eDNA results confirming great crested newt presence. The outstanding 22 standing water bodies were not subject to HSI assessments and eDNA sampling due to land access restrictions (10 waterbodies) or were unsuitable (12 waterbodies) at time of survey.

5.0 REFERENCES

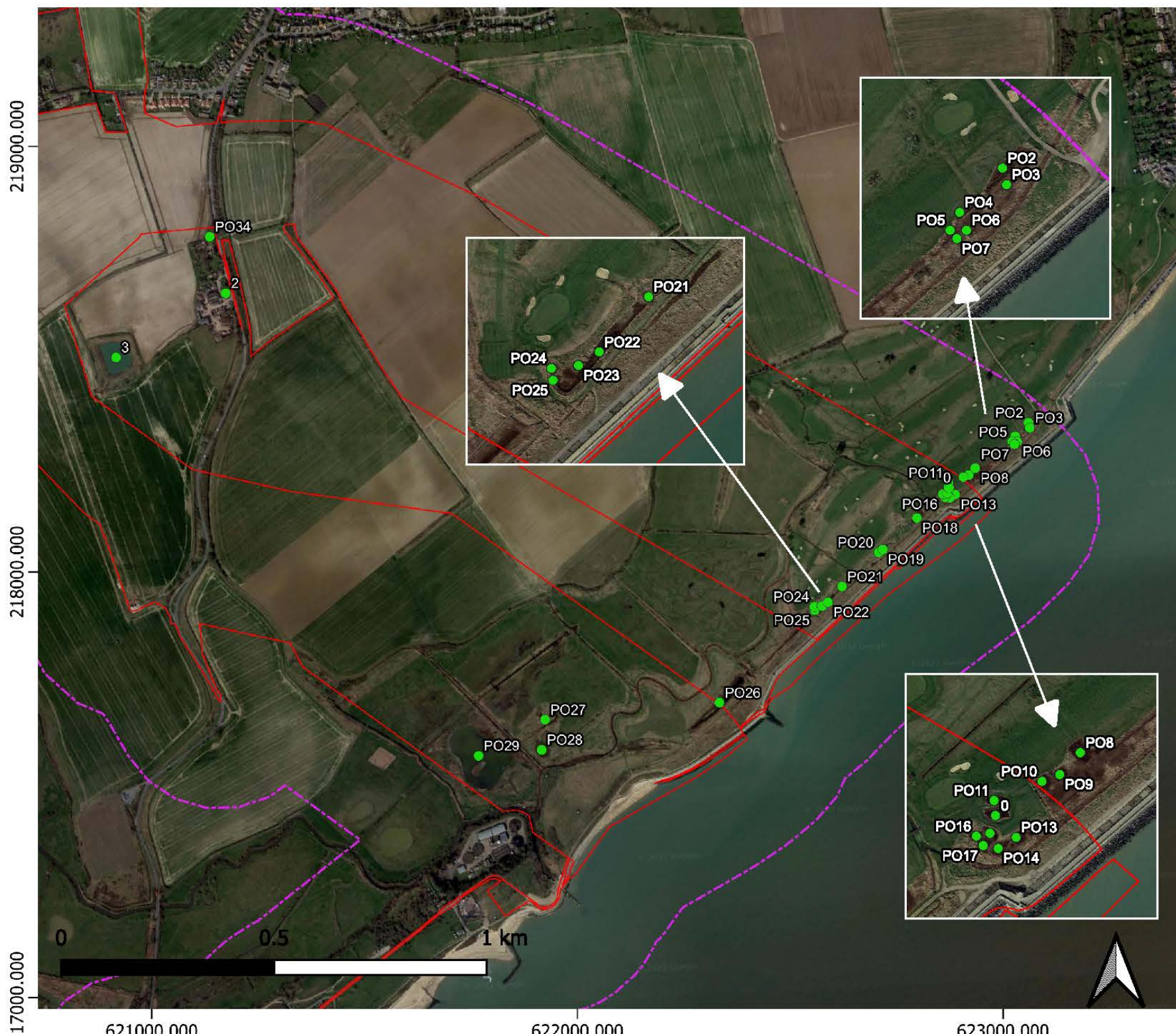
ARG UK Advice Note 5 (2010). *Great crested newt habitat suitability index*. Amphibian and Reptile Groups of the United Kingdom.

Biggs J, Ewald N, Valentini A, Gaboriaud C, Griffiths RA, Foster J, Wilkinson J, Arnett A, Williams P and Dunn F (2014). Analytical and methodological development for improved surveillance of the Great Crested Newt. Appendix 5. Technical advice note for field and laboratory sampling of great crested newt (*Triturus cristatus*) environmental DNA. Freshwater Habitats Trust, Oxford.

Oldham R.S, Keeble J., Swan M.J.S., & Jeffcote M., (2000). Evaluating the suitability of habitat for the great crested newt (*Triturus cristatus*) British Herpetological Society, pp.143-155

Royal HaskoningDHV, (2022). North Falls Extended Phase 1 Habitat Survey Report

APPENDIX A: Standing Water Body Location Plan



LEGEND

- GCN Absent
- GCN Present
- No Access Granted
- eDNA Not Complete
- Five Estuaries 250m Buffer
- Five Estuaries Onshore

**GREAT CRESTED NEWT WATERBODIES
FIGURE 1a**

PROJECT TITLE:
5 Estuaries Offshore Wind Farm

CLIENT:
Royal HaskoningDHV

DATE:
27/10/2022

PRODUCED BY:
F. Austin

REF: 22043



ECOLOGY
RESOURCES



LEGEND

- GCN Absent
- GCN Present
- No Access Granted
- eDNA Not Complete
- Five Estuaries 250m Buffer
- Five Estuaries Onshore

