MORGAN AND MORECAMBE OFFSHORE WIND FARMS: TRANSMISSION ASSETS

Environmental Statement

Volume 3, Annex 3.8: Great crested newt and reptile survey technical report

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Contents

Tables

Figures

Appendices

Glossary

Term	Meaning
Applicants	Morgan Offshore Wind Limited (Morgan OWL) and Morecambe Offshore Windfarm Ltd (Morecambe OWL).
Correlation graph	Graph showing relationship between variables.
eDNA	Environmental Deoxyribonucleic Acid is DNA that is collected from soil, water, or air rather than directly from an individual organism.
Environmental Impact Assessment	The process of identifying and assessing the significant effects likely to arise from a project. This requires consideration of the likely changes to the environment, where these arise as a consequence of a project, through comparison with the existing and projected future baseline conditions.
Environmental Statement	The document presenting the results of the Environmental Impact Assessment process.
Expert Working Group	A forum for targeted engagement with regulators and interested stakeholders through the Evidence Plan Process.
Evidence Plan Process	A voluntary consultation process with specialist stakeholders to agree the approach to, and information to support, the EIA and Habitats Regulations Assessment processes for certain topics.
Habitats Regulations	The Conservation of Habitats and Species Regulations 2017 (as amended) and the Conservation of Offshore Marine Habitats and Species Regulations 2017 (as amended)
Habitat Suitability Index	Habitat Suitability Index is a methodology used to assess and evaluate the suitability of a pond or ponds for great crested newts.
Herptile	Reptiles and amphibians.
Landfall	The area in which the offshore export cables make landfall (come on shore) and the transitional area between the offshore cabling and the onshore cabling. This term applies to the entire landfall area at Lytham St. Annes between Mean Low Water Springs and the transition joint bays inclusive of all construction works, including the offshore and onshore cable routes, intertidal working area and landfall compound(s).
Morgan and Morecambe Offshore Wind Farms: Transmission Assets	The offshore and onshore infrastructure connecting the Morgan Offshore Wind Project and the Morecambe Offshore Windfarm to the national grid. This includes the offshore export cables, landfall site, onshore export cables, onshore substations, 400 kV grid connection cables and associated grid connection infrastructure such as circuit breaker compounds. Also referred to in this report as the Transmission Assets, for ease of reading.
Onshore Infrastructure Area	The area within the Transmission Assets Order Limits landward of Mean High Water Springs. Comprising the offshore export cables from Mean High Water Springs to the transition joint bays, onshore export cables, onshore substations and 400 kV grid connection cables, and associated temporary and permanent infrastructure including temporary and permanent compound areas and accesses. Those parts of the Transmission Assets Order Limits proposed only for ecological mitigation/biodiversity benefit are excluded from this area.

Term	Meaning
Onshore Order Limits	See Transmission Assets Order Limits: Onshore (below).
Study area	This is an area which is defined for each environmental topic which includes the Transmission Assets Order Limits as well as potential spatial and temporal considerations of the impacts on relevant receptors. The study area for each topic is intended to cover the area within which an impact can be reasonably expected.
Survey area	The area within which each survey has been undertaken. This may differ from the Study Area as a Survey Area will be based on species or survey-specific guidance on the extent of survey required, which and may be limited by, for example, habitat conditions, or be defined in terms of buffer areas around an area of potential impact.
Transmission Assets	See Morgan and Morecambe Offshore Wind Farms: Transmission Assets (above).
Transmission Assets Order Limits	The area within which all components of the Transmission Assets will be located, including areas required on a temporary basis during construction and/or decommissioning
Transmission Assets Order Limits: Onshore	The area within which all components of the Transmission Assets landward of Mean High Water Springs will be located, including areas required on a temporary basis during construction and/or decommissioning (such as construction compounds).

Acronyms

Acronym	Meaning
ARG	Amphibian and Reptile Group
BAP	Biodiversity Action Plan
Defra	Department for Environment, Food & Rural Affairs
DLL	District Level Licensing
eDNA	Environmental Deoxyribonucleic Acid
EIA	Environmental Impact Assessment
ES	Environmental Statement
GCN	Great Crested Newt
HRegs2	The Conservation of Habitats and Species Regulations, Part 2
HSI	Habitat Suitability Index
IUCN	International Union for the Conservation of Nature
JNCC	Joint Nature Conservation Committee
LBAP	Local Biodiversity Action Plan
LERN	Lancashire Environmental Records Network
MAGIC	Multi-Agency Geographic Information for the Countryside

Acronym	Meaning
NERC	Natural Environment and Rural Communities
WCA5	Wildlife and Countryside Act 1981, Schedule 5

Units

Unit	Description
°C	Degrees Celsius
ha	Hectare
mph	Miles per hour
km	Kilometre
m	Metre
ml	Millilitre

1 Great crested newt and reptile survey technical report

1.1 Introduction

1.1.1.1 This document forms Volume 3, Annex 3.8: Great crested newt and reptile survey technical report of the Environmental Statement (ES), prepared for the Morgan and Morecambe Offshore Wind Farms: Transmission Assets (referred to hereafter as 'the Transmission Assets'). The ES presents the findings of the Environmental Impact Assessment (EIA) process for the Transmission Assets.

1.1.1.2 The purpose of this technical report is to present the results of the great crested newt and reptile desk studies and field surveys to inform Volume 3, Chapter 3: Onshore ecology and nature conservation of the ES. The field surveys comprised Great Crested Newt (GCN)*Triturus cristatus* Habitat Suitability Index (HSI) and environmental DNA (eDNA) surveys, and reptile field surveys undertaken between April 2022 and July 2024.

1.1.1.3 The desk studies and field surveys were designed to determine the presence or likely absence of GCN and reptiles within and surrounding the Onshore Infrastructure Area.

1.1.2 Study area

1.1.2.1 The study area is intended to cover the area within which an impact can be reasonably expected and describes the geographical extent subject to desk-based research. The study area for GCN and reptile comprises the Onshore Infrastructure Area and a 2 km buffer (referred to hereafter as 'the study area'). The 2 km buffer area has been selected to extend to the furthest areas which GCN and reptiles would be expected to travel and provide information on all populations which could be affected by the works.

1.1.2.2 The location and geographic extent of the study areas are presented in **Figure 1.1** and **Figure 1.2** of this technical report, respectively.

1.1.3 Survey area

1.1.3.1 As the project is using Natural England's District Level Licensing (DLL) scheme, the area subject to GCN HSI and eDNA surveys ('the GCN survey area') is defined as the Onshore Infrastructure Area, as shown in **Figure 1.1**. The DDL scheme negates the requirement for surveys outside of the direct impacts, typically up to at least 250 m from a site boundary.

1.1.3.2 The area subject to reptile surveys ('the reptile survey area') is defined as the Onshore Infrastructure Area, as shown in **Figure 1.2.**

1.1.3.3 The reptile survey area extends to the Onshore Infrastructure Area only. The requirements to assess habitats beyond the Onshore Infrastructure Area is not required for reptiles as, unlike GCN, reptiles breed in terrestrial habitat and any populations using the site would therefore be detected during the onsite survey.

1.1.4 Contextual data

1.1.4.1 Owing to the iterative design process of the Transmission Assets, some GCN HSI and eDNA surveys, and reptile surveys were undertaken outside the final Onshore Infrastructure area. These surveys were located within previous iterations of the Onshore Infrastructure Area. Nevertheless, information from these surveys have been included in this technical report because they provide context regarding the ecological sensitivity of the wider area and to inform Volume 3, Chapter 3: Onshore ecology and nature conservation of the ES (where relevant). Any contextual information (based on survey data collected from outside the survey area) is clearly marked throughout this annex.

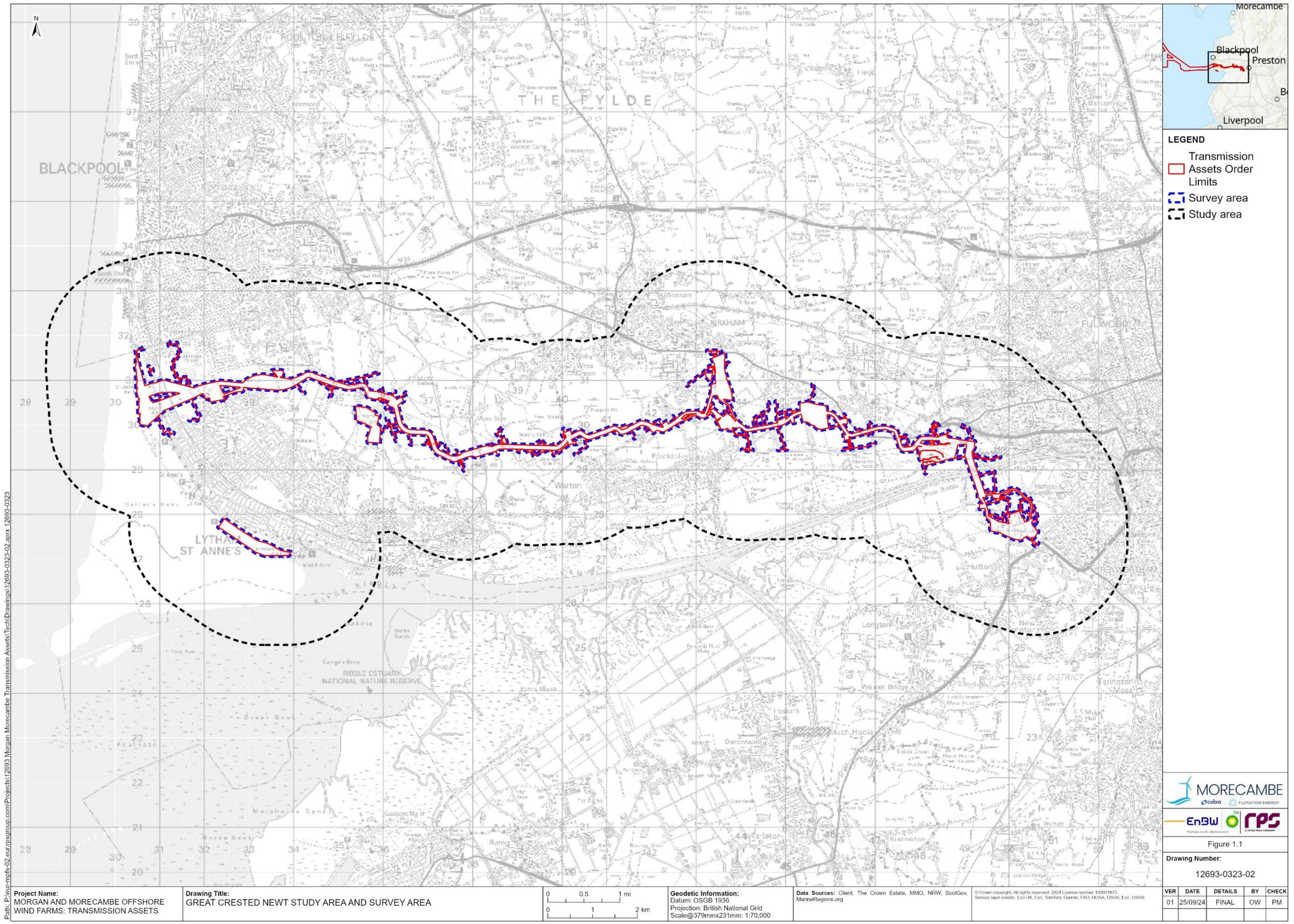

Figure 1.1: GCN study area and survey area

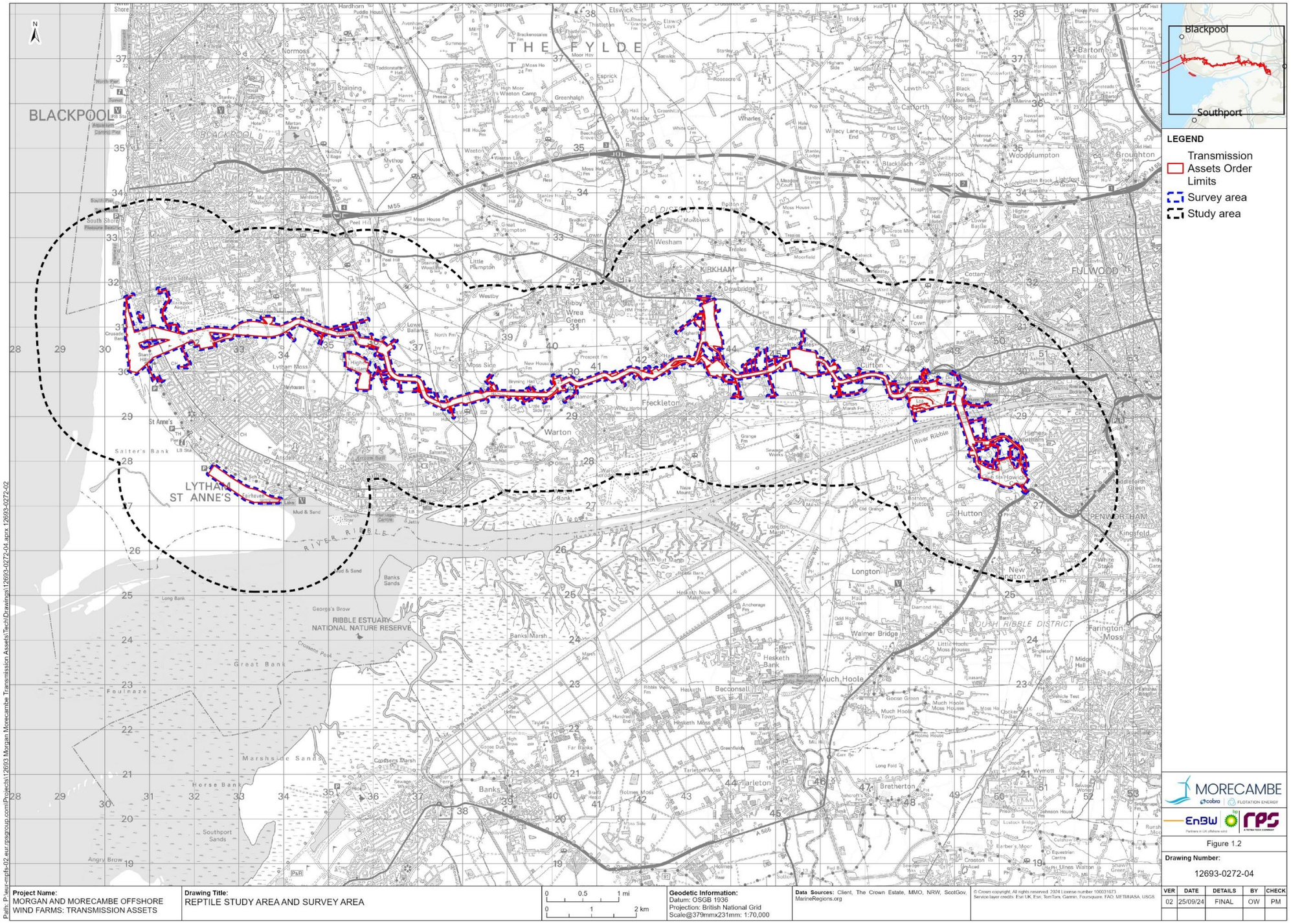

Figure 1.2: Reptile study area and survey area

1.1.5 Relevant legislation

1.1.5.1 Two key pieces of legislation are relevant for herptiles, a collective name for reptiles and amphibians, in the UK: the Conservation of Habitats and Species Regulations 2017 (the Habitats Regulations) and the Wildlife and Countryside Act 1981.

1.1.5.2 GCN are listed under Schedule 2 of the Conservation of Habitats and Species Regulations 2017 making the species a European protected species. This makes it an offence to:

- deliberately capture, injure or kill a GCN;
- deliberately disturb a GCN; and
- damage or destroy a breeding site or resting place of a GCN.

1.1.5.3 GCN are fully protected under Schedule 5 of the Wildlife and Countryside Act 1981 . Under Section 9 it is an offence to:

- intentionally or recklessly disturb a GCN while it is occupying a structure or place, which it uses for that purpose; and
- obstruct access to a place of shelter or protection.

1.1.5.4 Adder *Vipera berus*, grass snake *Natrix helvetica*, common lizard *Zootoca vivipara* and slow worm *Anguis fragilis* are protected under Schedule 5, Section 9 of the Wildlife and Countryside Act 1981, which prohibits the following with respect to reptiles:

- intentional or reckless injuring or killing;
- selling, offering, or exposing for sale, or having in possession or transporting for the purpose of sale, any live or dead wild animal or any part of, or anything derived from, such an animal; and
- publishing or causing to be published any advertisement likely to be understood as conveying buying or selling, or intending to buy or sell, any of those things.

1.1.5.5 Sand lizard *Lacerta agilis* and smooth snake *Coronella austriaca* are protected under Schedule 5, Section 9 of the Wildlife and Countryside Act 1981, which prohibits the following with respect to reptiles.

- Intentional disturbance while occupying a structure or place used for shelter or protection.
- Obstruction of access to any structure or place which they use for shelter or protection.
- Selling, offering, or exposing for sale, or having in possession or transporting for the purpose of sale, any live or dead wild animal or any part of, or anything derived from, such an animal.
- Publishing or causing to be published any advertisement likely to be understood as conveying buying or selling, or intending to buy or sell, any of those things.

1.1.5.6 In addition, smooth snake and sand lizard are afforded additional protections under Schedule 2 of the Habitats Regulations. This makes it an offence to:

- capture, injure or kill either species;
- disturb either species;
- take or destroy the eggs; and
- damage or destroy a breeding site or resting place of either species.

1.1.6 Consultation

1.1.6.1 In October 2022, the Applicants submitted an EIA Scoping Report to the Planning Inspectorate, which described the scope and methodology for the technical studies being undertaken to provide an assessment of any likely significant effects for the construction, operation and maintenance and decommissioning phases of the Transmission Assets.

1.1.6.2 The scope, methodology and findings of the herptile surveys, including those undertaken beyond the current GCN and reptile survey areas, were discussed, and agreed with stakeholders via regular Onshore Ecology Expert Working Group meetings. Further detail regarding consultation undertaken with respect to onshore ecology, including herptile surveys, can be found in Volume 3, Chapter 3: Onshore ecology and nature conservation of the ES.

1.1.6.3 District Level Licensing (DLL) is a new approach to authorising developments affecting GCN, introduced in 2016 by Natural England. It is a strategic mitigation licence granted in registered areas at a local authority level and allows developers to proceed with developments where there is a likelihood of GCN being present, based on the presence of ponds within 250 m of the development site. DLL shifts investment from site-based assessment and mitigation into strategic habitat improvements that will increase GCN populations at a county level.