**GREAT CRESTED NEWT WATERBODIES
FIGURE 1b**

PROJECT TITLE:
5 Estuaries Offshore Wind Farm

CLIENT:
Royal HaskoningDHV

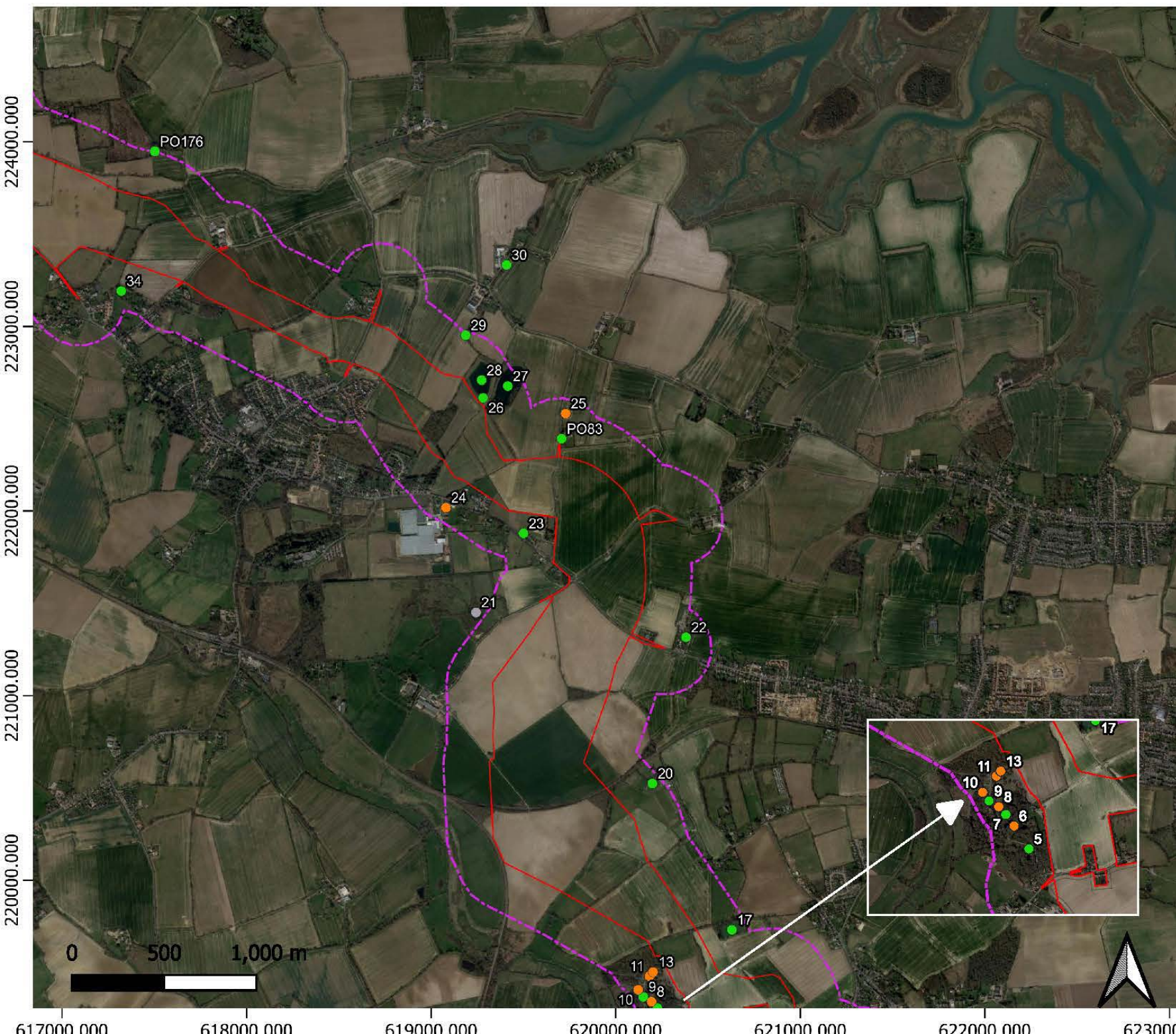
DATE:
27/10/2022

PRODUCED BY:
F. Austin

REF: 22043



ECOLOGY
RESOURCES



LEGEND

- GCN Absent
- GCN Present
- No Access Granted
- eDNA Not Complete
- Five Estuaries 250m Buffer
- Five Estuaries Onshore

**GREAT CRESTED NEWT WATERBODIES
FIGURE 1c**

PROJECT TITLE:
5 Estuaries Offshore Wind Farm

CLIENT:
Royal HaskoningDHV

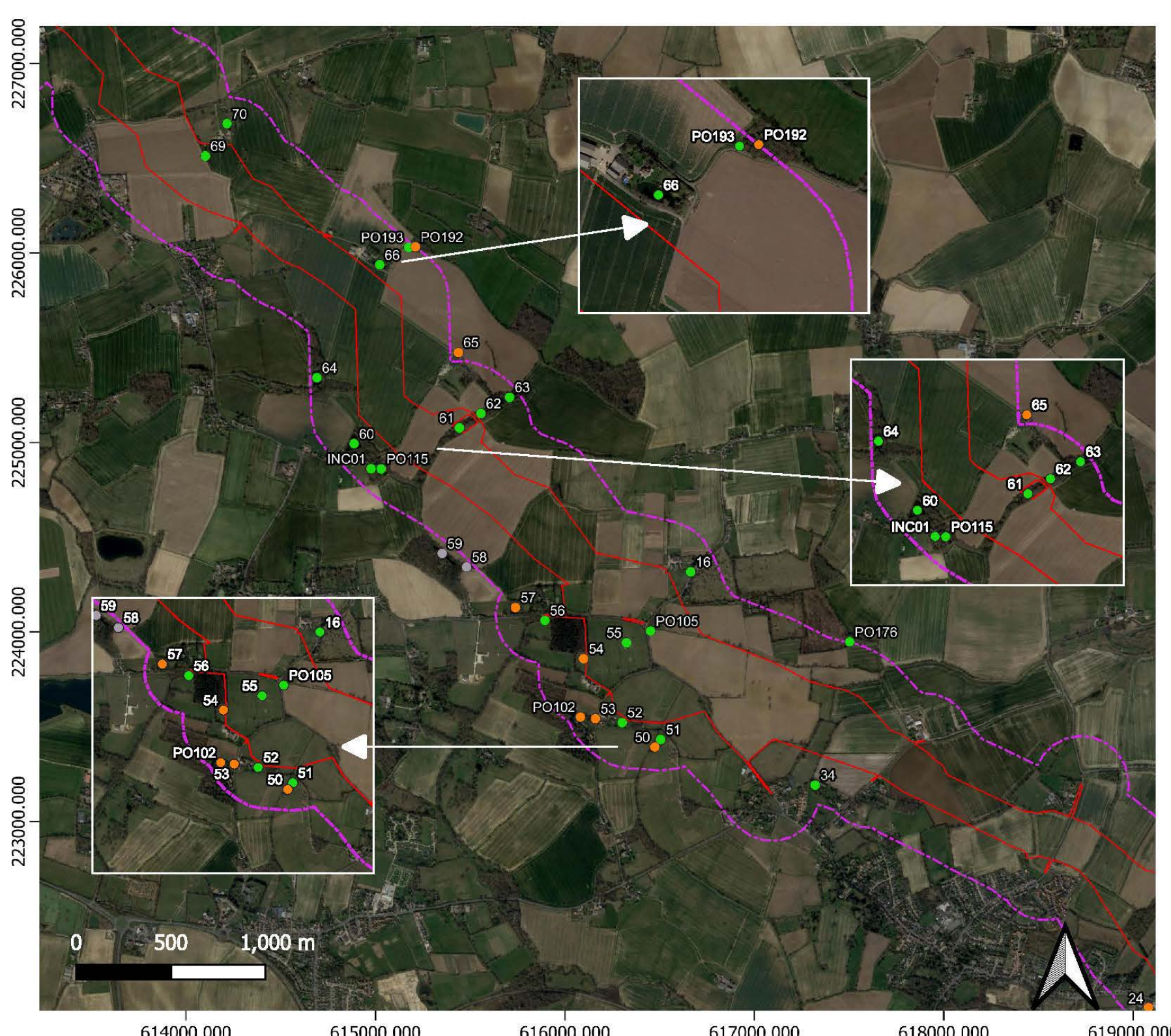
DATE:
27/10/2022

PRODUCED BY:
F. Austin

REF: 22043



ECOLOGY
RESOURCES



LEGEND

- GCN Absent
- GCN Present
- No Access Granted
- eDNA Not Complete
- Five Estuaries 250m Buffer
- Five Estuaries Onshore

**GREAT CRESTED NEWT WATERBODIES
FIGURE 1d**

PROJECT TITLE:
5 Estuaries Offshore Wind Farm

CLIENT:
Royal HaskoningDHV

DATE:
27/10/2022

PRODUCED BY:
F. Austin

REF: 22043



ECOLOGY
RESOURCES



LEGEND

- GCN Absent
- GCN Present
- No Access Granted
- eDNA Not Complete
- Five Estuaries 250m Buffer
- Five Estuaries Onshore

**GREAT CRESTED NEWT WATERBODIES
FIGURE 1e**

PROJECT TITLE:
5 Estuaries Offshore Wind Farm

CLIENT:
Royal HaskoningDHV

DATE:
27/10/2022



PRODUCED BY:
F. Austin



REF: 22043







**ECOLOGY
RESOURCES**



APPENDIX B: All Standing Water Body Results



Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
0		TM 22873 18198	1	0.1	1	0.67	1	1	0.67	0.65	0.33	0.9	0.62	Average	Negative	17/05/2022
2		TM 21174 18655	1	0.2	0.9	1	1	0.01	0.01	0.9	1	0.6	0.31	Poor	Negative	06/06/2022



Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
P02		TM 23060 18351	1	0.91 54	1	0.67	1	1	0.67	0.65	0.67	0.8	0.82	Excellent	Negative	17/05/2022
3	No photograph available	TM 20916 18504	1	0.87 7	0.9	0.67	1	0.67	0.67	0.72	0.67	0.4	0.73	Good	Negative	16/05/2022
P03		TM 23063 18339	1	0.91 54	1	0.67	1	1	0.67	0.65	0.67	0.8	0.82	Excellent	Negative	17/05/2022



Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
4		TM 20451 18437	1	0.80 01	0.9	0.67	1	0.67	0.67	0.69	0.67	0.35	0.71	Good	Negative	16/05/2022
P04	No photograph available	TM 23029 18319	1	0.91 54	1	0.67	1	1	0.67	0.65	0.67	0.8	0.82	Excellent	Negative	17/05/2022
5		TM 20329 19152	1	0.91 54	1	0.67	1	1	0.67	0.9	0.67	0.9	0.86	Excellent	Negative	16/05/2022
P05	No photograph available	TM 23022 18306	1	0.91 54	1	0.67	1	1	0.67	0.9	0.67	0.9	0.86	Excellent	Negative	17/05/2022


Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
6		TM 20262 19255	1	0.00	0.90	0.33	0.70	0.01	0.67	1	1.00	0.35	0.50	Below Average	Positive	16/05/22
P06	No photograph available	TM 23034 18306	1	0.91 54	1	0.67	1	1	0.67	0.9	0.67	0.9	0.86	Excellent	Negative	17/05/2022
7		TM 20225 19307	1	1.00	0.10	0.33	1.00	0.67	1.00	1	1.00	0.40	0.62	Average	Negative	16/05/22
P07	No photograph available	TM 23027 18300	1	0.91 54	1	0.67	1	1	0.67	0.9	0.67	0.9	0.86	Excellent	Negative	17/05/2022



Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
8		TM 20193 19342	1	0.21	0.90	0.33	0.50	0.67	0.67	1	1.00	0.30	0.58	Below Average	Positive	16/05/22
P08	No photograph available	TM 22935 18244	1	0.91 54	1	0.67	1	1	0.67	0.9	0.67	0.9	0.86	Excellent	Negative	17/05/2022
9		TM 20149 19368	1	0.4	1	1	0.7	1	1	0.95	1	0.9	0.86	Excellent	Negative	16/05/2022 17/05/2022



Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
P09	No photograph available	TM 22920 18228	1	0.91 54	1	0.67	1	1	0.67	0.9	0.67	0.9	0.86	Excellent	Negative	17/05/2022
10		TM 20121 19407	1	0.50	0.10	0.33	1.00	0.67	1.00	1	1.00	0.35	0.57	Below Average	Positive	16/05/22
P010		TM 22907 18223	1	0.91 54	1	0.67	1	1	0.67	0.90	0.67	0.90	0.86	Excellent	Negative	17/05/2022


Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
11		TM 20182 19480	1	0.1	0.1	0.33	1	1	1	0.65	0.33	1	0.49	Poor	Positive	08/06/2022
P011		TM 22872 18209	1	0.2	0.5	0.67	1	1	0.67	0.9	0.67	0.9	0.68	Average	Negative	17/05/2022

Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
13		TM 20202 19503	1	0.21	0.10	0.33	0.60	0.67	0.67	1	1.00	0.90	0.53	Below Average	Positive	16/05/2022
P013	No photograph available	TM 22869 18185	1	1	0.5	0.67	1	1	0.67	0.7	0.67	0.9	0.79	Excellent	Negative	17/05/22
14		TM 20205 19520	1	0.5	0.1	0.01	1	1	1	1	0.01	0.3	0.2	Poor	N/A	07/06/2022
P014	No photograph available	TM 61544 22507	1	1	0.5	0.67	1	1	0.67	0.7	0.67	0.9	1	Excellent	Negative	17/05/22




Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
15		TM 20228 19525	1	0.5	0.1	0.01	1	1	1	1	0.1	0.3	0.2	Poor	N/A	07/06/2022
P015	No photograph available	TM 62286 21818	1	1	0.5	0.67	1	1	0.67	0.7	0.67	0.9	1	Excellent	Negative	17/05/22
16	No photograph available	TM 16664 24317	1	0.60	0.90	0.33	1.00	0.67	0.67	0.69	0.67	0.30	0.64	Average	Negative	27/09/21 06/05/22
P016	No photograph available	TM 22859 18183	1	1	0.5	0.67	1	1	0.67	0.7	0.67	0.9	0.79	Excellent	Negative	17/05/2022
17	No photograph available	TM 20629 19731	1	0.80 01	0.9	0.67	1	0.67	0.67	1	0.67	0.5	0.76	Good	Negative	14/06/2022
P017	No photograph available	TM 22864 18176	1	1	0.5	0.67	1	1	0.67	0.7	0.67	0.9	0.79	Excellent	Negative	17/05/2022



Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
P018		TM 22798 18127	1	0.7	0.5	0.67	1	1	0.67	0.7	0.67	0.9	0.76	Good	Negative	17/05/2022
P019		TM 22718 18053	1	1	0.5	0.67	1	1	0.67	0.7	0.67	0.9	0.79	Excellent	Negative	17/05/2022
20	No photograph available	TM 20198 20524	1	0.2	0.9	1	1	0.01	0.01	0.95	0.67	0.6	0.3	Poor	Negative	14/06/2022




Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
P020		TM 22708 18046	1	1	0.5	0.67	1	1	0.67	0.7	0.67	1	0.79	Excellent	Negative	17/05/2022
P021		TM 22622 17966	1	0.2	1	0.67	1	1	0.67	0.7	0.67	1	0.72	Good	Negative	17/05/2022


Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
22		TM 20380 21316	1	0.5	0.9	0.67	1	10.6 7	0.67	0.66	0.67	0.4	0.68	Average	Negative	06/05/2022
P022		TM 22589 17929	1	0.3	0.5	0.67	1	0.67	0.67	0.78	0.67	0.9	0.68	Average	Negative	17/05/2022



Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
23		TM 19500 21879	1	0.3	0.5	0.67	1	1	1	0.9	0.67	0.5	0.7	Good	Negative	10/05/2022
P023		TM 22575 17920	1	0.3	0.5	0.67	1	0.67	0.67	0.8	0.67	0.9	0.68	Average	Negative	17/05/2022
24	No photograph available	TM 19080 22018	1	0.3	1	0.67	1	1	0.67	0.8	0.67	0.45	0.7	Good	Positive	09/06/2022

Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
P024		TM 22557 17918	1	0.3	0.5	0.67	1	0.67	0.67	0.78	0.67	0.9	0.68	Average	Negative	17/05/2022
25		TM 19728 22526	1	0.80	0.90	0.33	0.60	0.67	0.67	0.85	0.67	1.00	0.72	Good	Positive	10/05/22
P025		TM 22558 17910	1	03	0.5	0.67	1	0.67	0.67	0.78	0.67	0.9	0.68	Average	Negative	17/05/2022



Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
26	No photograph available	TM 19281 22613	1	0.9	0.9	0.67	1	1	0.67	0.84	0.67	0.4	0.77	Good	Negative	01/06/2022
P026	No photograph available	TM 22334 17693	1	0.80 01	1	0.67	1	0.67	0.67	1	0.67	0.9	0.82	Excellent	Negative	18/05/2022
27		TM 19415 22677	1	0.00	0.90	0.67	1.00	0.67	0.67	0.9	1.00	0.35	0.80	Excellent	Negative	10/05/2022
P027		TM 21924 17653	1	0.1	0.1	0.33	1	0.67	1	0.7	1	0.95	0.52	Below Average	Negative	20/05/2022



Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
28		TM 19273 22709	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Negative	10/05/2022
P028		TM 21916 17582	1	0.6	1	0.67	1	1	0.67	0.7	0.67	0.7	0.5	Good	Negative	20/05/2022
29		TM 19189 22953	1	0.90	0.90	0.33	1.00	0.67	0.67	0.85	0.67	0.30	0.68	Average	Negative	10/05/22



Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
P029		TM 21768 17568	1	0	1	0.67	1	0.67	0.67	0.95	0.67	0.3	0.72	Good	Negative	18/05/2022
30	*Image not available for this pond*	TM 19408 23333	1	0.3	1	0.67	1	1	1	0.68	0.67	0.35	0.7	Good	Negative	01/06/2022



Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
34		TM 17321 23192	1	0.3	0.9	1	1	0.01	0.01	0.9	0.67	0.7	0.32	Poor	Negative	08/06/2022
P034	No photograph available	TM 21136 18788	1	0.2	1	0.67	0.6	0.67	0.67	0.65	0.67	0.3	0.58	Below Average	Negative	16/05/2022
50		TM 16475 23393	1	1.00	0.90	0.67	0.60	0.67	0.67	0.98	1.00	0.80	0.81	Excellent	Positive	12/05/2022



Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
51		TM 16505 23432	1	0.98	0.90	0.67	1.00	0.01	0.67	0.96	1.00	0.50	0.53	Below Average	Negative	12/05/2022
52		TM 16302 23522	1	1.10	0.90	0.33	1.00	0.67	0.33	0.93	1.00	0.45	0.70	Good	Negative	11/05/2022
53		TM 16161 23542	1	0.21	0.90	0.67	0.60	0.67	0.67	0.9	1.00	0.30	0.63	Average	Positive	11/05/2022



Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
54		TM 16098 23859	1	0.3	0.5	0.33	1	0.67	1	0.92	0.67	0.35	0.61	Average	Positive	11/05/2022
55		TM 16325 23943	1	0.83	0.90	0.33	1.00	0.67	1.00	0.93	1.00	0.30	0.73	Good	Negative	12/10/21 16/06/22
56		TM 15894 24061	1	0.1	0.5	0.67	1	1	1	0.55	1	1	0.67	Average	Negative	26/04/2022

Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
57		TM 15739 24129	1	0.1	1	0.67	1	1	1	0.7	1	0.35	0.66	Average	Positive	01/06/2022
60		TM 14887 24994	1	0	0.9	0.67	1	0.67	0.67	0.75	0.67	0.4	0.72	Good	Negative	26/04/2022



Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
61		TM 15443 25077	1	0.95 39	0.5	0.33	0.6	1	1	0.65	0.67	0.35	0.65	Average	Negative	10/05/2022
62		TM 15557 25153	1	0.88 47	1	0.33	0.6	0.67	0.67	0.7	1	0.4	0.68	Average	Negative	11/05/2022 12/07/2022



Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
63		TM 15708 25239	1	0	0.9	1	1	0.67	0.67	0.65	1	0.3	0.75	Good	Negative	26/04/2022
64		TM 14691 25342	1	0.00	0.90	0.67	1.00	0.67	0.01	0.85	1.00	0.30	0.54	Below Average	Negative	26/04/2022



Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
65		TM 15438 25475	1	0.2	0.5	0.33	1	1	1	0.65	0.67	0.9	0.65	Average	Positive	10/05/2022
66		TM 15022 25938	1	N/A	0.90	0.67	1.00	0.67	0.33	0.85	0.33	0.50	0.70	Good	Negative	26/04/2022



Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
69		TM 14102 26512	1	0.92	0.50	0.33	0.20	0.67	0.67	0.9	0.33	0.50	0.54	Below Average	Negative	26/04/2022
70		TM 14216 26683	1	0.2	0.9	0.67	1	0.67	1	0.69	0.67	0.35	0.64	Average	Negative	12/05/2022



Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
P070		TM 20232 19499	1	0.05	0.1	0.01	1	1	1	1	0.01	0.3	0.2	Poor	Negative	07/06/2022
72		TM 12979 26811	1	0.80 01	0.9	0.67	1	0.67	0.67	1	0.67	0.65	0.72	Good	Positive	01/06/2022
73	No photograph available	TM 13093 27091	1	0.60	0.90	0.67	1.00	0.67	0.67	0.58	1.00	0.35	0.71	Good	Negative	28/04/2022

Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
74	No photograph available	TM 12555 28104	1	0.31	0.90	0.33	1.00	0.67	0.67	0.58	0.33	0.40	0.56	Below Average	Negative	28/04/2022
P083		TM 19706 22392	1	0.80 01	0.9	0.67	1	0.67	0.67	0.68	0.67	0.35	0.69	Average	Negative	10/05/2022
P0102		TM 16082 23551	1	0.98	0.90	0.67	1.00	0.67	0.67	0.96	1.00	0.40	0.80	Excellent	Positive	11/05/2022

Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
P0105		TM 16452 24005	1	0.31	0.90	0.33	0.20	0.67	0.67	0.76	1.00	0.35	0.54	Average	Negative	27/09/21 26/04/22
P0115	No photograph available	TM 15030 24861	1	0.2	0.5	0.33	1	0.67	1	0.65	1	0.95	0.65	Average	Excellent	26/04/2022
P0142		TM 11851 27529	1	0.80 01	0.9	0.67	1	0.67	0.67	0.68	0.67	0.6	0.75	Good	Negative	11/05/2022

Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
P0176		TM 17503 23948	1	0.50	0.90	0.33	1.00	0.67	0.67	0.96	1.00	0.90	0.75	Good	Negative	07/06/22
P0183		TM 11680 27882	1	0.80 01	0.9	0.67	1	0.67	0.67	0.68	0.67	0.35	0.71	Good	Negative	11/05/2022

Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
P0192		TM 15211 26033	1	0.8	1	0.67	1	0.67	0.67	0.89	1	0.4	0.78	Good	Positive	11/05/2022
IP0192		TM 15283 26070	1	0.1	0.1	0.67	1	1	1	0.6	0.67	1	0.55	Below Average	N/A	11/05/2022

Water body Ref.	Water body Photograph	Grid Ref	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	HSI Score	HSI Category	eDNA Result	HSI and eDNA Survey Date
P0193		TM 15175 26030	1	0.1	0.1	0.33	1	0.67	1	0.88	1	0.7	0.51	Below Average	Negative	12/05/2022
P0195		TM 11643 28069	1	0.81 54	1	0.67	1	0.67	0.67	0.7	0.67	0.35	0.72	Good	Negative	11/05/2022
INC01	No photograph available	TM 14977 24862	1	0.3	1	0.67	1	0.67	0.33	0.75	1	0.6	0.68	Average	Negative	26/04/2022

APPENDIX C: Surescreen Scientifics – eDNA Results

Folio No: E13559
 Report No: 1
 Purchase Order: 173EM1204/22042
 Client: ECOLOGY RESOURCES
 Contact: Elliot Mack

TECHNICAL REPORT

ANALYSIS OF ENVIRONMENTAL DNA IN POND WATER FOR THE DETECTION OF GREAT CRESTED NEWTS (*TRITURUS CRISTATUS*)

SUMMARY

When great crested newts (GCN), *Triturus cristatus*, inhabit a pond, they continuously release small amounts of their DNA into the environment. By collecting and analysing water samples, we can detect these small traces of environmental DNA (eDNA) to confirm GCN habitation or establish GCN absence.

RESULTS

Date sample received at Laboratory: 13/05/2022
Date Reported: 23/05/2022
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
1737	PO83	TM 19706 22392	Pass	Pass	Pass	Negative	0
1738	PO84	TM 19728 22526	Pass	Pass	Pass	Positive	1
1739	PO86	TM 19273 22709	Pass	Pass	Pass	Negative	0
1740	PO85	TM 19415 22677	Pass	Pass	Pass	Negative	0
1741	PO87	TM 19189 22953	Pass	Pass	Pass	Negative	0
1742	PO88	TM 19326 23209	Pass	Pass	Pass	Negative	0
1744	PO143	TM 11123 27625	Pass	Pass	Pass	Negative	0



1747	PO124	TM 14102 26511	Pass	Pass	Pass	Negative	0
1749	PO129	TM 13255 28265	Pass	Pass	Pass	Negative	0
1751	PO128	TM 12555 28100	Pass	Pass	Pass	Negative	0
1752	PO120	TM 15022 25938	Pass	Pass	Pass	Negative	0
1753	PO118	TM 14691 25342	Pass	Pass	Pass	Negative	0
1754	PO115	TM 15030 24861	Pass	Pass	Pass	Negative	0
1756	PO105	TM 16452 24005	Pass	Pass	Pass	Negative	0
1757	POIncol	614980 224854	Pass	Pass	Pass	Negative	0
1758	PO116	TM 14887 24994	Pass	Pass	Pass	Negative	0
1759	PO107	TM 15894 24061	Pass	Pass	Pass	Negative	0
1760	PO112	TM 15709 25239	Pass	Pass	Pass	Negative	0
1857	PO106	TM 16664 24317	Pass	Pass	Pass	Negative	0
1859	PO132	TM 13265 29325	Pass	Pass	Pass	Negative	0
1860	PO138	TM 10198 30183	Pass	Pass	Pass	Negative	0
1861	PO131	TM 13205 29298	Pass	Pass	Pass	Negative	0
1862	PO130	TM 13137 29295	Pass	Pass	Pass	Negative	0
1863	PO127	TM 13093 27091	Pass	Pass	Pass	Negative	0

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

Reported by: Esther Strafford

Approved by: Gabriela Danickova



METHODOLOGY

The samples detailed above have been analysed for the presence of GCN eDNA following the protocol stated in DEFRA WC1067 'Analytical and methodological development for improved surveillance of the Great Crested Newt, Appendix 5.' (Biggs et al. 2014). Each of the 6 sub-sample tubes are first centrifuged and pooled together into a single sample which then undergoes DNA extraction. The extracted sample is then analysed using real time PCR (qPCR), which uses species-specific molecular markers to amplify GCN DNA within a sample. These markers are unique to GCN DNA, meaning that there should be no detection of closely related species.

If GCN DNA is present, the DNA is amplified up to a detectable level, resulting in positive species detection. If GCN DNA is not present then amplification does not occur, and a negative result is recorded.

Analysis of eDNA requires scrupulous attention to detail to prevent risk of contamination. True positive controls, negative controls and spiked synthetic DNA are included in every analysis and these have to be correct before any result is declared and reported. Stages of the DNA analysis are also conducted in different buildings at our premises for added security.

SureScreen Scientifics Ltd is ISO9001 accredited and participate in Natural England's proficiency testing scheme for GCN eDNA testing. We also carry out regular inter-laboratory checks on accuracy of results as part of our quality control procedures.