1.1.6.4 The suitability of the Transmission Assets for application to the DLL scheme was discussed with Natural England, who have confirmed that they support this approach for the Transmission Assets in principle. As such, the Project is applying to the DLL scheme, and HSI and eDNA surveys have been undertaken to support the application.

1.1.6.5 There were no comments on the methods for GCN surveys in the statutory consultation responses.

1.1.6.6 Comments from the Section 42 consultation were received with specific reference to reptiles. It was stated by Natural England that sand lizard *Lacerta agilis* surveys should be undertaken due to the presence of sand dunes and re-introduction of sand lizards within the Onshore Order Limits.

1.1.6.7 The sand lizard populations have been monitored by the Fylde Sand Dune Project Steering Group between 2021 and 2023. Surveys show the lizards are still present at Fylde Dunes, although population numbers and exact locations have not been made available due to the protection of the species. Desk study results, including sand lizard information from the Fylde Sand Dune Project, are shown in **section 1.3.1** and Volume 3, Annex 3.1: Onshore

ecology desk study technical report of the ES. These desk study results have informed the assessment presented in Volume 3, Chapter 3: Onshore ecology and nature conservation of the ES.

1.1.6.8 Further surveys are not considered necessary for the Transmission Assets due to existing survey information available being sufficient to assess the potential impacts to the species.

1.2 Methodology

1.2.1 Overview

1.2.1.1 A combination of desk studies and field surveys were undertaken to ascertain the presence or likely absence of notable herptile species and the diversity of these species within the study and survey areas.

1.2.1.2 The results of the desk study are presented in Volume 3, Annex 3.1: Onshore ecology desk study technical report of the ES and summarised below.

1.2.2 Desk study

1.2.2.1 Herptile species data was collected from existing desk studies and datasets. These are summarised in **Table 1.1** below.

Table 1.1: Summary of key desktop sources for Transmission Assets relevant to herptiles

Title	Source	Year	Author
Lancashire Environmental Records Network (LERN)	LERN data share site.	2024	LERN
Multi Agency Geographic Information for the Countryside (MAGIC)	Department for Environment, Food and Rural Affairs (Defra).	2024	Defra
UK Protected Area Joint Nature Conservation Committee (JNCC)	JNCC website.	2024	JNCC
Red List	International Union for the Conservation of Nature (IUCN)	2023	IUCN
Fylde Sand Dunes Project	Fylde Sand Dune Project Steering Group	2021-2023	Fylde Sand Dunes Project Steering Group

1.2.3 Site-specific surveys: GCN

Extended phase 1 habitat survey

1.2.3.1 In order to inform the baseline and the existing environmental conditions to be reported within the ES, as well as to inform any identified mitigation,

extended phase 1 surveys have been carried out for the Transmission Assets.

1.2.3.2 A combination of desk study and extended phase 1 habitat surveys undertaken between May 2022 and May 2024 identified 555 waterbodies, 61 within the Onshore Infrastructure Area, and 494 within a 250 m buffer. Due to the decision to apply to the DLL scheme, the requirement for surveys of ponds outside of the Onshore Infrastructure Area was negated. Instead, survey effort was focused on those ponds within the Onshore Infrastructure Area (i.e. those ponds subject to direct impacts).

1.2.3.3 All accessible waterbodies were visited and subject to a Habitat Suitability Index (HSI) assessment in the field by competent surveyors. The HSI assessment assessed the potential suitability of the waterbodies for GCN. HSI assessments were undertaken in 2022 and 2024.

1.2.3.4 eDNA assessments were undertaken of some waterbodies in 2022, based on accessibility and the Preliminary Environmental Information Report stage draft Onshore Order Limits (see Volume 1, Chapter 4: Site selection and consideration of alternatives of the ES for a description of the design evolution of the Transmission Assets).

1.2.3.5 In 2024, eDNA surveys were carried out of all accessible waterbodies within the Onshore Infrastructure Area except for any waterbodies in the sand dunes where trenchless techniques are being employed and there is negligible potential for adverse impacts on GCN.

HSI assessment

1.2.3.6 The suitability of a pond to support GCN was assessed following the HSI assessment methodology described in the UK Amphibian and Reptile Group's (ARG) Advice Note 5 (ARG UK, 2010). This method is a modified version of the original HSI methodology described in Oldham *et al*, 2000. The methodology can be found in Appendix F of Volume 3, Annex 3.4 Onshore ecology survey methodologies of the ES.

1.2.3.7 The HSI method involves the assessment of ten key habitat parameters, listed in

1.2.3.8 **Table** 1.2 below, which are typically associated with ponds used by GCN. Each parameter is given a score from zero to one based on the descriptions and HSI scoring system provided in Advice Note 5 (ARG UK, 2010).

1.2.3.9 HSI surveys can be carried out at any time of year, although it is recommended that the surveys are carried out between March and June (inclusive) to capture the condition of ponds within the GCN breeding season.

1.2.3.10 These assessments were used to inform the eDNA surveys and will be used to inform the DLL approach discussed below.

Table 1.2: HSI indices

Suitability index	Topic	Description
SI1	Location	Sites scored according to UK zone in which they occur.

Suitability index	Topic	Description
SI2	Pond area	Surface area of the pond when water is at its highest level (excluding flooding events); usually in the spring. For ponds smaller than 50 m^2 a score of 0.05 is used. For ponds larger than 2,000 m^2 this factor is omitted. Index score measured from a correlation graph.
SI3	Permanence	Local knowledge and personal judgement. Four category scale: never dries, rarely dries, sometimes dries, dries annually.
SI4	Water quality	Based on invertebrate diversity, presence of submerged plants, knowledge of the water sources. Not to be confused with water clarity. Four point scale: good, moderate, poor, bad.
SI5	Shade	Estimate percentage of pond perimeter shaded, to at least 1 m from shore, excluding emergent vegetation. May to September inclusive. Score taken from correlation graph.
SI6	Waterfowl	Three point scale of impact: absent, minor, major.
SI7	Fish	Local knowledge and site observations. Four point scale: absent, possible, minor, major.
SI8	Pond count	Number of ponds within 1 km. Score taken from correlation graph.
SI9	Terrestrial habitat	Require understanding of newt requirements. Habitat within 250 m of a pond, not separated by a significant barrier to newt movement. 4 point scale: good, moderate, poor, none.
SI10	Macrophytes	Estimate of percentage pond surface area covered by macrophytes (including emergent, floating (not duckweed) and submerged plants reaching the surface). May to September inclusive. Score taken from correlation graph.

1.2.3.11 An overall HSI score was calculated from the scores for each habitat parameter listed in **Table 1.2**, using the following equation:

$$HSI\ Score = (SI1 \times SI2 \times SI3 \times SI4 \times SI5 \times SI6 \times SI7 \times SI8 \times SI9 \times SI10)^{1/10}$$

1.2.3.12 The overall HSI score was then translated into a classification of habitat suitability, as listed in **Table 1.3** below. Data for HSI surveys was recorded using the Arc GIS Field Maps application.

Table 1.3: HSI classification

HSI score	Suitability for GCN
>0.8	Excellent
0.7 – 0.79	Good
0.6 – 0.69	Average
0.5 – 0.59	Below average
<0.5	Poor

eDNA analysis

1.2.3.13 An eDNA analysis of water samples collected from ponds was undertaken following the method set out in the document Analytical and Methodological Development for Improved Surveillance of the Great Crested Newt (Biggs et al., 2014). This method has been developed for standing waterbodies only, due to the potential for eDNA to be washed downstream from a sample location before samples can be collected in flowing waterbodies/watercourses.

1.2.3.14 Collection of eDNA can be undertaken between 15 April and 30 June. Water samples were collected by GCN licensed ecologists using sampling kits provided by a laboratory approved in the use of this survey method. Samples were not collected in heavy rain to avoid potential risk of contamination.

1.2.3.15 Surveyors collected 30 ml of water samples from 20 locations along the margins of a waterbody, using a sterile ladle. The samples were collected from the bank edge without entering or touching the water to prevent contamination of samples. Where access allowed, water samples were collected from points evenly spaced along the banks. When collecting the water samples, the surveyors used a ladle to gently agitate the water and mix the water column before collecting each sample, whilst taking care not to disturb any sediment.

1.2.3.16 The 20 samples collected from each waterbody were emptied into a sterile plastic bag and homogenised by gently shaking the bag to ensure eDNA is evenly mixed through the sample. A pipette was then used to transfer six 15 ml sub-samples of the water from the bag into sterile tubes containing 35 ml of ethanol to preserve the eDNA samples.

1.2.3.17 The samples were then stored in a refrigerator before being couriered to the lab for analysis to confirm presence or absence of GCN eDNA.

1.2.3.18 The results of the eDNA surveys will be used to inform DLL approach discussed below.

District level licensing

1.2.3.19 As set out in section 3.3 of Volume 3, Chapter 3: Onshore ecology and nature conservation of the ES, the possibility of the Transmission Assets applying for the DLL scheme was discussed with the Expert Working Group. Natural England responded that they have no objection in principle and requested the Applicants make a formal request.

1.2.3.20 The Natural England Open Data Geoportal was used to review GCN risk zones with respect to the DLL scheme (Natural England, 2024). The Transmission Assets GCN survey area lies within both amber and green zones for DLL (as provided by the NE Geoportal detailed above), as can be seen in **Figure 1.3**. Amber zones are those that contain suitable habitat and GCN are likely to be present. Green zones are those that contain moderate habitat suitability and therefore GCN may be present.

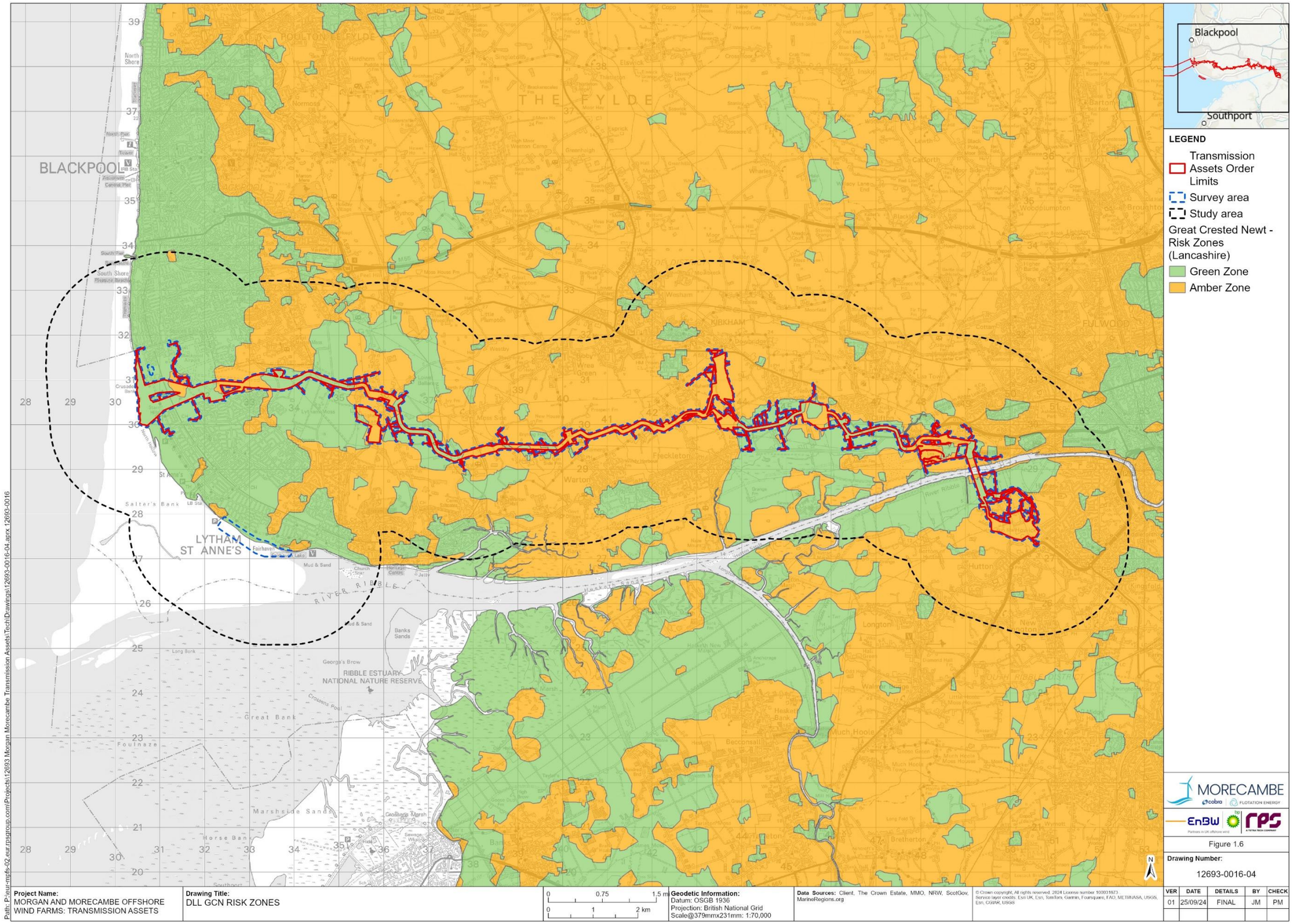

Figure 1.3: DLL GCN risk zones

1.2.4 Site-specific surveys: reptiles

Extended phase 1 habitat survey

1.2.4.1 In order to inform the baseline and the existing environmental conditions to be reported within the ES, as well as to inform any identified mitigation, extended phase 1 surveys have been carried out for the Transmission Assets. Sites were scoped in for reptile surveys if they provided suitable habitat to support a breeding population of reptiles. This was based on guidance in the Reptile Habitat Management Handbook (Edgar, Foster, and Baker, 2010).

1.2.4.2 Where phase 1 surveys identified the requirement for further reptile surveys, these were carried out following the guidelines set out in Froglife Advice Sheet 10 (Froglife, 1999) and the Herpetofauna Workers Manual (Gent and Gibson, 2003).

Reptile survey

1.2.4.3 At each site selected for reptile survey, a combination of corrugated iron and roofing felt refugia measuring a minimum of 0.5 metres (m) x 0.5 m was placed in areas identified as suitable habitat. At sites where the habitat assessment identified potential for grass snake to occur, surveyors deployed an appropriate number (based on extent of suitable habitat) of larger refugia, to increase the likelihood of detecting this species.

1.2.4.4 High quality habitats for reptiles are those which provide structural variation to accommodate a range of reptile behaviours, including foraging, breeding, basking, and sheltering.

1.2.4.5 High quality habitats that are important for reptiles (including rare species) such as rough grassland, scrub, and woodland edge are usually surveyed at a density of 100 refugia per hectare (ha). However, for presence/absence surveys for common species, the recommended survey density is 5 to 10 refugia per ha as per guidance by Froglife (1999). This could be further reduced to a density of 2.5 per ha upon inspection by the surveyor if the majority of the site comprises intensively farmed arable fields and/or close-grazed pasture. As these habitats are unsuitable for reptiles, the actual density of refugia per ha of suitable reptile habitat substantially would exceed the recommended density, and as such the number of refugia placed would be reduced accordingly.

1.2.4.6 All refugia were number marked using spray paint and their location recorded using the ArcGIS Field Maps application. Once placed, artificial refugia was left to settle for seven to ten days prior to conducting the first check.

1.2.4.7 Each site containing refugia was checked for reptiles on seven occasions during the optimal survey season (April to May, and September),

1.2.4.8 Where any survey visits were conducted during the sub-optimal months of June and July, additional visits were required if surveys were undertaken during unsuitable conditions until at least seven visits under suitable conditions were conducted.

1.2.4.9 Binoculars were used to check for reptiles between refugia, as well as careful checks by lifting each refugium. Checks were spaced apart by at least two days.

1.2.4.10 Each refugia check was conducted during the following conditions.

- Time: conducted between 07:00 and 18:00.
- Air temperature: 9°c to 20°c.
- Wind: still to moderate (equivalent to up to Beaufort 4; 13 – 17 mph.
- Rain: no or light rain only at time of survey. Surveys between periods of heavy rain (when all other conditions are suitable) are also acceptable.

1.2.4.11 During each check the surveyor recorded details of all reptiles encountered during the survey, including refugia number, species, number, life stage (adult, subadult, juvenile) and when possible, sex.

1.2.4.12 All records of reptiles were recorded using the ArcGIS Field Maps application which will link the results to the location of the reptile refugia.

1.2.5 Incidental records

1.2.5.1 Any sightings of non-target species (or evidence of) recorded during surveys were reported in the separate incidental records form on the ArcGIS Field Maps application.

1.2.6 Limitations

1.2.6.1 Access was not granted to all identified waterbodies within the GCN survey area and as a result, all could not be fully surveyed. However, as GCN mitigation will be provided via the District Level Licensing Scheme, the lack of survey data in any ponds will be taken into account when the contribution required by Transmission Assets is calculated by Natural England and therefore any absent survey data does not affect the validity of mitigation measures.

1.2.6.2 Due to late grant of access, some reptile survey visits were carried out in suboptimal months. However, surveys were carried out in optimal weather conditions and at least seven visits were completed for all but two locations scoped in for reptile survey. Therefore, these surveys are considered representative of the baseline, as far as seasonally possible.

1.2.6.3 Due to a combination of lack of access and livestock destroying reptile mats only 2 of 6 visits were completed in the Lea Marsh area (part of area 6). However, this area is outside of the Onshore Permanent Infrastructure area and considered to not have a significant impact with regards to informing the impact assessment for reptiles.

1.3 Results

1.3.1 Desk study

LERN desk study

1.3.1.1 The desk study data provided by LERN contained three reptile species and five amphibian species recorded in the last ten years within the GCN and reptile study area.

1.3.1.2 Five amphibian species, including GCN, common frog *Rana temporaria*, common toad *Bufo bufo*, palmate newt *Lissotriton helveticus*, and smooth newt *Lissotriton vulgaris*, have been recorded within the Onshore Infrastructure Area as well as the wider study area.

1.3.1.3 Eggs or juvenile GCN indicating breeding populations of GCN have been recorded at approximately 500 m from the Onshore Infrastructure Area. Several adults of both sexes have been identified, as well as eggs. As various life stages have been recorded this indicates that this population of GCN is a breeding population.

1.3.1.4 Three species of reptile, including sand lizard *Lacerta agilis,* slow-worm *Anguis fragilis*, and common lizard *Zootoca vivipara*, have been recorded within 2 km of the Onshore Infrastructure Area. Of these species, sand lizard and common lizard have been recorded within the Onshore Infrastructure Area in sand dunes close to Lytham St Annes at the west end of the Onshore Infrastructure Area (refer to Volume 3, Annex 3.1: Onshore ecology desk study technical report of the ES).

1.3.1.5 The results of protected or otherwise notable herptile records within the study area is presented in **Table 1.4** below.

Table 1.4: Desk study results

Group	Taxon name	Common name	Date ranges of records held by LERN	Number of records	Conservation status
Reptile	*Anguis fragilis*	Slow-worm	2012 to 2015	7	• Local Biodiversity Action Plan (LBAP). • Natural Environment and Rural Communities (NERC). • Wildlife and Countryside Act 1981, Schedule 5 (WCA5).
Reptile	*Zootoca vivipara*	Common lizard	2015 to 2019	4	• WCA5

Group	Taxon name	Common name	Date ranges of records held by LERN	Number of records	Conservation status
Reptile	*Lacerta agilis*	Sand lizard	2020	28	• NERC. • The Conservation of Habitats and Species Regulations, Part 2 (HRegs2). • LBAP. • WCA5
Amphibian	*Triturus cristatus*	Great crested newt	2012 to 2020	475	• Biodiversity Action Plan. • HRegs2. • WCA5.
Amphibian	*Rana temporaria*	Common frog	2012 to 2020	116	• Habitats Directive, Annex 5. • WCA5.
Amphibian	*Bufo bufo*	Common toad	2012 to 2019	205	• WCA5.
Amphibian	*Lissotriton helveticus*	Palmate newt	2012 to 2019	20	• WCA5.
Amphibian	*Lissotriton vulgaris*	Smooth newt	2012 to 2019	202	• WCA5.

Sand lizards

1.3.1.6 Data from the Fylde Sand Dune Project Steering Group showed that sand lizards are present on the Fylde Sand Dunes site where an introduction scheme was implemented between 2017 and 2020. The steering group results did not provide exact locations and population sizes but provided ‘heat maps’ of survey observations made via transect surveys undertaken in 2021, 2022 and 2023. These indicate that sand lizards are present in the part of the sand dunes within the Onshore Infrastructure Area.

1.3.2 Site-specific surveys: GCN

HSI surveys

1.3.2.1 A total of 97 waterbodies were subject to HSI assessment in total, in surveys undertaken in 2022 and 2024. Of these, 60 ponds were within the GCN survey area and 37 were outside the survey area and are included for contextual information.

1.3.2.2 HSI scores for ponds within the survey area are presented in **Table 1.5** and **Table 1.6** and provide HSI scores for ponds which were included within the HSI surveys but which lie outside of the defined GCN survey area. Although outside of the defined survey area, these results have been included to provide contextual information about the presence of GCN within the surrounding area.

1.3.2.3 In 2022, 36 contextual ponds were surveyed. Two ponds were classified as excellent, five ponds were classified as good, 13 ponds were classified as average, four ponds as below average and 13 ponds as poor. In 2024, one contextual pond was surveyed, and was classified as good.

1.3.2.4 The full (raw) HSI survey data is provided in **Appendix A** at the end of this technical report.

Table 1.5: HSI survey results (2022)

Waterbody reference	Location (grid reference)	HSI Score	Suitability	Within Onshore Infrastructure Area
4	SD3104130602	0.53	Below average	No
11	SD3134030731	0.70	Average	Yes
12	SD3137430735	0.70	Good	Yes
14	SD3154630848	0.78	Good	Yes
15	SD3155430602	0.77	Good	Yes
55	SD3577130290	0.69	Average	Yes
62	SD3590829999	0.49	Poor	No
63	SD3591330127	0.50	Poor	No
67	SD3609430716	0.73	Good	No
99	SD3755029726	0.50	Poor	No
143	SD3942129486	0.44	Poor	Yes
146	SD4000529691	0.29	Poor	No
159	SD4091729900	0.72	Good	No
163	SD4125730014	0.70	Good	No
164	SD4136630111	0.70	Good	No
165	SD4144929946	0.63	Average	No
166	SD4148929806	0.59	Below average	No
167	SD4155930041	0.47	Poor	No
169	SD4157630224	0.70	Good	No
170	SD4163130164	0.36	Poor	No
172	SD4173529887	0.45	Poor	No

Waterbody reference	Location (grid reference)	HSI Score	Suitability	Within Onshore Infrastructure Area
176	SD4214129993	0.53	Below average	No
178	SD4223730246	0.50	Below average	No
180	SD4226529820	0.81	Excellent	No
184	SD4246629804	0.65	Average	No
185	SD4249029672	0.48	Poor	No
186	SD4255730333	0.80	Good	No
188	SD4259630221	0.29	Poor	No
190	SD4280730054	0.72	Good	No
193	SD4284929922	0.78	Good	No
213	SD4321130064	0.60	Average	No
261	SD4391430688	0.41	Poor	No
262	SD4391830816	0.66	Average	No
266	SD4403730670	0.56	Below average	No
340	SD4522430139	0.68	Average	No
354	SD4540930606	0.60	Average	No
373	SD4570930965	0.69	Average	No
504	SD4940527478	0.62	Average	No
68	SD3619631516	0.86	Excellent	No
113	SD3783030252	0.63	Average	No
137	SD3894729733	0.48	Poor	No
144	SD3958630097	0.81	Excellent	No
153	SD4068630068	0.43	Poor	No
162	SD4115730240	0.59	Below Average	No
168	SD4156130347	0.43	Poor	No
174	SD4195230340	0.66	Average	No
175	SD4206230376	0.60	Average	No
182	SD4238830555	0.31	Poor	No
187	SD4258330499	0.79	Good	No
265	SD4400230970	0.67	Average	No
267	SD4404630343	0.67	Average	No
271	SD4413030356	0.64	Average	No

Waterbody reference	Location (grid reference)	HSI Score	Suitability	Within Onshore Infrastructure Area
272	SD4413630688	0.43	Poor	No
273	SD4414630710	0.41	Poor	No
277	SD4420030565	0.75	Good	No
281	SD4430530788	0.71	Good	No
282	SD4436127202	0.72	Good	No
397	SD4668926844	0.40	Poor	No
409	SD4691527383	0.64	Average	No
410	SD4691929087	0.45	Poor	No
417	SD4724227200	0.52	Below average	No
420	SD4728627453	0.45	Poor	No
430	SD4738126985	0.65	Average	No
431	SD4739827474	0.61	Average	No
432	SD4739828949	0.64	Average	No
434	SD4741726901	0.56	Below average	No
435	SD4741928988	0.64	Average	No
441	SD4760426716	0.75	Good	No
450	SD4778926669	0.68	Average	No
496	SD4903627684	0.38	Poor	No
497	SD4909727655	0.44	Poor	No
501	SD4921627489	0.58	Below average	No
505	SD4941327404	0.64	Average	No
506	SD4943027224	0.39	Poor	No

Table 1.6: HSI survey results (2024)

Waterbody reference	Location (grid reference)	HSI Score	Suitability	Within Onshore Infrastructure Area
89	SD3726129391	0.57	Below average	Yes
125	SD3828229384	0.60	Below average	Yes
133	SD3869129461	0.71	Good	Yes
154	SD4068729811	0.71	Good	Yes
179	SD4226429870	0.44	Poor	Yes
233	SD4343331113	0.43	Poor	Yes
235	SD4348930983	0.68	Average	Yes
238	SD4351630992	0.71	Good	Yes
240	SD4352930901	0.52	Below average	Yes
241	SD4356630777	0.49	Poor	Yes
243	SD4363930755	0.68	Average	Yes
246	SD4367929861	0.68	Average	No
249	SD4369429904	0.65	Average	No
285	SD4439029988	0.38	Poor	No
472	SD4843929650	0.49	Poor	Yes
510	SD4961527904	0.62	Average	Yes
511	SD4965027861	0.65	Average	Yes
519	SD4995527635	0.69	Average	Yes
527	SD5024728264	0.76	Good	Yes
533	SD5044927512	0.75	Good	Yes
522	SD5000529427	0.700359	Good	No