INTERPRETATION OF RESULTS

- SIC:** **Sample Integrity Check** [Pass/Fail]
When samples are received in the laboratory, they are inspected for any tube leakage, suitability of sample (not too much mud or weed etc.) and absence of any factors that could potentially lead to inconclusive results.
- DC:** **Degradation Check** [Pass/Fail]
Analysis of the spiked DNA marker to see if there has been degradation of the kit or sample between the date it was made to the date of analysis. Degradation of the spiked DNA marker may lead indicate a risk of false negative results.
- IC:** **Inhibition Check** [Pass/Fail]
The presence of inhibitors within a sample are assessed using a DNA marker. If inhibition is detected, samples are purified and re-analysed. Inhibitors cannot always be removed, if the inhibition check fails, the sample should be re-collected.
- Result:** **Presence of GCN eDNA** [Positive/Negative/Inconclusive]
Positive: GCN DNA was identified within the sample, indicative of GCN presence within the sampling location at the time the sample was taken or within the recent past at the sampling location.
Positive Replicates: Number of positive qPCR replicates out of a series of 12. If one or more of these are found to be positive the pond is declared positive for GCN presence. It may be assumed that small fractions of positive analyses suggest low level presence, but this cannot currently be used for population studies. In accordance with Natural England protocol, even a score of 1/12 is declared positive. 0/12 indicates negative GCN presence.
Negative: GCN eDNA was not detected or is below the threshold detection level and the test result should be considered as evidence of GCN absence, however, does not exclude the potential for GCN presence below the limit of detection.



Folio No: E13850
Report No: 1
Purchase Order: 173EM1204/22042
Client: ECOLOGY RESOURCES
Contact: Elliot Mack

TECHNICAL REPORT

ANALYSIS OF ENVIRONMENTAL DNA IN POND WATER FOR THE DETECTION OF GREAT CRESTED NEWTS (*TRITURUS CRISTATUS*)

SUMMARY

When great crested newts (GCN), *Triturus cristatus*, inhabit a pond, they continuously release small amounts of their DNA into the environment. By collecting and analysing water samples, we can detect these small traces of environmental DNA (eDNA) to confirm GCN habitation or establish GCN absence.

RESULTS

Date sample received at Laboratory: 27/05/2022
Date Reported: 09/06/2022
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
1745	PO98	TM 16505 23432	Pass	Pass	Pass	Negative	0
1746	PO141	TM 10858 28469	Pass	Pass	Pass	Negative	0
1761	PO101	TM 16161 23542	Pass	Pass	Pass	Positive	4
1762	PO103	TM 16098 23862	Pass	Pass	Pass	Positive	3
1764	PO102	TM 16082 23551	Pass	Pass	Pass	Positive	10
1765	PO142	TM 11851 27529	Pass	Pass	Pass	Negative	0
1766	PO183	TM 11680 27882	Pass	Pass	Pass	Negative	0



1767	PO195	TM 11643 28069	Pass	Pass	Pass	Negative	0
1768	PO100	TM 16303 23523	Pass	Pass	Pass	Negative	0
1769	PO114	TM 15442 25077	Pass	Pass	Pass	Negative	0
1770	PO113	TM 15557 25153	Pass	Pass	Pass	Negative	0
1772	PO99	TM 16475 23393	Pass	Pass	Pass	Positive	12
1774	PO193	TM 15175 26030	Pass	Pass	Pass	Negative	0
1775	PO124	TM 14102 26511	Pass	Pass	Pass	Negative	0
1781	PO117	TM 15438 25475	Pass	Pass	Pass	Positive	1
1782	PO81	TM 19500 21879	Pass	Pass	Pass	Negative	0
1812	PO18	TM 22798 18127	Pass	Pass	Pass	Negative	0
1816	PO03	TM 23063 18339	Pass	Pass	Pass	Negative	0
1817	PO11	TM 22872 18209	Pass	Pass	Pass	Negative	0
1819	PO02	TM 23060 18351	Pass	Pass	Pass	Negative	0
1822	PO01	TM 23238 18578	Pass	Pass	Pass	Negative	0
1823	PO13-17	TM 22869 18185	Pass	Pass	Pass	Negative	0
1824	PO12	TM 22873 18193	Pass	Pass	Pass	Negative	0
1858	PO125	TM 14217 26683	Pass	Pass	Pass	Negative	0

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

Reported by: Esther Strafford

Approved by: Chris Troth



METHODOLOGY

The samples detailed above have been analysed for the presence of GCN eDNA following the protocol stated in DEFRA WC1067 'Analytical and methodological development for improved surveillance of the Great Crested Newt, Appendix 5.' (Biggs et al. 2014). Each of the 6 sub-sample tubes are first centrifuged and pooled together into a single sample which then undergoes DNA extraction. The extracted sample is then analysed using real time PCR (qPCR), which uses species-specific molecular markers to amplify GCN DNA within a sample. These markers are unique to GCN DNA, meaning that there should be no detection of closely related species.

If GCN DNA is present, the DNA is amplified up to a detectable level, resulting in positive species detection. If GCN DNA is not present then amplification does not occur, and a negative result is recorded.

Analysis of eDNA requires scrupulous attention to detail to prevent risk of contamination. True positive controls, negative controls and spiked synthetic DNA are included in every analysis and these have to be correct before any result is declared and reported. Stages of the DNA analysis are also conducted in different buildings at our premises for added security.

SureScreen Scientifics Ltd is ISO9001 accredited and participate in Natural England's proficiency testing scheme for GCN eDNA testing. We also carry out regular inter-laboratory checks on accuracy of results as part of our quality control procedures.

INTERPRETATION OF RESULTS

SIC: **Sample Integrity Check** [Pass/Fail]

When samples are received in the laboratory, they are inspected for any tube leakage, suitability of sample (not too much mud or weed etc.) and absence of any factors that could potentially lead to inconclusive results.

DC: **Degradation Check** [Pass/Fail]

Analysis of the spiked DNA marker to see if there has been degradation of the kit or sample between the date it was made to the date of analysis. Degradation of the spiked DNA marker may lead indicate a risk of false negative results.

IC: **Inhibition Check** [Pass/Fail]

The presence of inhibitors within a sample are assessed using a DNA marker. If inhibition is detected, samples are purified and re-analysed. Inhibitors cannot always be removed, if the inhibition check fails, the sample should be re-collected.

Result: **Presence of GCN eDNA** [Positive/Negative/Inconclusive]

Positive: GCN DNA was identified within the sample, indicative of GCN presence within the sampling location at the time the sample was taken or within the recent past at the sampling location.

Positive Replicates: Number of positive qPCR replicates out of a series of 12. If one or more of these are found to be positive the pond is declared positive for GCN presence. It may be assumed that small fractions of positive analyses suggest low level presence, but this cannot currently be used for population studies. In accordance with Natural England protocol, even a score of 1/12 is declared positive. 0/12 indicates negative GCN presence.

Negative: GCN eDNA was not detected or is below the threshold detection level and the test result should be considered as evidence of GCN absence, however, does not exclude the potential for GCN presence below the limit of detection.