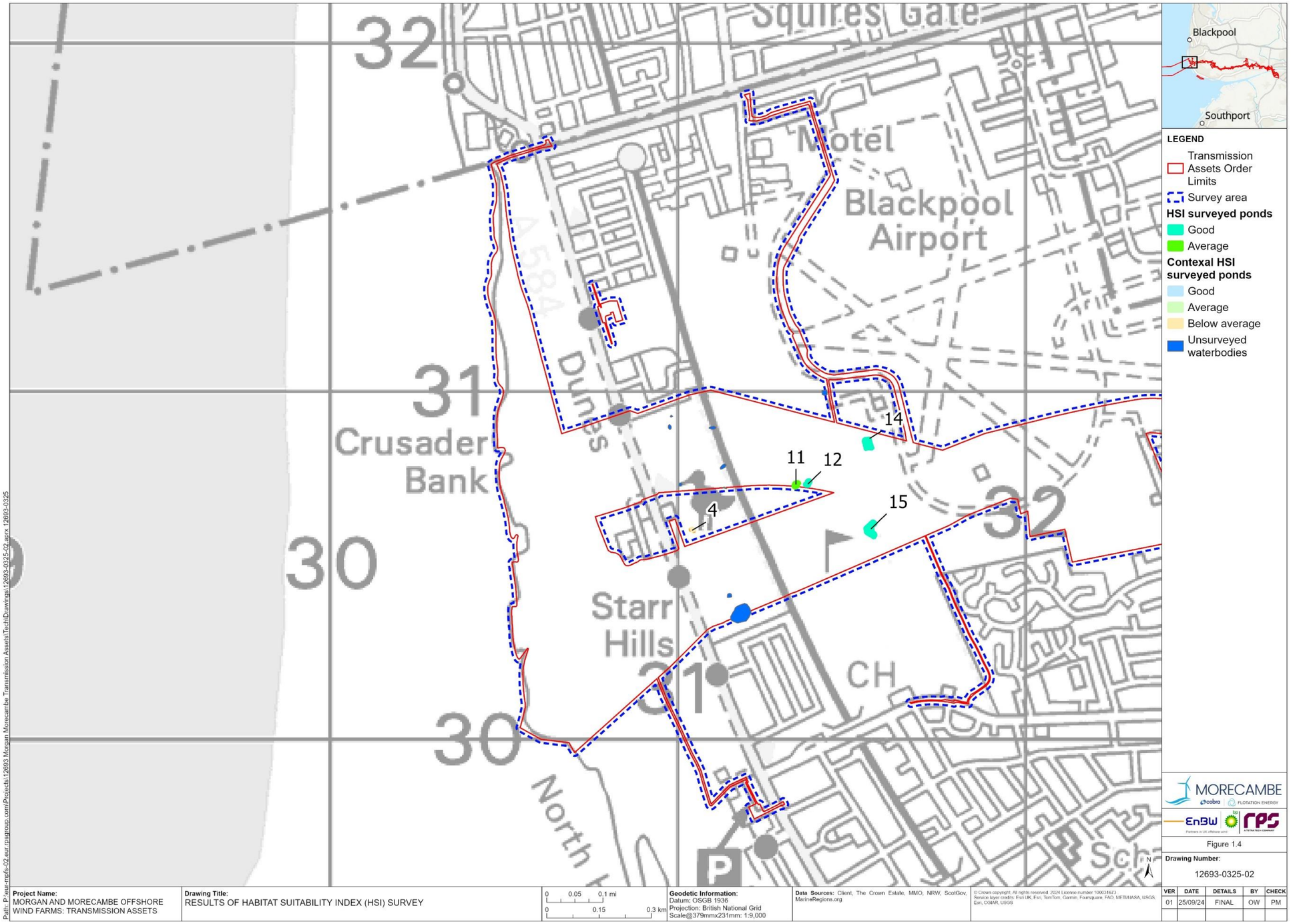

Figure 1.4: Results of HSI survey - Sheet 1

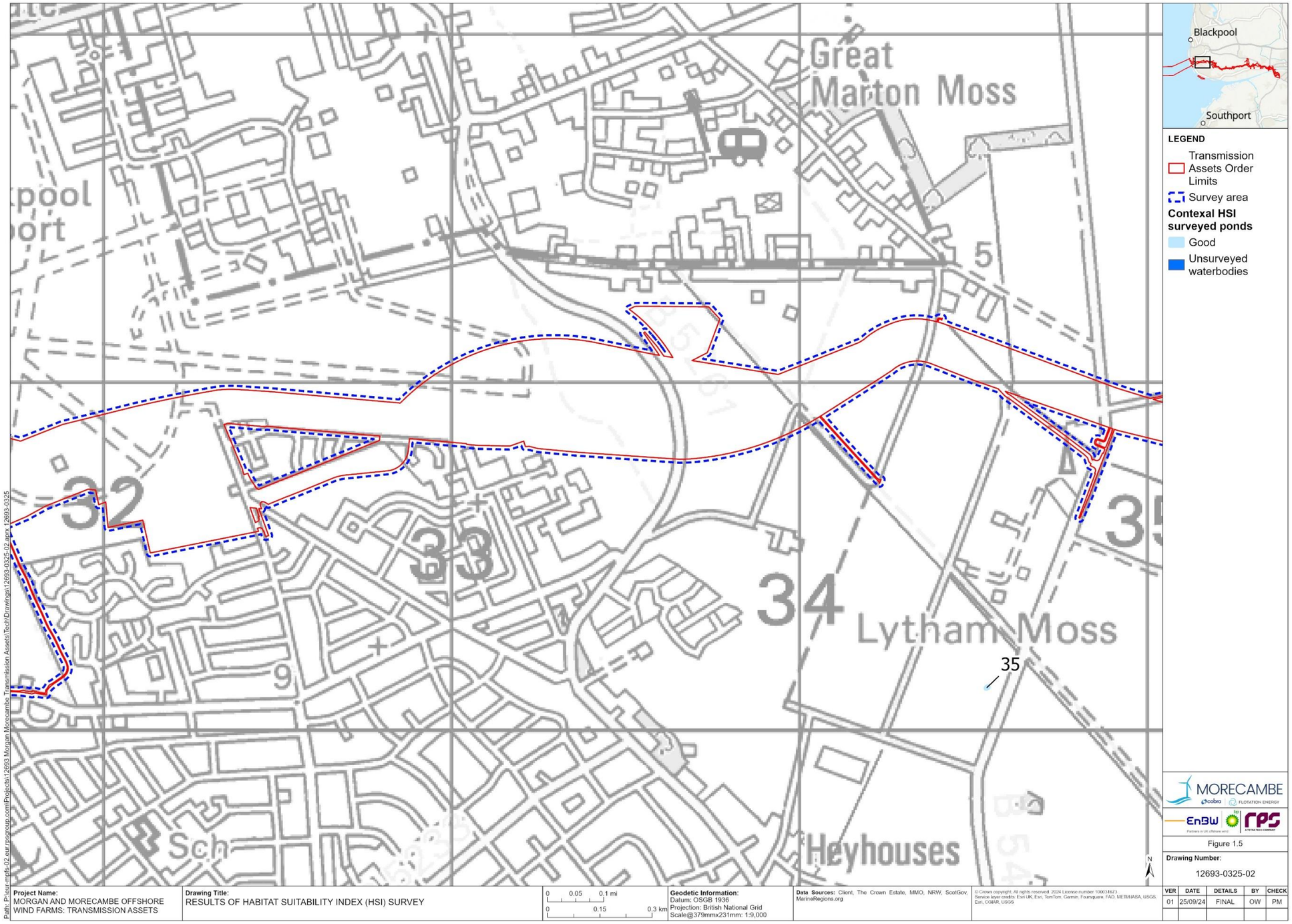

Figure 1.5: Results of HSI survey - Sheet 2

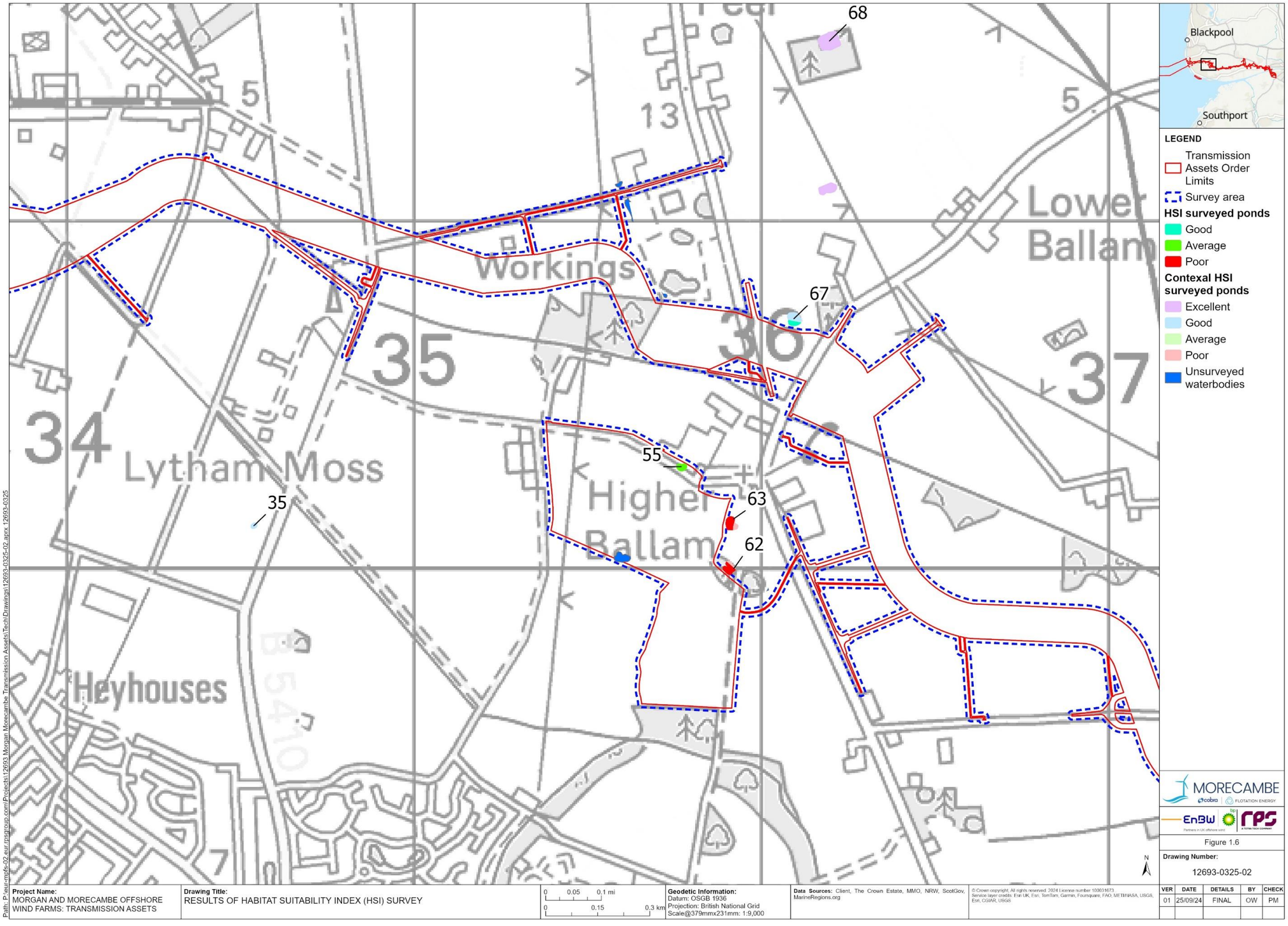

Figure 1.6: Results of HSI survey - Sheet 3

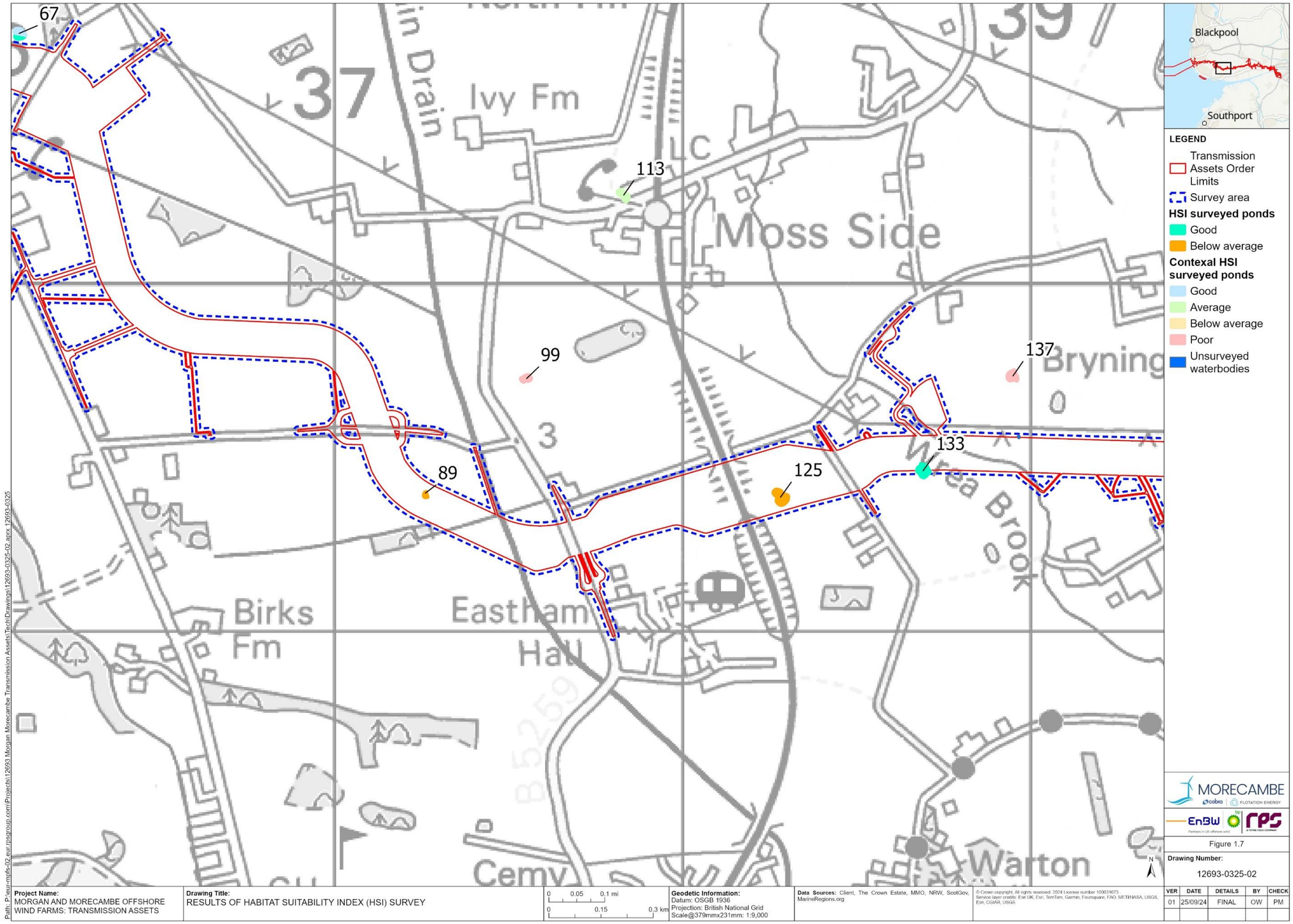

Figure 1.7: Results of HSI survey - Sheet 4

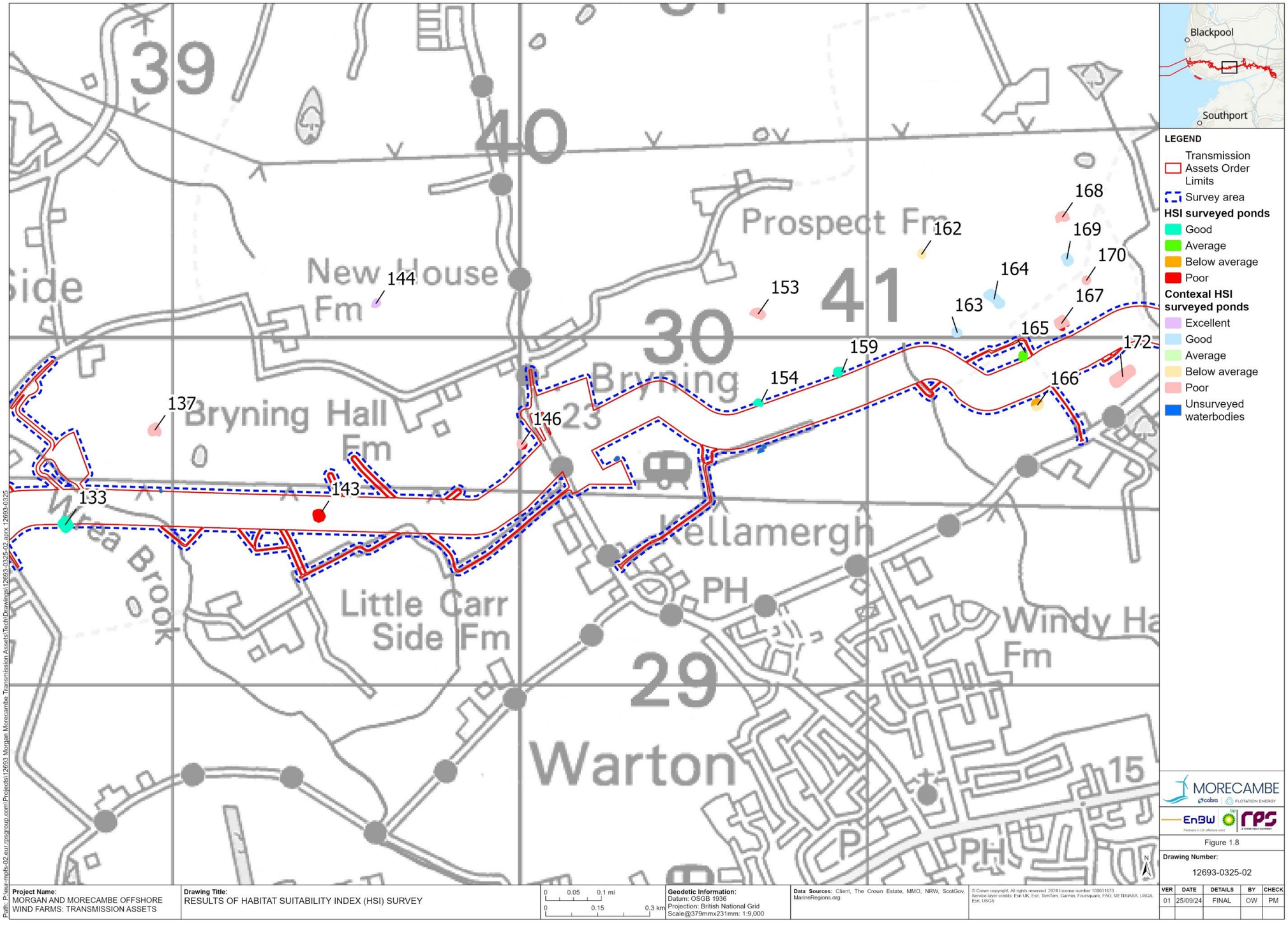

Figure 1.8: Results of HSI survey - Sheet 5

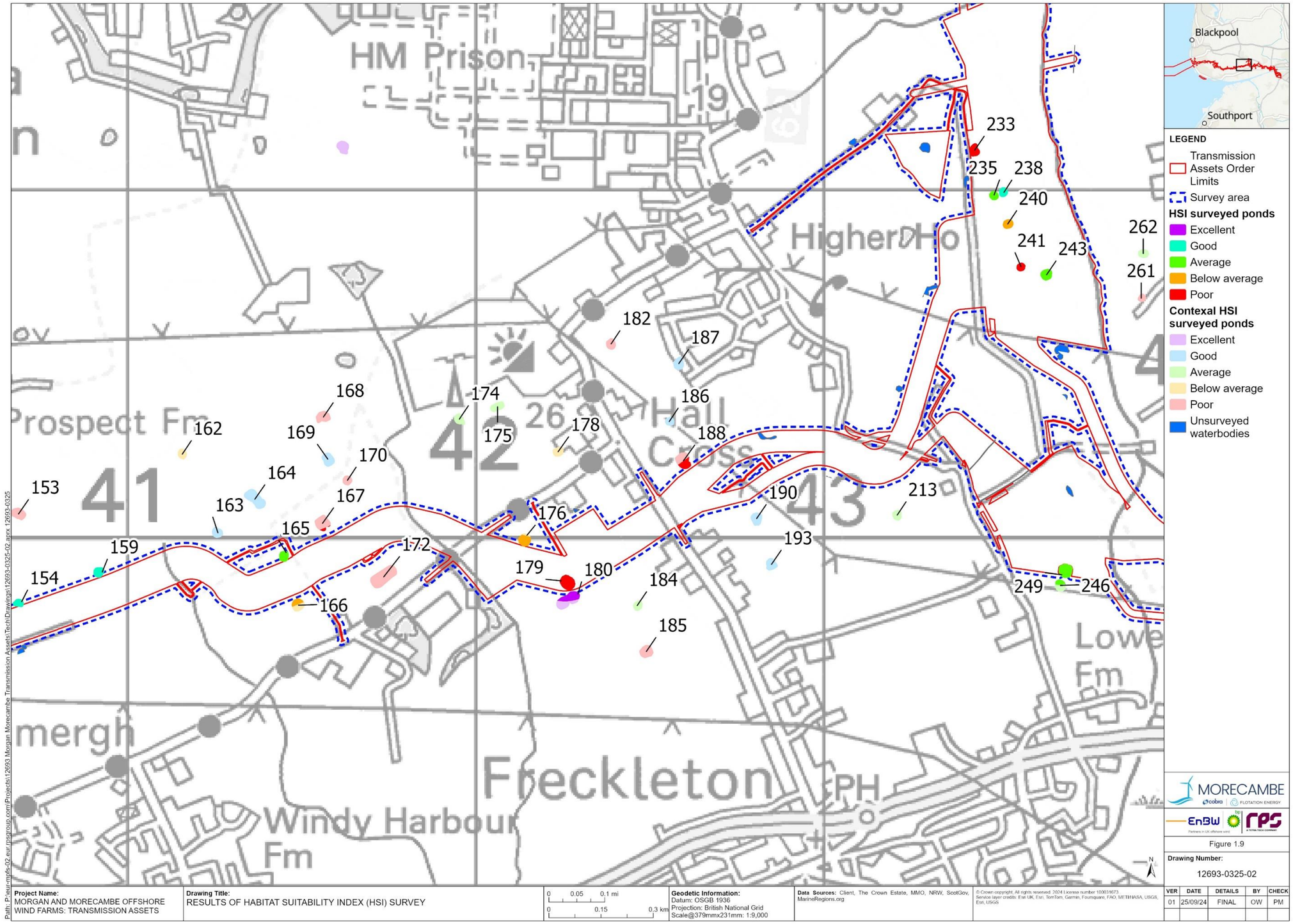

Figure 1.9: Results of HSI survey - Sheet 6

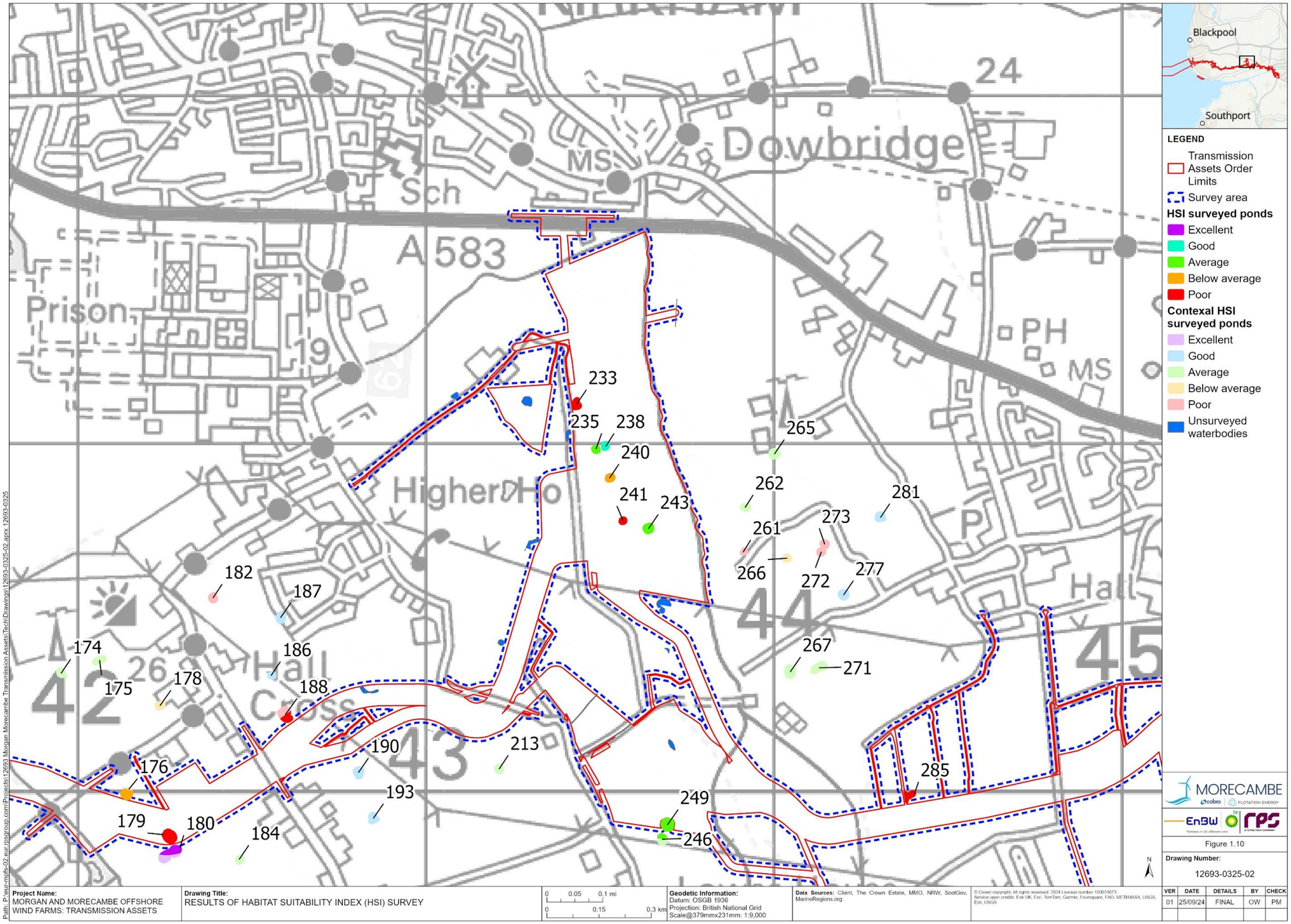

Figure 1.10: Results of HSI survey - Sheet 7

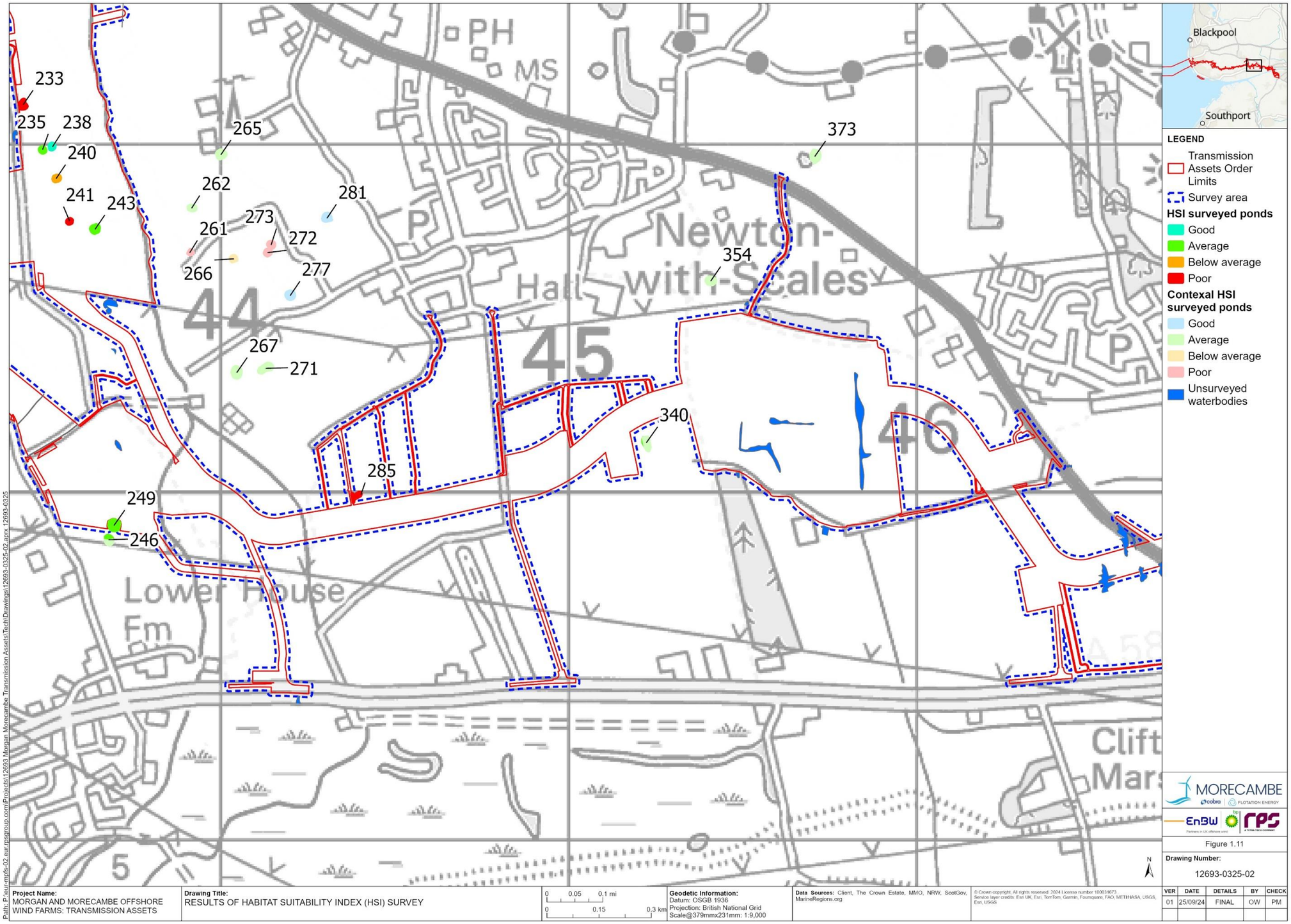

Figure 1.11: Results of HSI survey - Sheet 8

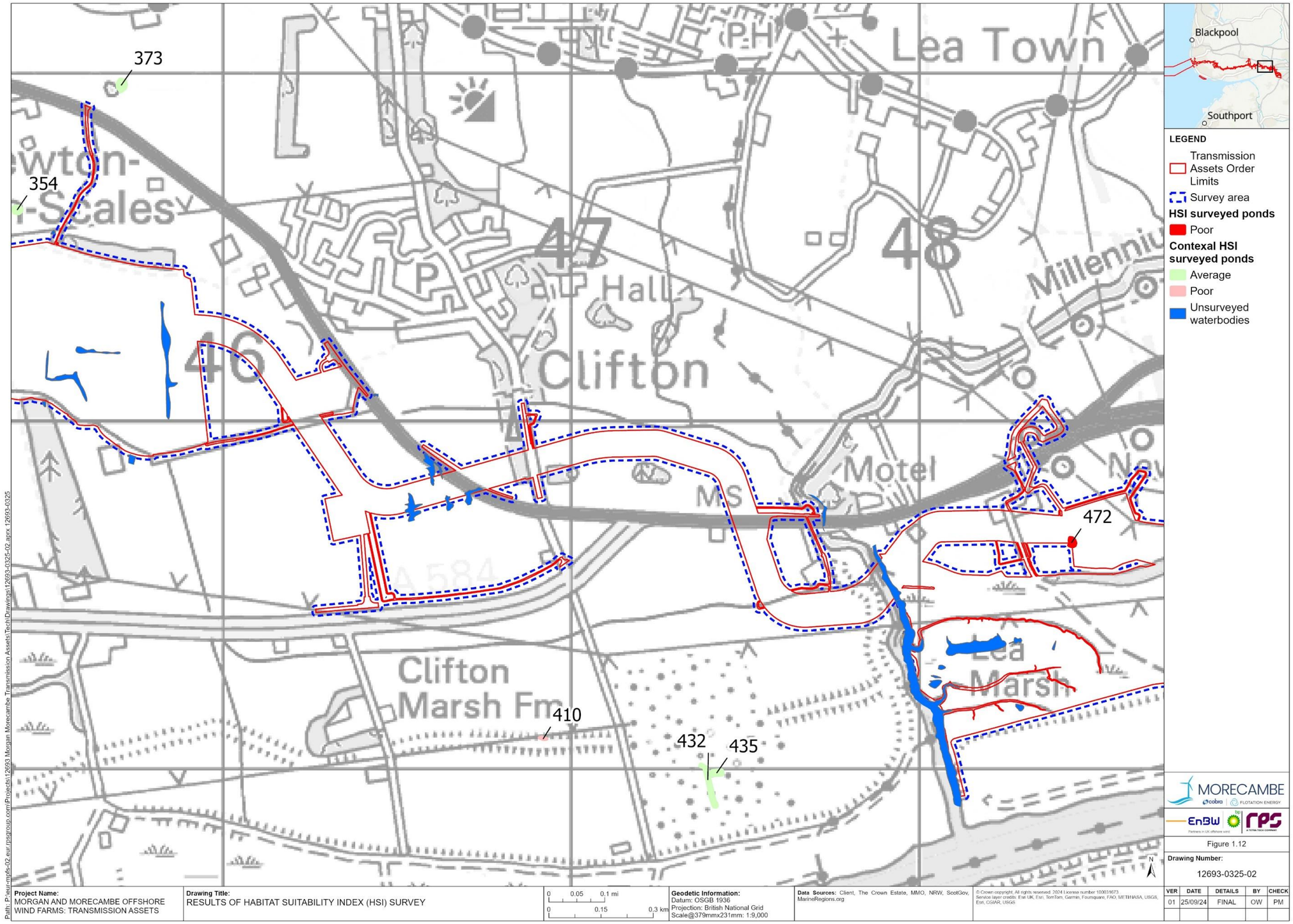

Figure 1.12: Results of HSI survey - Sheet 9

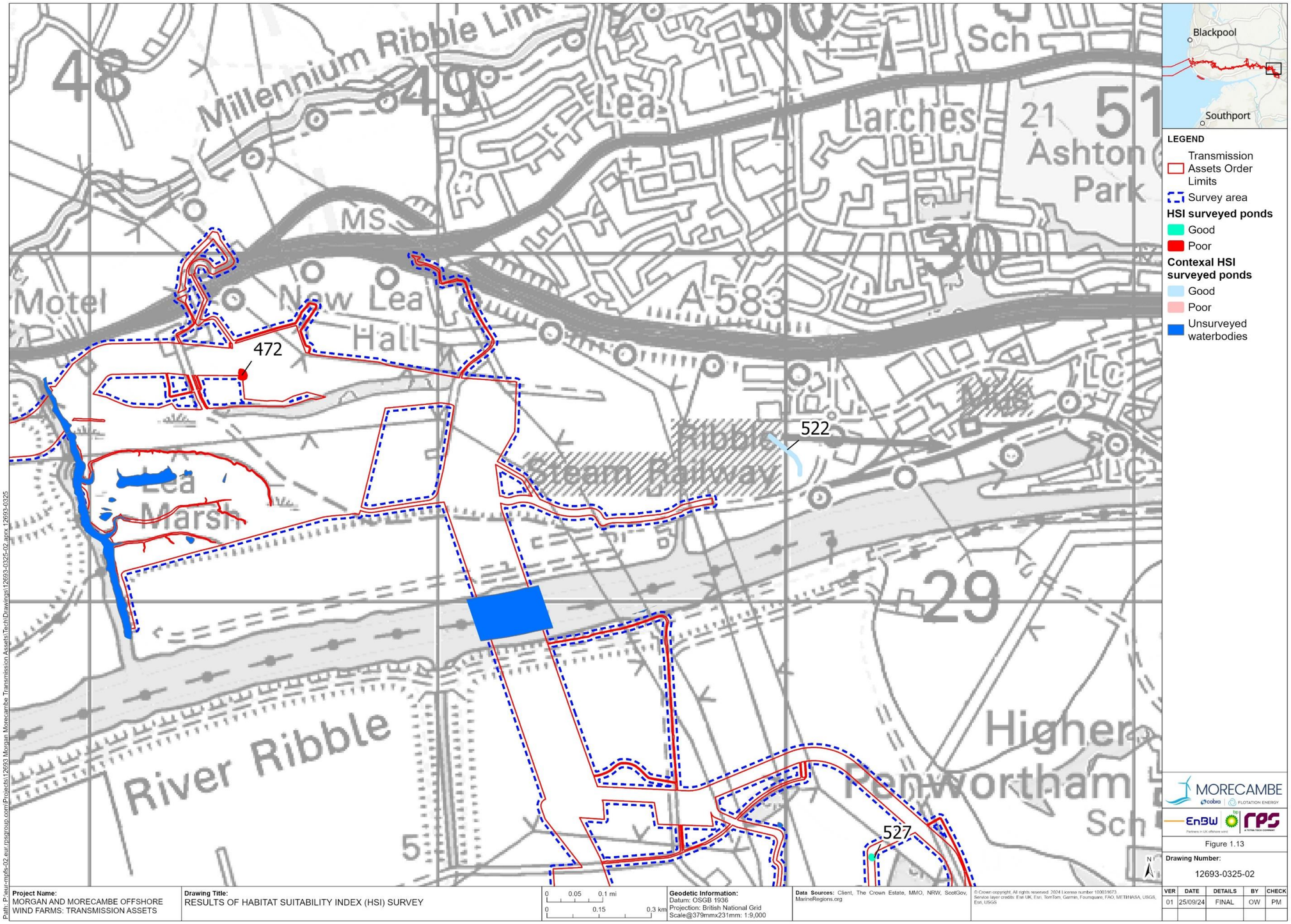

Figure 1.13: Results of HSI survey - Sheet 10

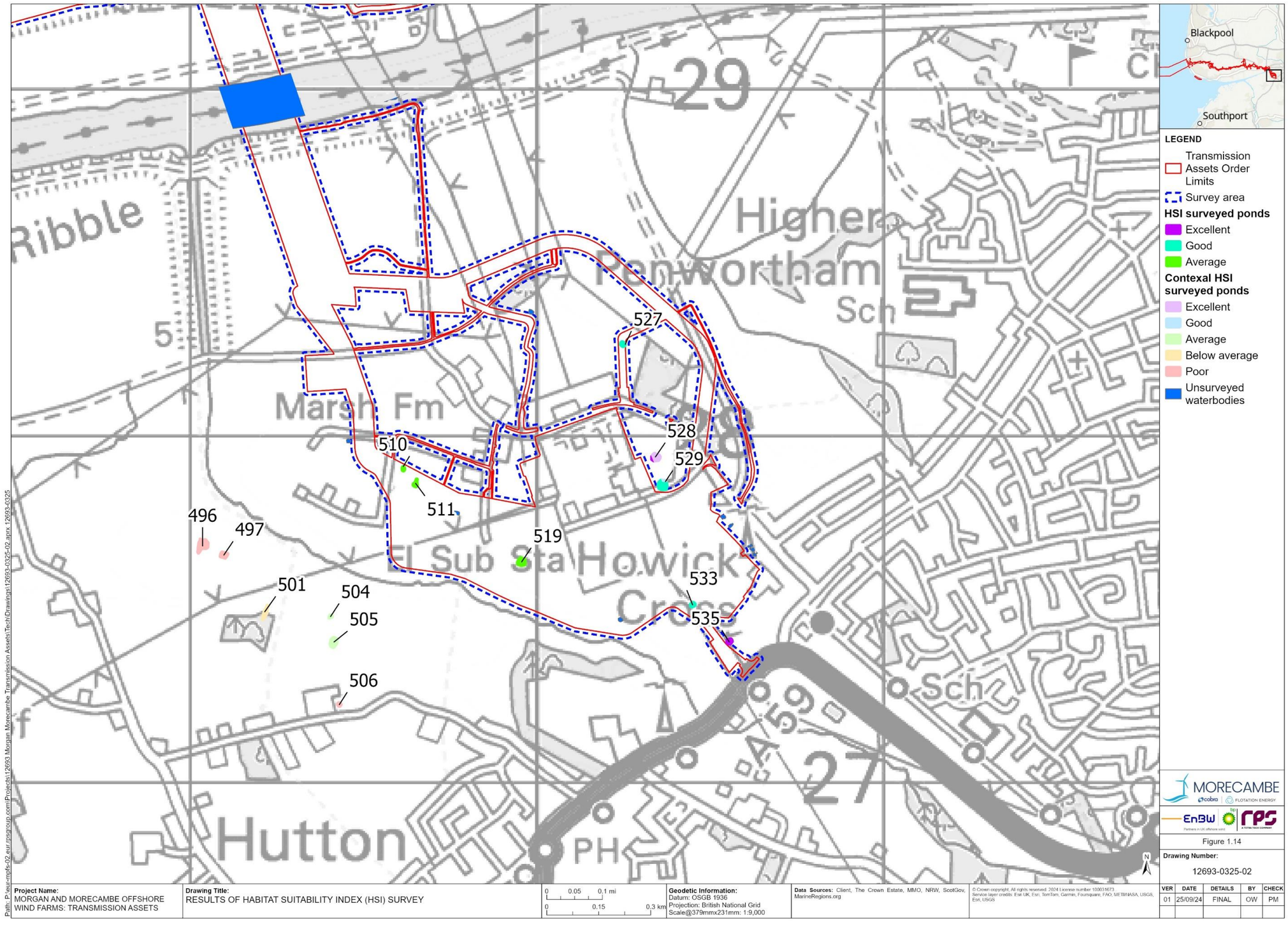

Figure 1.14: Results of HSI survey - Sheet 11

eDNA surveys

1.3.2.5 A total of 57 waterbodies in total were subject to further assessment in the form of eDNA testing.

1.3.2.6 Of these, 42 waterbodies were inside the survey area. Results of the eDNA surveys are provided in **Table 1.7** and **Table 1.8**.

1.3.2.7 No surveyed waterbodies visited in 2022 within the survey area returned a positive result for GCN.

1.3.2.8 Seven waterbodies visited in 2024 within the survey area returned a positive result for GCN.

1.3.2.9 Fourteen waterbodies were subject to eDNA testing that are outside of the GCN survey area but have been included as contextual data which is summarised in **Table 1.7**. Three of these ponds returned a positive result. Locations of the surveyed waterbodies are shown on **Figure 1.15** to **Figure 1.23**.

1.3.2.10 The eDNA analysis results are provided in **Appendix B** at the end of this technical report.

Table 1.7: eDNA survey results (2022)

Waterbody reference	Location	eDNA Result	Within Onshore Infrastructure Area
67	SD3609430716	Absent	No
137	SD3894729733	Absent	No
143	SD3942129486	Absent	Yes
146	SD4000529691	Absent	No
153	SD4068630068	Absent	No
159	SD4091729900	Absent	No
163	SD4125730014	Absent	No
164	SD4136630111	Absent	No
165	SD4144929946	Absent	No
166	SD4148929806	Absent	No
170	SD4163130164	Absent	No
232	SD4340831026	Absent	No
261	SD4391430688	Absent	No
266	SD4403730670	Absent	No
267	SD4404630343	Absent	No
68	SD3619631516	Present	No
144	SD3958630097	Absent	No
162	SD4115730240	Absent	No

271	SD4413030356	Absent	No
272	SD4413630688	Absent	No
273	SD4414630710	Absent	No
556	SD3619231094	Present	No
557	SD4161931123	Present	No
558	SD4075230266	Absent	No
559	SD4078430308	Absent	No
560	SD4097330321	Absent	No
561	SD4006230554	Absent	No
562	SD4100330789	Absent	No
563	SD4138531139	Absent	No

Table 1.8: eDNA survey results (2024)

Waterbody reference	Location	eDNA Result	Within Onshore Infrastructure Area
89	SD3726129391	Absent	Yes
125	SD3828229384	Absent	Yes
133	SD3869129461	Absent	Yes
143	SD3942129486	Absent	Yes
154	SD4068729811	Absent	Yes
179	SD4226429870	Absent	Yes
188	SD4259630221	Absent	No
233	SD4343331113	Absent	Yes
235	SD4348930983	Present	Yes
238	SD4351630992	Absent	Yes
240	SD4352930901	Present	Yes
241	SD4356630777	Absent	Yes
243	SD4363930757	Present	Yes
246	SD4367929861	Absent	No
249	SD4369429904	Absent	No
285	SD4439029988	Absent	No
371	SD4560629897	Absent	No
451	SD4782729244	Absent	No
472	SD4843929650	Absent	Yes
510	SD4962227915	Absent	Yes
511	SD4965027861	Absent	Yes
519	SD4995527635	Absent	Yes
527	SD5024728264	Present	Yes
528	SD5034227937	Absent	No
529	SD5036227857	Present	No
533	SD5044927512	Present	Yes
535	SD5055527406	Absent	No
544	SD5075927287	Present	No
35	SD 34539 30122	Negative	No