Folio No: E13885
 Report No: 1
 Purchase Order: 173EM1204/22042
 Client: ECOLOGY RESOURCES
 Contact: Elliot Mack

TECHNICAL REPORT

ANALYSIS OF ENVIRONMENTAL DNA IN POND WATER FOR THE DETECTION OF GREAT CRESTED NEWTS (TRITURUS CRISTATUS)

SUMMARY

When great crested newts (GCN), *Triturus cristatus*, inhabit a pond, they continuously release small amounts of their DNA into the environment. By collecting and analysing water samples, we can detect these small traces of environmental DNA (eDNA) to confirm GCN habitation or establish GCN absence.

RESULTS

Date sample received at Laboratory: 30/05/2022
Date Reported: 09/06/2022
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
1743	PO147	TM 09673 27216	Pass	Pass	Pass	Negative	0
1771	PO192	TM 15211 26033	Pass	Pass	Pass	Positive	1
1777	PO27	TM 21924 17653	Pass	Pass	Pass	Negative	0
1783	PO28	TM 21916 17582	Pass	Pass	Pass	Negative	0
1801	PO26	TM 22334 17693	Pass	Pass	Pass	Negative	0
1806	PO29	TM 21768 17568	Pass	Pass	Pass	Negative	0
1809	PO25	TM 22558 17910	Pass	Pass	Pass	Negative	0



1810	PO19	TM 22718 18053	Pass	Pass	Pass	Negative	0
1811	PO20	TM 22708 18046	Pass	Pass	Pass	Negative	0
1813	PO22	TM 22589 17929	Pass	Pass	Pass	Negative	0
1814	PO24	TM 22557 17918	Pass	Pass	Pass	Negative	0
1815	PO23	TM 22575 17920	Pass	Pass	Pass	Negative	0
1818	PO21	TM 22622 17966	Pass	Pass	Pass	Negative	0
1820	PO37	TM 20452 18437	Pass	Pass	Pass	Negative	0
1821	PO64	TM 20225 19307	Pass	Pass	Pass	Negative	0
1825	PO69	TM 20202 19503	Pass	Pass	Pass	Positive	12
1826	PO59	TM 20329 19152	Pass	Pass	Pass	Negative	0
1827	PO67	TM 20121 19408	Pass	Pass	Pass	Positive	9
1828	PO65	TM 20193 19342	Pass	Pass	Pass	Positive	2
1829	PO36	TM 20916 18505	Pass	Pass	Pass	Negative	0
1830	PO34	TM 21136 18788	Pass	Pass	Pass	Negative	0
1831	PO60	TM 20262 19254	Pass	Pass	Pass	Positive	7
1832	PO66	TM 20149 19368	Pass	Pass	Pass	Negative	0

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

Reported by: Esther Strafford

Approved by: Chris Troth

METHODOLOGY



The samples detailed above have been analysed for the presence of GCN eDNA following the protocol stated in DEFRA WC1067 'Analytical and methodological development for improved surveillance of the Great Crested Newt, Appendix 5.' (Biggs et al. 2014). Each of the 6 sub-sample tubes are first centrifuged and pooled together into a single sample which then undergoes DNA extraction. The extracted sample is then analysed using real time PCR (qPCR), which uses species-specific molecular markers to amplify GCN DNA within a sample. These markers are unique to GCN DNA, meaning that there should be no detection of closely related species.

If GCN DNA is present, the DNA is amplified up to a detectable level, resulting in positive species detection. If GCN DNA is not present then amplification does not occur, and a negative result is recorded.

Analysis of eDNA requires scrupulous attention to detail to prevent risk of contamination. True positive controls, negative controls and spiked synthetic DNA are included in every analysis and these have to be correct before any result is declared and reported. Stages of the DNA analysis are also conducted in different buildings at our premises for added security.

SureScreen Scientifics Ltd is ISO9001 accredited and participate in Natural England's proficiency testing scheme for GCN eDNA testing. We also carry out regular inter-laboratory checks on accuracy of results as part of our quality control procedures.

INTERPRETATION OF RESULTS

- SIC:** **Sample Integrity Check** [Pass/Fail]
When samples are received in the laboratory, they are inspected for any tube leakage, suitability of sample (not too much mud or weed etc.) and absence of any factors that could potentially lead to inconclusive results.
- DC:** **Degradation Check** [Pass/Fail]
Analysis of the spiked DNA marker to see if there has been degradation of the kit or sample between the date it was made to the date of analysis. Degradation of the spiked DNA marker may lead indicate a risk of false negative results.
- IC:** **Inhibition Check** [Pass/Fail]
The presence of inhibitors within a sample are assessed using a DNA marker. If inhibition is detected, samples are purified and re-analysed. Inhibitors cannot always be removed, if the inhibition check fails, the sample should be re-collected.
- Result:** **Presence of GCN eDNA** [Positive/Negative/Inconclusive]
Positive: GCN DNA was identified within the sample, indicative of GCN presence within the sampling location at the time the sample was taken or within the recent past at the sampling location.
Positive Replicates: Number of positive qPCR replicates out of a series of 12. If one or more of these are found to be positive the pond is declared positive for GCN presence. It may be assumed that small fractions of positive analyses suggest low level presence, but this cannot currently be used for population studies. In accordance with Natural England protocol, even a score of 1/12 is declared positive. 0/12 indicates negative GCN presence.
Negative: GCN eDNA was not detected or is below the threshold detection level and the test result should be considered as evidence of GCN absence, however, does not exclude the potential for GCN presence below the limit of detection.



Folio No: E14177
 Report No: 1
 Purchase Order: 173EM1204/22042
 Client: ECOLOGY RESOURCES
 Contact: Elliot Mack

TECHNICAL REPORT

ANALYSIS OF ENVIRONMENTAL DNA IN POND WATER FOR THE DETECTION OF GREAT CRESTED NEWTS (*TRITURUS CRISTATUS*)

SUMMARY

When great crested newts (GCN), *Triturus cristatus*, inhabit a pond, they continuously release small amounts of their DNA into the environment. By collecting and analysing water samples, we can detect these small traces of environmental DNA (eDNA) to confirm GCN habitation or establish GCN absence.