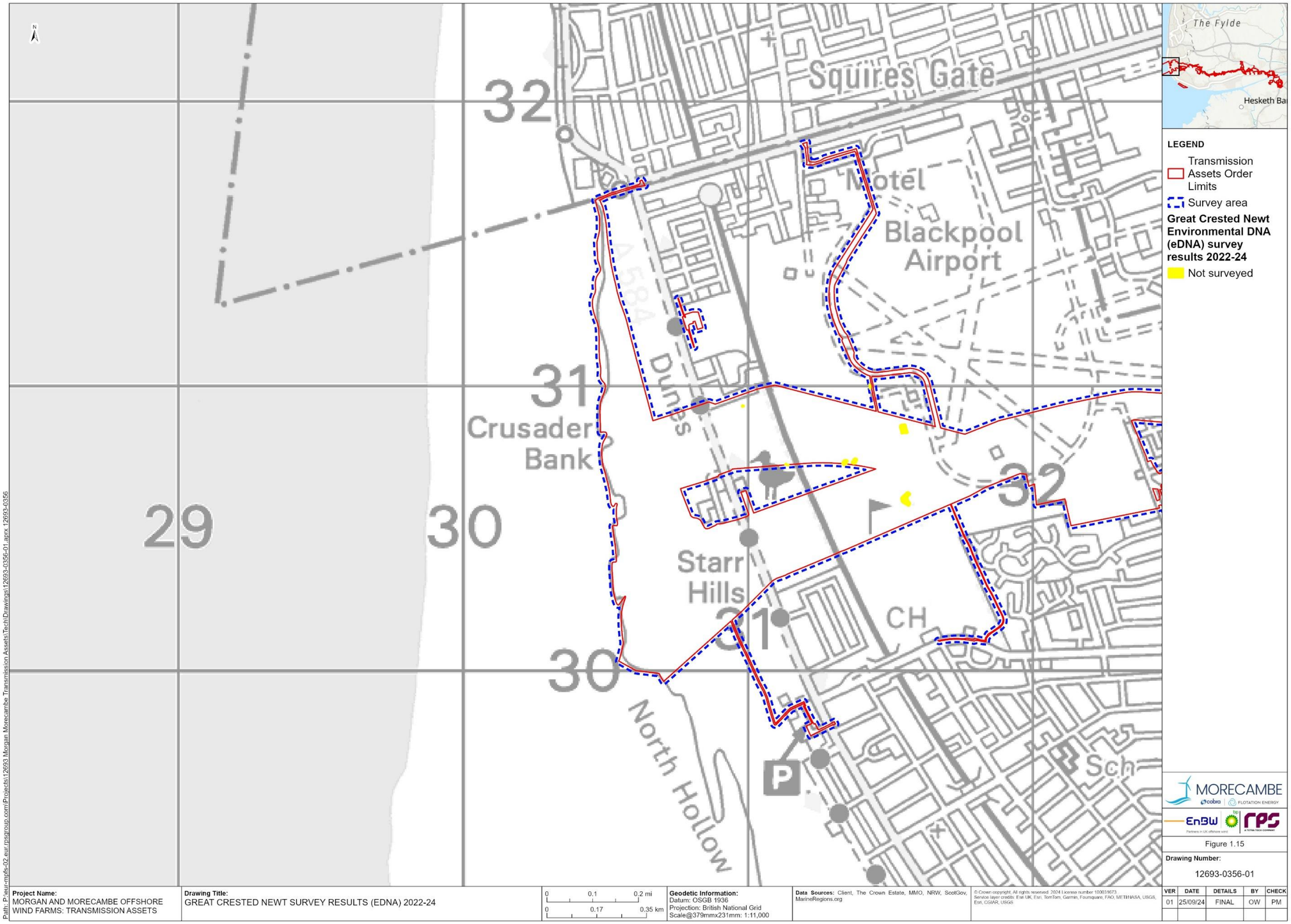

Figure 1.15: eDNA survey results – Sheet 1

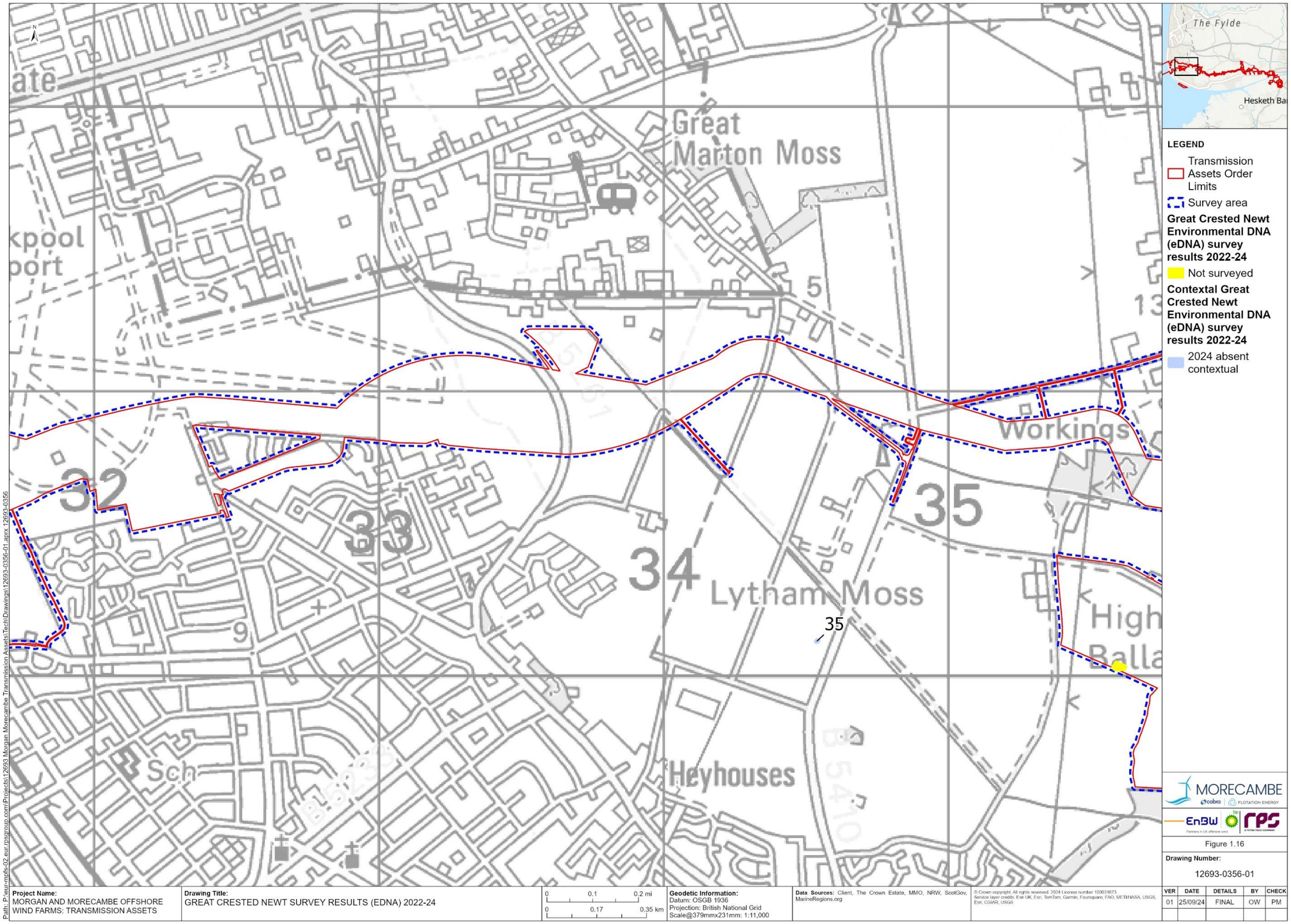

Figure 1.16: eDNA survey results – Sheet 2

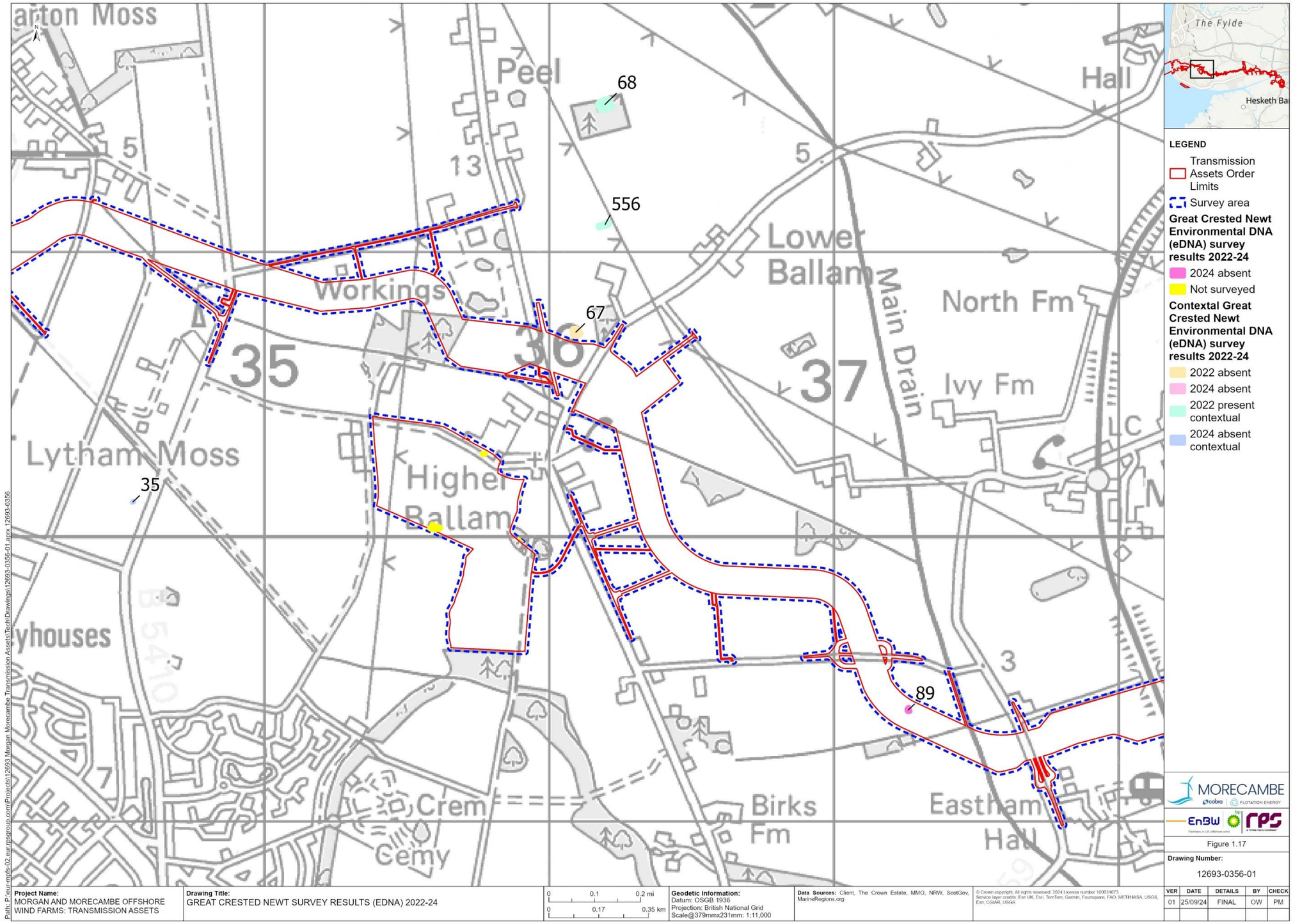

Figure 1.17: eDNA survey results – Sheet 3

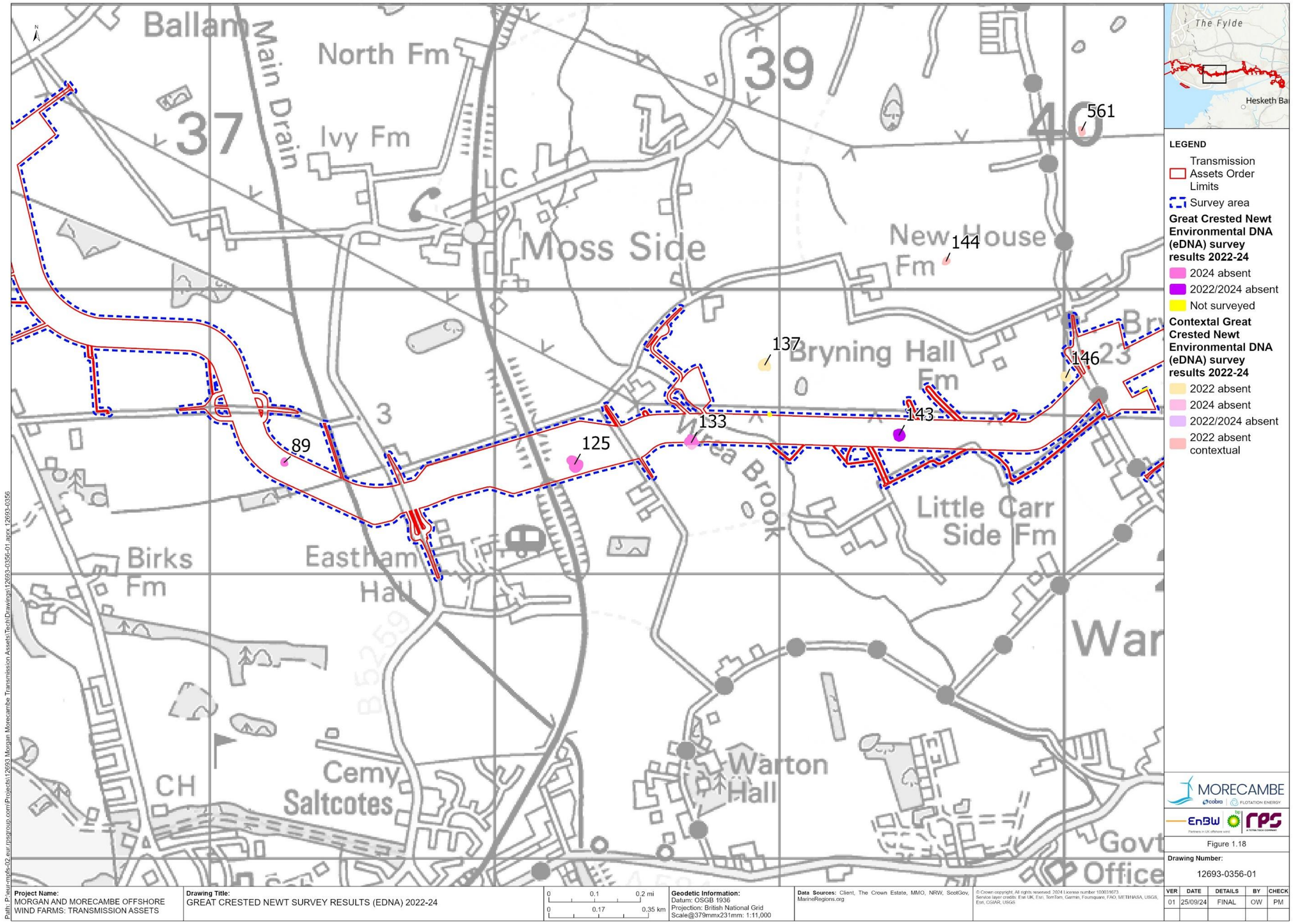

Figure 1.18: eDNA survey results – Sheet 4

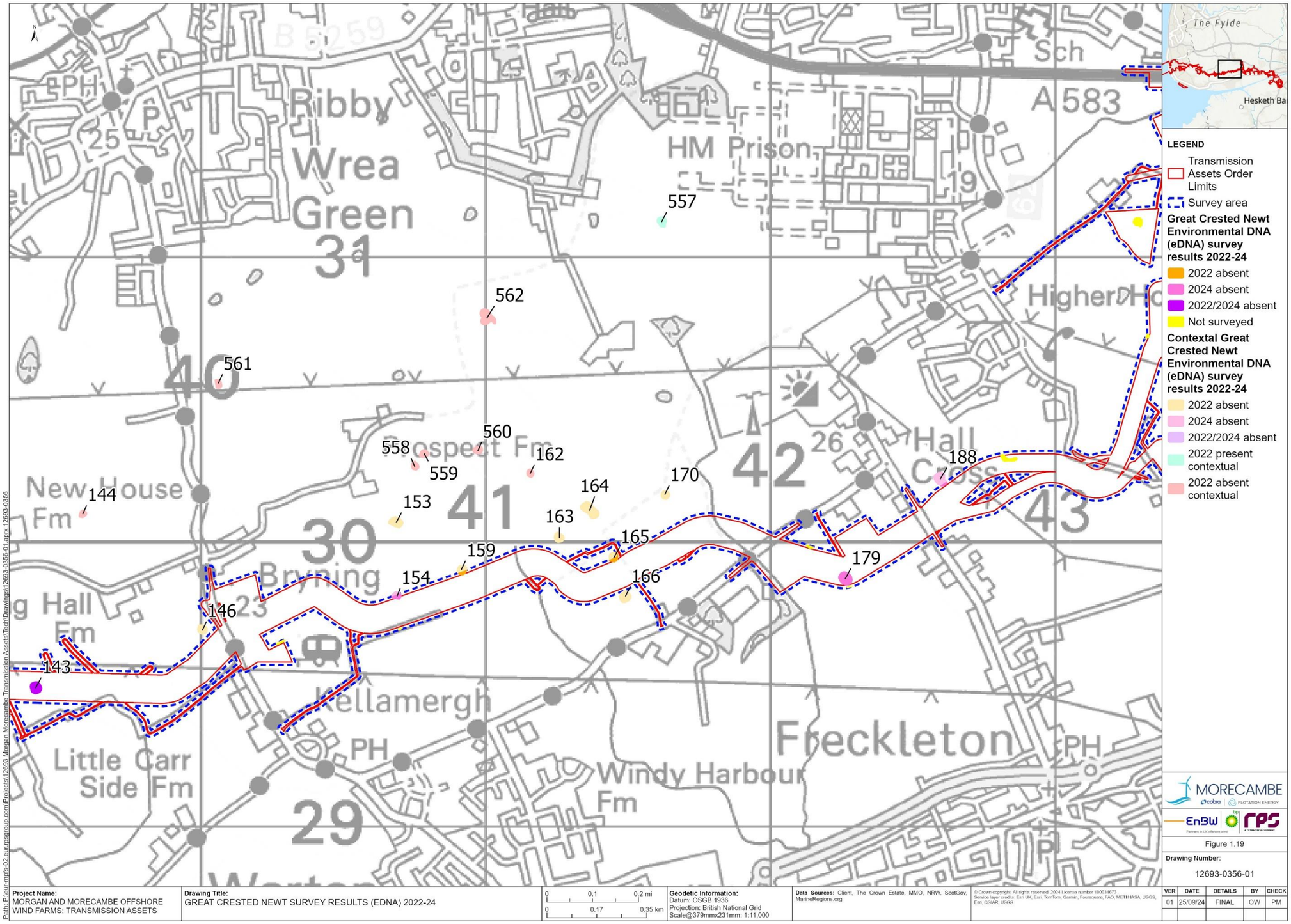

Figure 1.19: eDNA survey results – Sheet 5

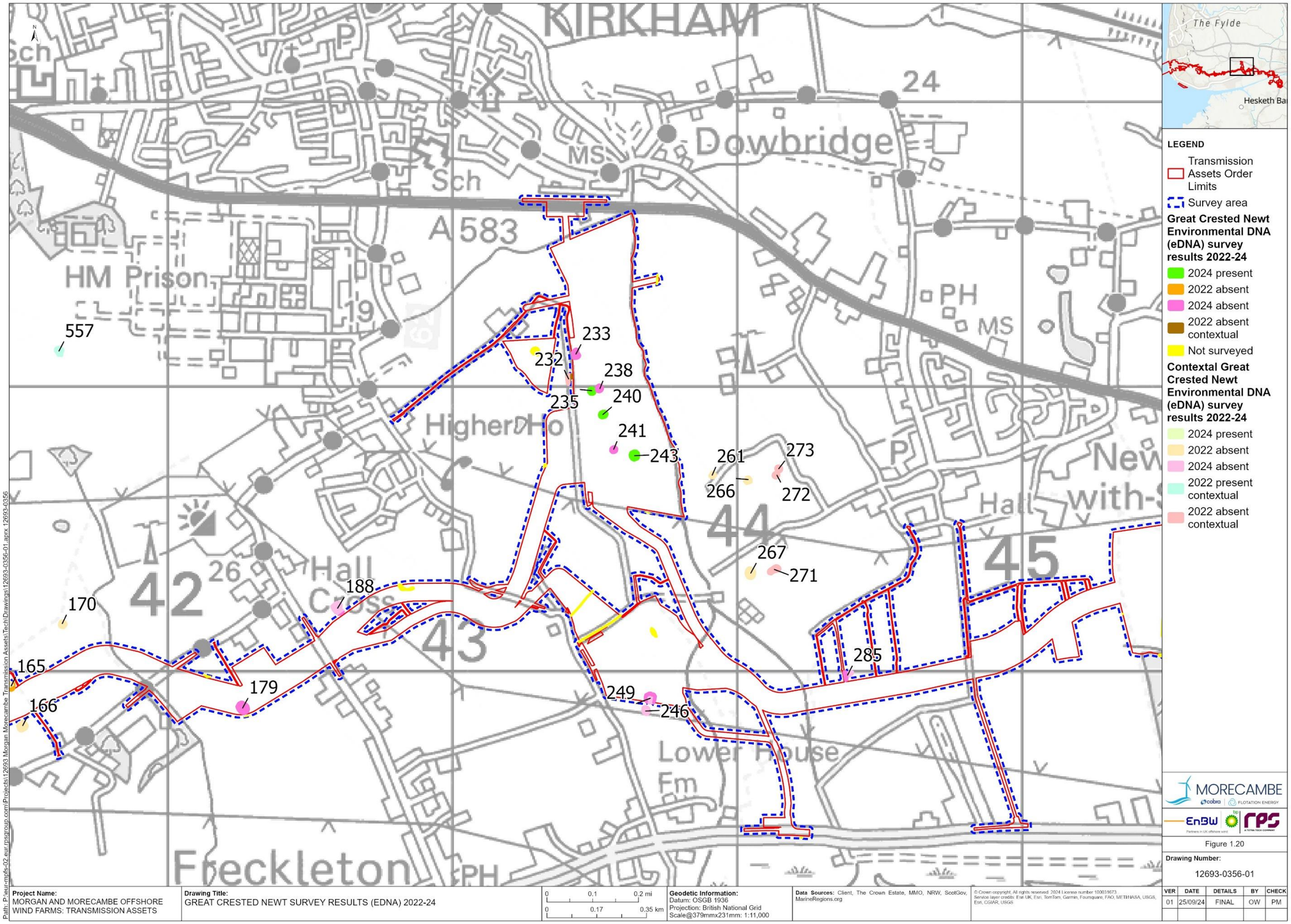

Figure 1.20: eDNA survey results – Sheet 6

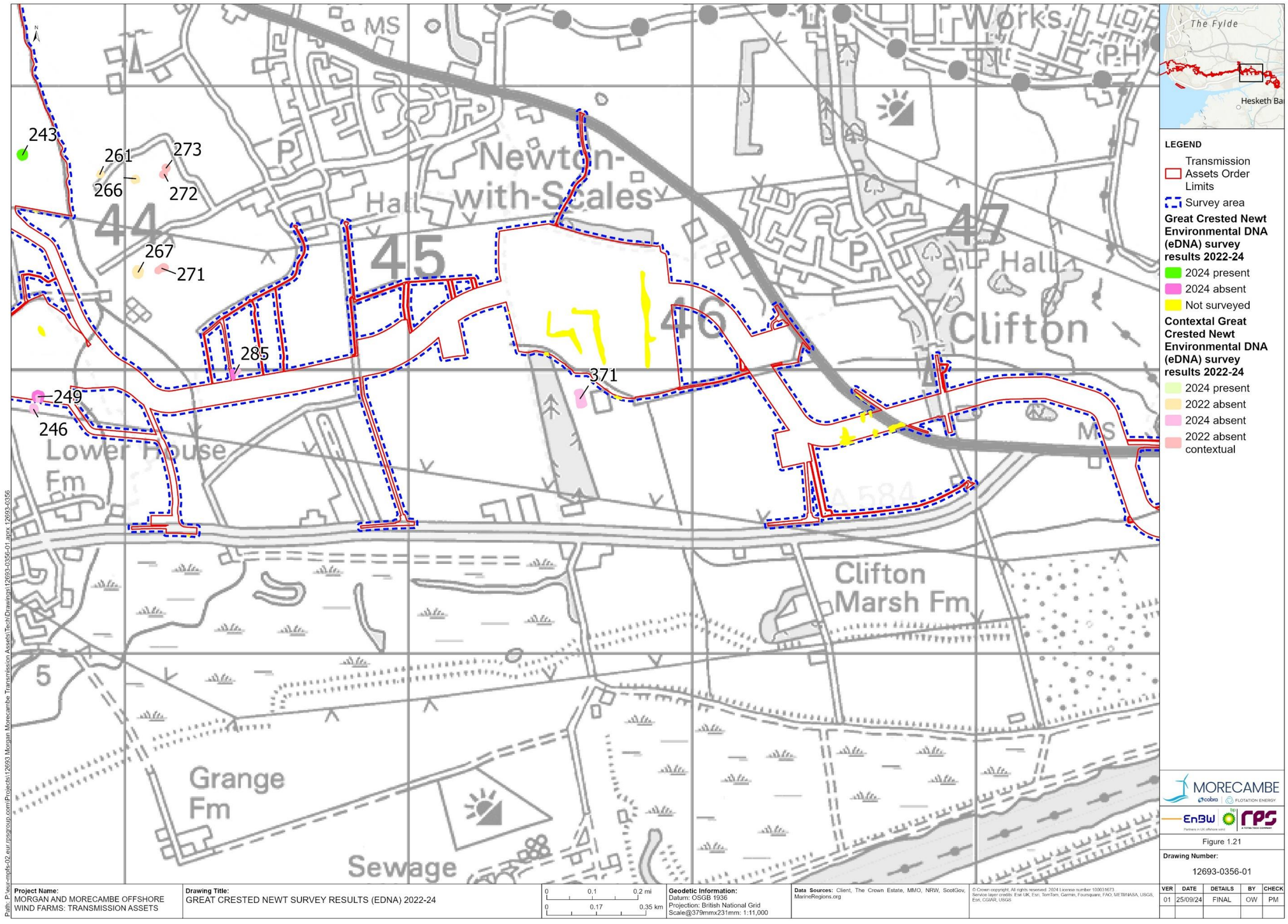

Figure 1.21: eDNA survey results – Sheet 7

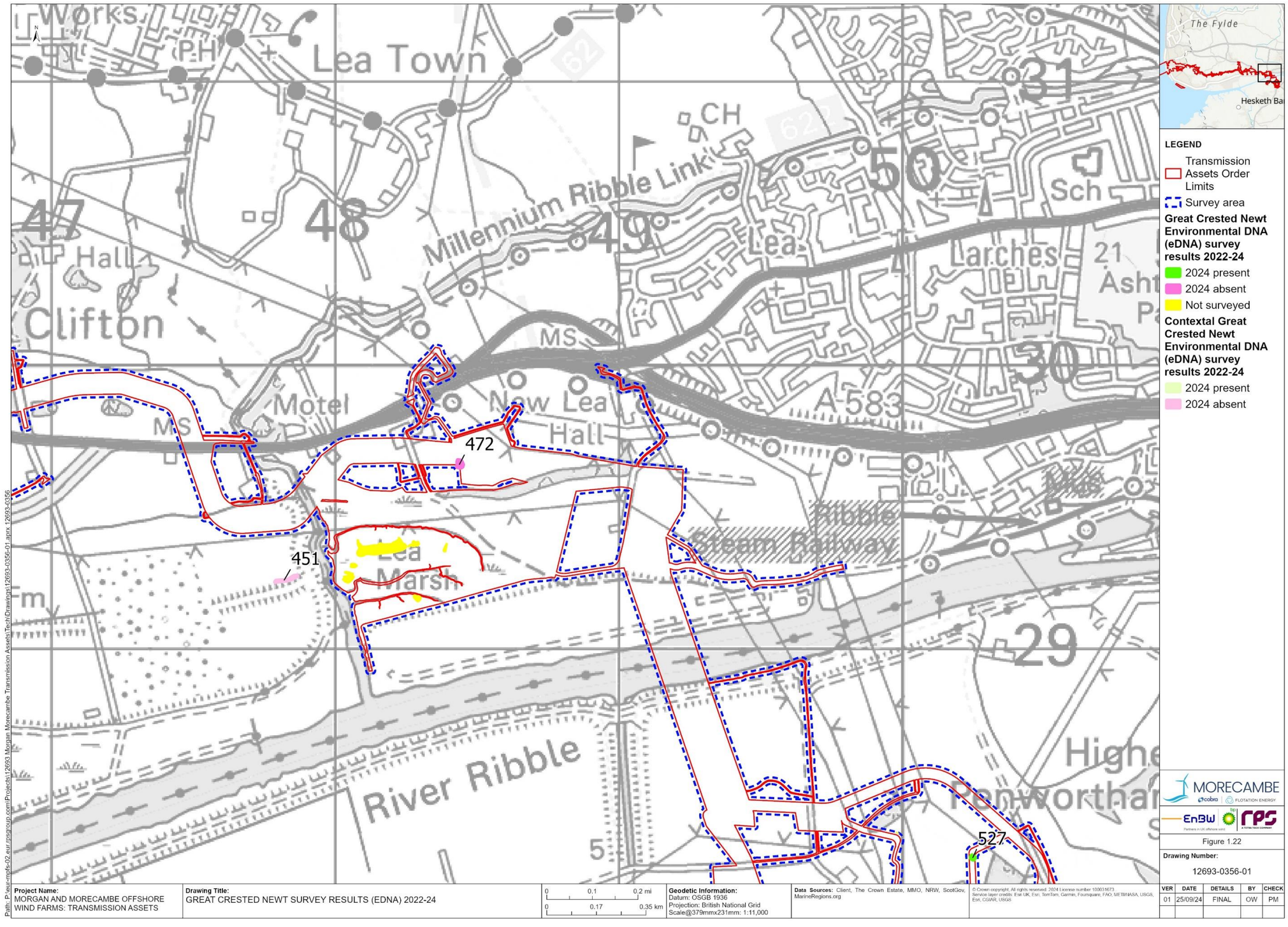

Figure 1.22: eDNA survey results – Sheet 8

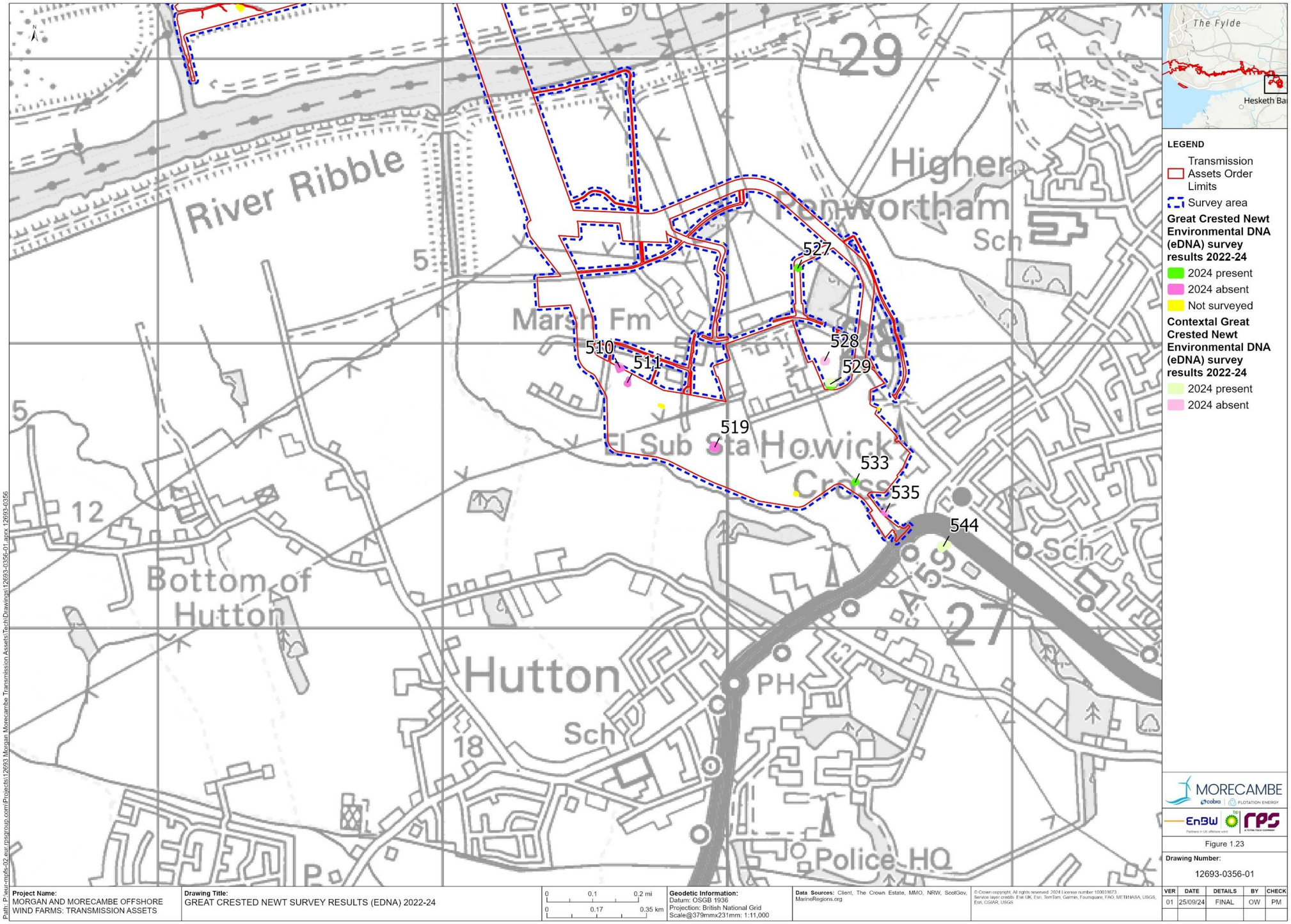

Figure 1.23: eDNA survey results – Sheet 9

1.3.3 Site-specific surveys: reptiles

Presence/absence surveys

1.3.3.1 The locations of surveyed areas are shown in **Figure 1.24** to **Figure 1.26**.

1.3.3.2 Five areas within the reptile survey area were surveyed for reptiles. Reptile refugia densities, number of visits and results are summarised in **Table 1.9**.

1.3.3.3 **Table 1.10** summarises survey results from two areas outside the Order Limits. No reptiles have been recorded during the surveys. Surveys have been completed in areas 1-3, 5, part of 6 and 7.

1.3.3.4 Two common amphibian species, common toad and common frog *Rana temporaria*, were recorded during the surveys. Full reptile survey results including dates and weather conditions during surveys are provided in **Appendix C** at the end of this technical report.

Table 1.9: Reptile survey results

Site reference	Site description	Number of refugia	Number of visits	Reptile species recorded	Other species
Area 3	Peel Rd, Branch Drain	10	8	None	Common toad - 2 Common shrew *Sorex anaeus* - 1
Area 4	Pegs Ln, Salcotes Rd SW	10	5 (two remaining)	None	None
Area 5	Cartmell Ln, Huck Ln East	30	8	None	Common toad - 6
Area 6	Blackpool Rd, Riversway South	75	2 (5 remaining)	None	Common toad - 4
Area 7	Howick Cross Ln South	40	8	None	Common frog - 1 Common toad - 10

Table 1.10: Contextual reptile survey results

Site reference	Site description	Number of refugia	Number of visits	Reptile species recorded	Other species
Area 1	East Runway, North (Queensway)	42	7	None	Common toad - 1 Common frog - 2
Area 2	East Runway, South (Queensway)	36	7	None	Common toad - 6