RESULTS

Date sample received at Laboratory: 15/06/2022
Date Reported: 23/06/2022
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
1763	PO 068	TM 20182 19480	Pass	Pass	Pass	Positive	1
1773	PO 176	TM 17503 23948	Pass	Pass	Pass	Negative	0
1776	ID391-LP49 5E	TM 192 226	Pass	Pass	Pass	Negative	0
1780	ID222-LP239 5 Estuaries	TM 157 241	Pass	Pass	Pass	Positive	12
1794	PO82	TM 19080 22018	Pass	Pass	Pass	Positive	4
1796	PO 134	TM 12275 29994	Pass	Pass	Pass	Negative	0



1797	PO135	TM 11336 29737	Pass	Pass	Pass	Negative	0
1798	PO178	TM 11446 29778	Pass	Pass	Pass	Negative	0
1799	PO94	TM 17317 23195	Pass	Pass	Pass	Negative	0
1800	PO35	TM 21174 18656	Pass	Pass	Pass	Negative	0
1803	PO79	TM 20381 21316	Pass	Pass	Pass	Negative	0
1807	10260 - LP996 5E	TM 130 268	Pass	Pass	Pass	Positive	1
1808	ID145 - LP75 5 Estuaries	TM 193 233	Pass	Pass	Pass	Negative	0

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

Reported by: Esther Strafford

Approved by: Chelsea Warner

METHODOLOGY

The samples detailed above have been analysed for the presence of GCN eDNA following the protocol stated in DEFRA WC1067 'Analytical and methodological development for improved surveillance of the Great Crested Newt, Appendix 5.' (Biggs et al. 2014). Each of the 6 sub-sample tubes are first centrifuged and pooled together into a single sample which then undergoes DNA extraction. The extracted sample is then analysed using real time PCR (qPCR), which uses species-specific molecular markers to amplify GCN DNA within a sample. These markers are unique to GCN DNA, meaning that there should be no detection of closely related species.

If GCN DNA is present, the DNA is amplified up to a detectable level, resulting in positive species detection. If GCN DNA is not present then amplification does not occur, and a negative result is recorded.

Analysis of eDNA requires scrupulous attention to detail to prevent risk of contamination. True positive controls, negative controls and spiked synthetic DNA are included in every analysis and these have to be correct before any result is declared and reported. Stages of the DNA analysis are also conducted in different buildings at our premises for added security.

SureScreen Scientifics Ltd is ISO9001 accredited and participate in Natural England's proficiency testing scheme for GCN eDNA testing. We also carry out regular inter-laboratory checks on accuracy of results as part of our quality control procedures.

INTERPRETATION OF RESULTS

SIC: **Sample Integrity Check** [Pass/Fail]



When samples are received in the laboratory, they are inspected for any tube leakage, suitability of sample (not too much mud or weed etc.) and absence of any factors that could potentially lead to inconclusive results.

DC: Degradation Check [Pass/Fail]

Analysis of the spiked DNA marker to see if there has been degradation of the kit or sample between the date it was made to the date of analysis. Degradation of the spiked DNA marker may lead indicate a risk of false negative results.

IC: Inhibition Check [Pass/Fail]

The presence of inhibitors within a sample are assessed using a DNA marker. If inhibition is detected, samples are purified and re-analysed. Inhibitors cannot always be removed, if the inhibition check fails, the sample should be re-collected.

Result: Presence of GCN eDNA [Positive/Negative/Inconclusive]

Positive: GCN DNA was identified within the sample, indicative of GCN presence within the sampling location at the time the sample was taken or within the recent past at the sampling location.

Positive Replicates: Number of positive qPCR replicates out of a series of 12. If one or more of these are found to be positive the pond is declared positive for GCN presence. It may be assumed that small fractions of positive analyses suggest low level presence, but this cannot currently be used for population studies. In accordance with Natural England protocol, even a score of 1/12 is declared positive. 0/12 indicates negative GCN presence.

Negative: GCN eDNA was not detected or is below the threshold detection level and the test result should be considered as evidence of GCN absence, however, does not exclude the potential for GCN presence below the limit of detection.



Folio No: E14893
Report No: 1
Purchase Order: 173EM/204/22042
Client: ECOLOGY RESOURCES
Contact: Elliot Mack

TECHNICAL REPORT

ANALYSIS OF ENVIRONMENTAL DNA IN POND WATER FOR THE DETECTION OF GREAT CRESTED NEWTS (*TRITURUS CRISTATUS*)

SUMMARY

When great crested newts (GCN), *Triturus cristatus*, inhabit a pond, they continuously release small amounts of their DNA into the environment. By collecting and analysing water samples, we can detect these small traces of environmental DNA (eDNA) to confirm GCN habitation or establish GCN absence.

RESULTS

Date sample received at Laboratory: 05/07/2022
Date Reported: 19/07/2022
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
1787	PO 174	TM 22864 18176	Pass	Pass	Pass	Negative	0
1788	PO 104	TM 16325 23943	Pass	Pass	Pass	Negative	0
1791	PO 76	TM 20614 20151	Pass	Pass	Pass	Negative	0

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

Reported by: Chelsea Warner

Approved by: Chelsea Warner



METHODOLOGY

The samples detailed above have been analysed for the presence of GCN eDNA following the protocol stated in DEFRA WC1067 'Analytical and methodological development for improved surveillance of the Great Crested Newt, Appendix 5.' (Biggs et al. 2014). Each of the 6 sub-sample tubes are first centrifuged and pooled together into a single sample which then undergoes DNA extraction. The extracted sample is then analysed using real time PCR (qPCR), which uses species-specific molecular markers to amplify GCN DNA within a sample. These markers are unique to GCN DNA, meaning that there should be no detection of closely related species.

If GCN DNA is present, the DNA is amplified up to a detectable level, resulting in positive species detection. If GCN DNA is not present then amplification does not occur, and a negative result is recorded.

Analysis of eDNA requires scrupulous attention to detail to prevent risk of contamination. True positive controls, negative controls and spiked synthetic DNA are included in every analysis and these have to be correct before any result is declared and reported. Stages of the DNA analysis are also conducted in different buildings at our premises for added security.

SureScreen Scientifics Ltd is ISO9001 accredited and participate in Natural England's proficiency testing scheme for GCN eDNA testing. We also carry out regular inter-laboratory checks on accuracy of results as part of our quality control procedures.

INTERPRETATION OF RESULTS

- SIC:** **Sample Integrity Check** [Pass/Fail]
When samples are received in the laboratory, they are inspected for any tube leakage, suitability of sample (not too much mud or weed etc.) and absence of any factors that could potentially lead to inconclusive results.
- DC:** **Degradation Check** [Pass/Fail]
Analysis of the spiked DNA marker to see if there has been degradation of the kit or sample between the date it was made to the date of analysis. Degradation of the spiked DNA marker may lead indicate a risk of false negative results.
- IC:** **Inhibition Check** [Pass/Fail]
The presence of inhibitors within a sample are assessed using a DNA marker. If inhibition is detected, samples are purified and re-analysed. Inhibitors cannot always be removed, if the inhibition check fails, the sample should be re-collected.
- Result:** **Presence of GCN eDNA** [Positive/Negative/Inconclusive]
Positive: GCN DNA was identified within the sample, indicative of GCN presence within the sampling location at the time the sample was taken or within the recent past at the sampling location.
Positive Replicates: Number of positive qPCR replicates out of a series of 12. If one or more of these are found to be positive the pond is declared positive for GCN presence. It may be assumed that small fractions of positive analyses suggest low level presence, but this cannot currently be used for population studies. In accordance with Natural England protocol, even a score of 1/12 is declared positive. 0/12 indicates negative GCN presence.
Negative: GCN eDNA was not detected or is below the threshold detection level and the test result should be considered as evidence of GCN absence, however, does not exclude the potential for GCN presence below the limit of detection.





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