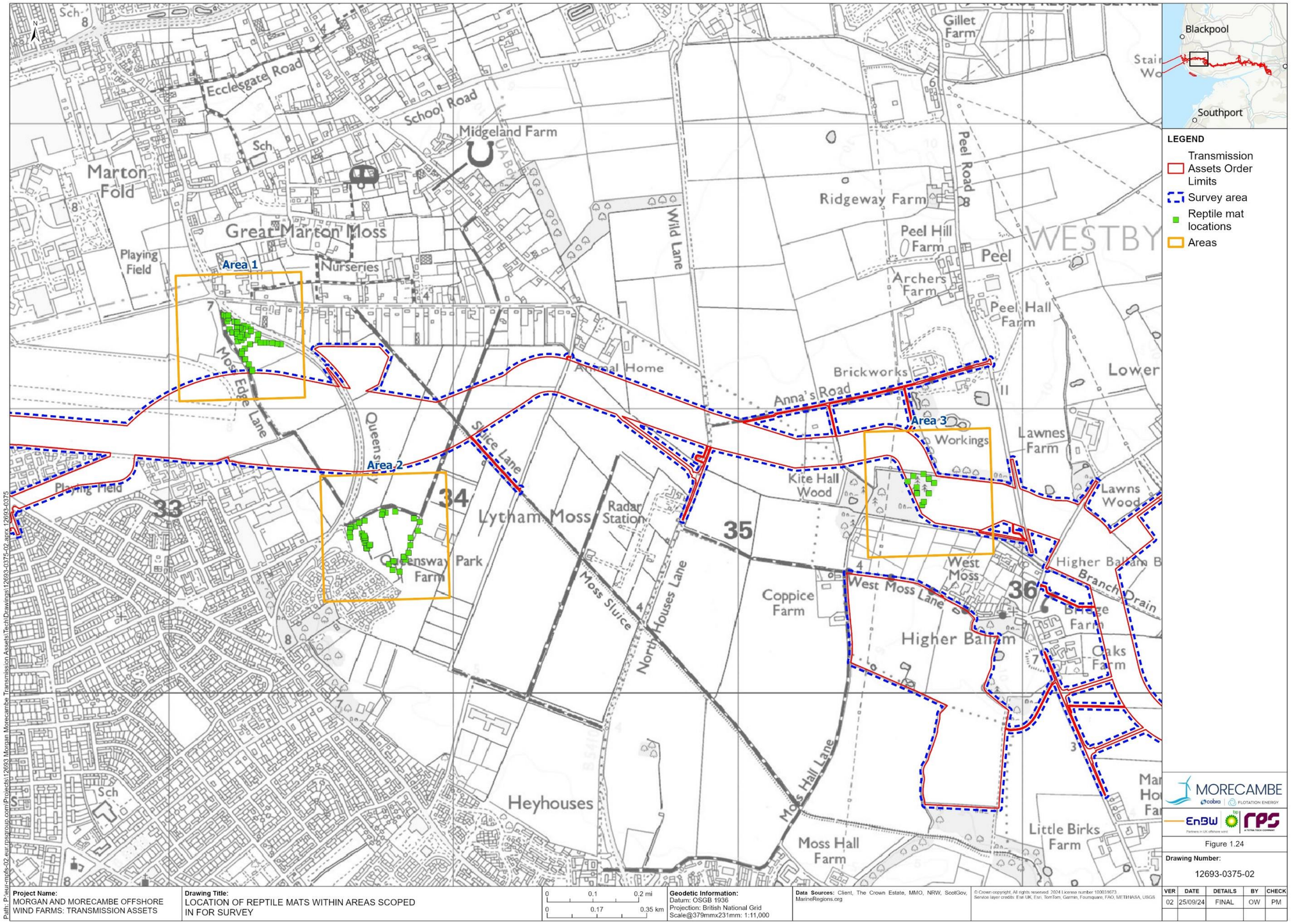

Figure 1.24: Reptile survey locations – Sheet 1

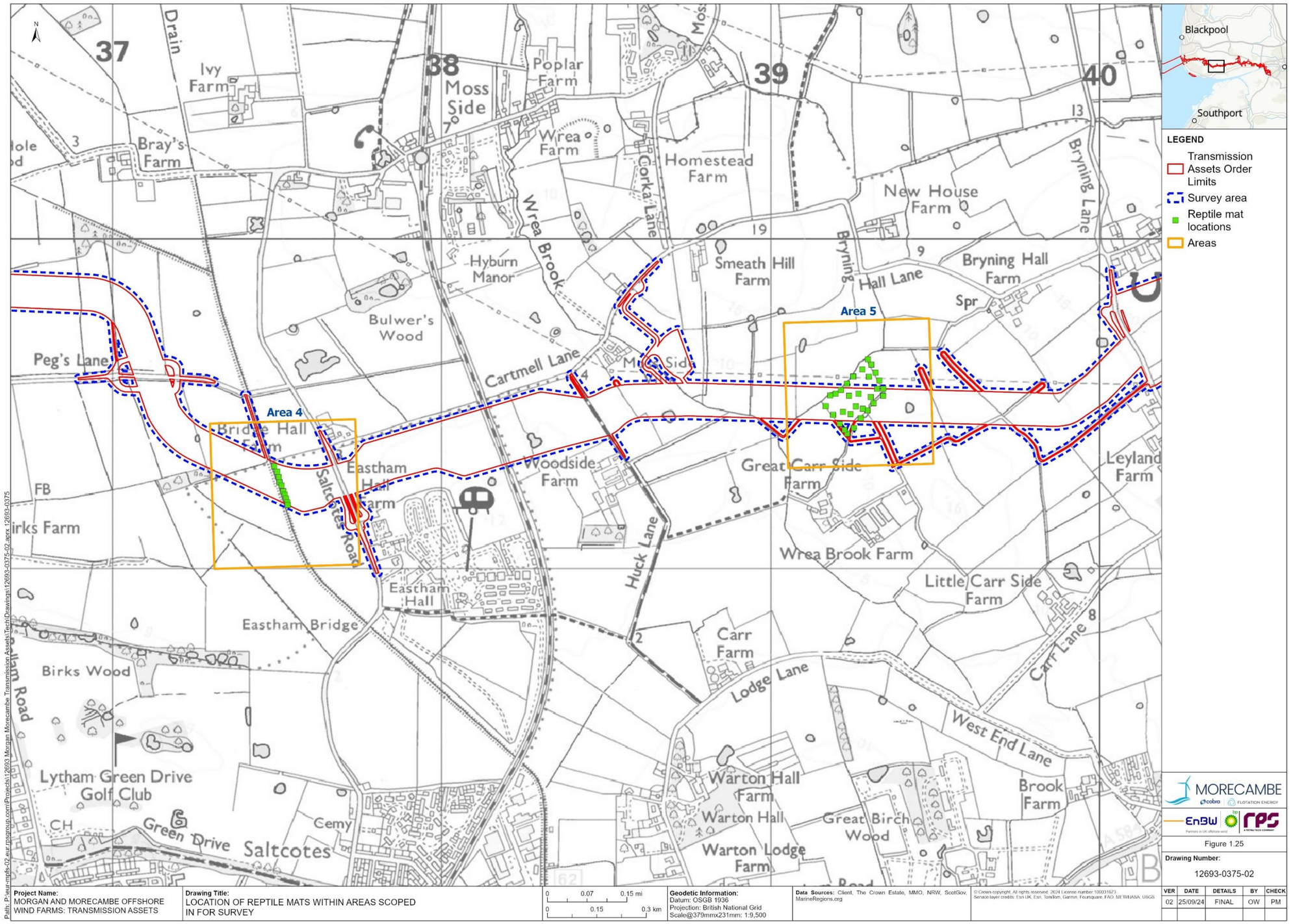

Figure 1.25: Reptile survey locations – Sheet 2

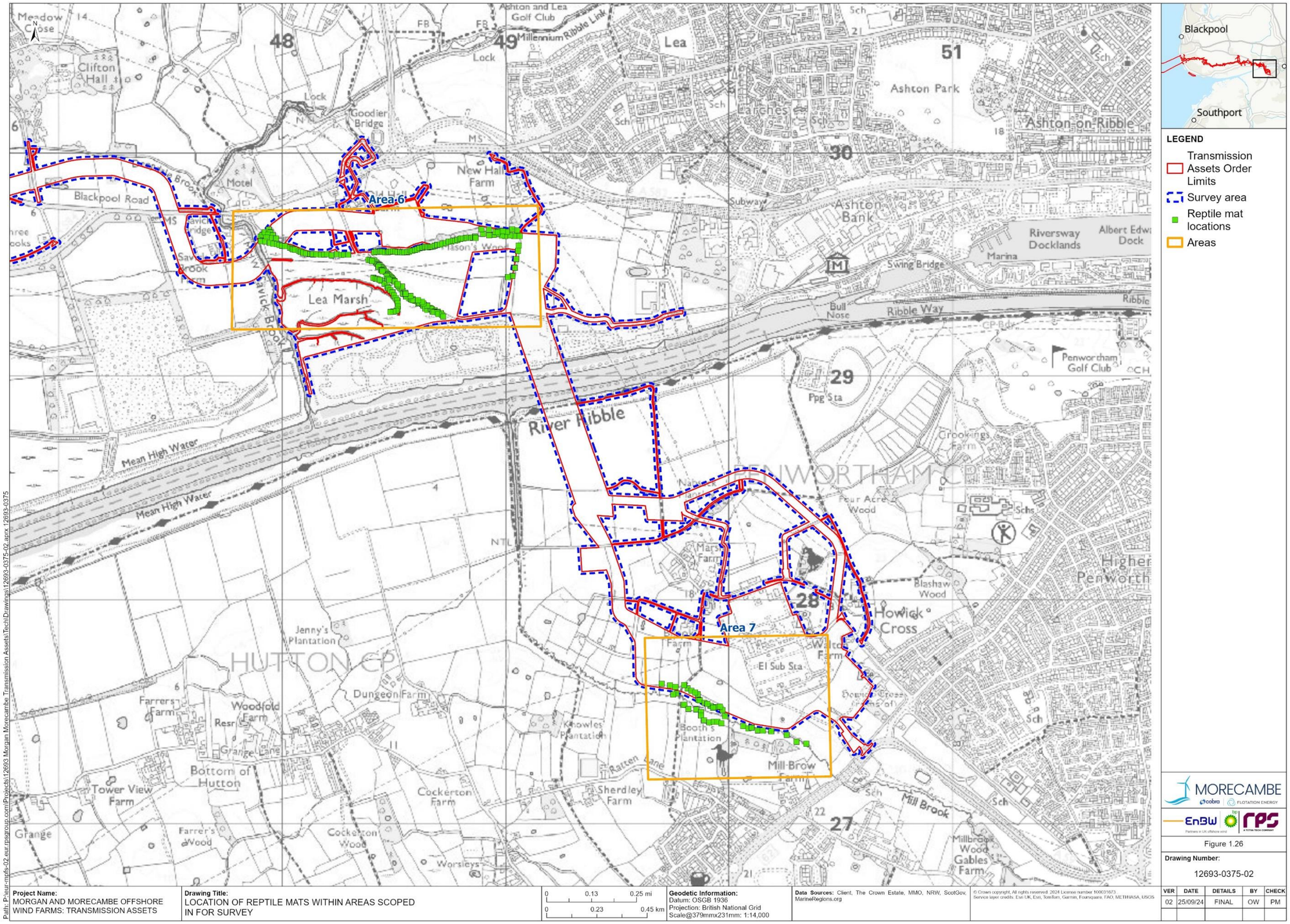

Figure 1.26: Reptile survey locations – Sheet 3

1.4 Summary

1.4.1.1 This technical report presents the results of the reptile and GCN desk study and field studies undertaken between April 2022 and June 2024 to inform Volume 3: Chapter 3: Onshore ecology and nature conservation of the ES.

1.4.1.2 Three reptile species and five amphibian species were recorded in the last ten years from the desk study, within 2 km of the Transmission Assets Order Limits, including sand lizard. Sand lizards occur on the sand dunes within the Onshore Infrastructure Area, shown in the desk study data reviewed in **section 1.3.1**. However, the sand dunes did not require survey for this report, as discussed in **section 1.1.6** and section 3.3 of Volume 3, Chapter 3: Onshore ecology and nature conservation of the ES..

1.4.1.3 Sixty waterbodies within the GCN survey area were subject to HSI assessment. In 2022, one pond was classified as excellent, 11 ponds were classified as good, 10 ponds were classified as average, five ponds as below average and 11 ponds as poor.

1.4.1.4 In 2024, one pond was classified as excellent, five ponds were classified as good, seven ponds were classified as average, three ponds as below average and five ponds as poor.

1.4.1.5 Forty-three ponds within the Transmission Assets order limits were sampled for eDNA, of which seven returned a positive result. A further three positive results were returned from the contextual data from ponds outside of the defined survey area.

1.4.1.6 No reptiles have been found during the reptile surveys.

1.4.1.7 Incidental sightings of common toad and common frog were recorded during the reptile surveys.

1.5 References

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Appendix A: HSI raw survey data

HSI scores for ponds within the survey area

Pond ID	Grid reference	Year of survey	Geographic location	Pond area	Permanence	Water quality	Shade	Waterflow	Fish	No. ponds within 1km	Terrestrial habitat	Macrophytes	HSI score	Suitability for GCN
4	SD3104130602	2022	Zone A	50-100m2	Sometimes dries	Poor	1	Absent	Absent	8	Good	0.3	0.53	Below average
11	SD3134030731	2022	Zone A	275m2	Never dries	Moderate	1	Absent	Possible	10	Poor	0.5	0.70	Average
12	SD3137430735	2022	Zone A	200m2	Never dries	Moderate	1	Absent	Possible	10	Poor	0.6	0.70	Good
14	SD3154630848	2022	Zone A	500-700m2	Never dries	Good	1	Minor	Possible	10	Poor	0.7	0.78	Good
15	SD3155430602	2022	Zone A	500-700m2	Never dries	Good	1	Absent	Possible	10	Poor	0.4	0.77	Good
55	SD3577130290	2022	Zone A	200m2	Sometimes dries	Moderate	1	Absent	Possible	10	Good	0.3	0.69	Average
62	SD3590829999	2022	Zone A	1300m2	Never dries	Moderate	0-60%	Absent	Major	>12	Poor	6-10%	0.49	Poor
63	SD3591330127	2022	Zone A	500-700m2	Never dries	Moderate	1	Major	Possible	11	Moderate	0.4	0.50	Poor
67	SD3609430716	2022	Zone A	1200m2	Rarely dries	Poor	0-60%	Minor	Absent	>12	Poor	26-30%	0.73	Good
89	SD3726129391	2024	Zone A	425.0996m2	Dries annually	Poor	0	Absent	Absent	>=13	Poor	0.1	0.57	Below average
99	SD3755029726	2022	Zone A	500-700m2	Sometimes dries	Moderate	1	Major	Absent	>12	Moderate	0.4	0.50	Poor
125	SD3828229384	2024	Zone A	3604.0796m2	Never dries	Poor	0.8	Minor	Possible	10	Poor	0.1	0.60	Below average
133	SD3869129461	2024	Zone A	2848.0698m2	Never dries	Moderate	0.1	Minor	Possible	10	Poor	0.25	0.71	Good
143	SD3942129486	2022	Zone A	<50m2	Dries annually	Poor	66-70%	Minor	Absent	>12	Poor	81-85%	0.44	Poor
146	SD4000529691	2022	Zone A	<50m2	Dries annually	Bad	0-60%	Absent	Absent	>12	Poor	<1%	0.29	Poor
154	SD4068729811	2024	Zone A	678.4329m2	Never dries	Poor	0.3	Absent	Possible	>=13	Poor	0.2	0.71	Good
159	SD4091729900	2022	Zone A	125m2	Sometimes dries	Moderate	0-60%	Minor	Absent	>12	Moderate	66-80%	0.72	Good
163	SD4125730014	2022	Zone A	125m2	Sometimes dries	Moderate	0-60%	Minor	Absent	>12	Moderate	46-50%	0.70	Good
164	SD4136630111	2022	Zone A	500-700m2	Rarely dries	Poor	0-60%	Minor	Absent	>12	Poor	6-10%	0.70	Good
165	SD4144929946	2022	Zone A	100m2	Sometimes dries	Poor	0-60%	Absent	Absent	>12	Good	<1%	0.63	Average
166	SD4148929806	2022	Zone A	>2000m2	Sometimes dries	Poor	76-80%	Minor	Absent	>12	Poor	<1%	0.59	Below average
167	SD4155930041	2022	Zone A	500-700m2	Never dries	Bad	1	Minor	Absent	>12	Poor	0.3	0.47	Poor
169	SD4157630224	2022	Zone A	500-700m2	Never dries	Poor	1	Absent	Absent	>12	Poor	0.3	0.70	Good
170	SD4163130164	2022	Zone A	350m2	Dries annually	Bad	96-100%	Absent	Absent	>12	Poor	96-100%	0.36	Poor
172	SD4173529887	2022	Zone A	400m2	Never dries	Moderate	1	Minor	Major	>12	Poor	0.35	0.45	Poor
176	SD4214129993	2022	Zone A	500-700m2	Never dries	Good	1	Minor	Major	>12	Poor	0.9	0.53	Below average
178	SD4223730246	2022	Zone A	300m2	Never dries	Good	1	Major	Absent	>12	Poor	0.6	0.50	Below average
179	SD4226429870	2024	Zone A	500-700m2	Never dries	Good	1	Minor	Major	>12	Moderate	0.8	0.56	Below average
180	SD4226529820	2022	Zone A	450m2	Never dries	Good	1	Minor	Minor	>12	Good	0.7	0.81	Excellent
184	SD4246629804	2022	Zone A	450m2	Rarely dries	Poor	1	Minor	Possible	>12	Poor	0.3	0.65	Average
185	SD4249029672	2022	Zone A	500-700m2	Never dries	Good	1	Minor	Major	>12	Poor	0.3	0.48	Poor
186	SD4255730333	2022	Zone A	500-700m2	Rarely dries	Moderate	0-60%	Minor	Absent	>12	Poor	36-40%	0.80	Good

Page 51

Pond ID	Grid reference	Year of survey	Geographic location	Pond area	Permanence	Water quality	Shade	Waterflow	Fish	No. ponds within 1km	Terrestrial habitat	Macrophytes	HSI score	Suitability for GCN
188	SD4259630221	2022	Zone A	200m2	Dries annually	Bad	96-100%	Absent	Absent	>12	Poor	<1%	0.29	Poor
190	SD4280730054	2022	Zone A	500-700m2	Never dries	Moderate	1	Absent	Possible	>12	Poor	0.3	0.72	Good
193	SD4284929922	2022	Zone A	450m2	Never dries	Good	1	Minor	Possible	>12	Poor	0.7	0.78	Good
213	SD4321130064	2022	Zone A	250m2	Never dries	Moderate	1	Absent	Absent	>12	Poor	0.3	0.70	Good
213	SD4321130064	2022	Zone A	200m2	Sometimes dries	Poor	66-70%	Minor	Absent	>12	Poor	21-25%	0.60	Average
233	SD4343331113	2024	Zone A	25.0044m2	Dries annually	Moderate	1	Absent	Absent	>=13	Poor	60 or 90	0.43	Poor
235	SD4348930983	2024	Zone A	715.2617m2	Sometimes dries	Moderate	0.8	Absent	Absent	>=13	Poor	0	0.68	Average
238	SD4351630992	2024	Zone A	708.1763m2	Sometimes dries	Moderate	0.6	Absent	Absent	>=13	Poor	0	0.71	Good
240	SD4352930901	2024	Zone A	896.6621m2	Never dries	Moderate	0	Major	Absent	>=13	Poor	0.45	0.52	Below average
241	SD4356630777	2024	Zone A	580.5479m2	Sometimes dries	Poor	0.35	Minor	Absent	>12	None	50 or 100	0.49	Poor
243	SD4363930755	2024	Zone A	1289.8611m2	Rarely dries	Poor	0.2	Minor	Possible	>=13	Poor	0.2	0.68	Average
246	SD4367929861	2024	Zone A	1300.7954m2	Never dries	Poor	0.4	Minor	Possible	>=13	Poor	0.2	0.68	Average
249	SD4369429904	2024	Zone A	2206.1313m2	Never dries	Poor	0.2	Minor	Possible	>=13	Poor	0.2	0.65	Average
261	SD4391430688	2022	Zone A	150m2	Sometimes dries	Bad	1	Absent	Absent	>12	Poor	0.3	0.41	Poor
262	SD4391830816	2022	Zone A	300m2	Never dries	Moderate	1	Minor	Possible	>12	Poor	0.3	0.66	Average
266	SD4403730670	2022	Zone A	250m2	Sometimes dries	Poor	0.4	Absent	Absent	>12	Poor	0.3	0.56	Below average
267	SD4404630343	2022	Zone A	500-700m2	Never dries	Good	1	Minor	Possible	>12	Poor	0.3	0.72	Good
285	SD4439029988	2024	Zone A	300m2	Dries annually	Bad	1	Absent	Absent	>12	Poor	0.3	0.38	Poor
340	SD4522430139	2022	Zone A	375m2	Rarely dries	Poor	0-60%	Minor	Absent	>12	Poor	6-10%	0.68	Average
354	SD4540930606	2022	Zone A	500-700m2	Never dries	Poor	86-90%	Minor	Possible	>12	Poor	1-5%	0.60	Average
373	SD4570930965	2022	Zone A	500-700m2	Never dries	Moderate	1	Minor	Possible	>12	Poor	0.3	0.69	Average
472	SD4843929650	2024	Zone A	1073.1306m2	Never dries	Moderate	0.35	Major	Possible	>=13	Moderate	0	0.49	Poor
504	SD4940527478	2022	Zone A	125m2	Never dries	Moderate	1	Absent	Absent	>12	Poor	0.4	0.62	Average
510	SD4961527904	2024	Zone A	862.3984m2	Dries annually	Moderate	0	Absent	Absent	>=13	Poor	0.1	0.62	Average
511	SD4965027861	2024	Zone A	372.1226m2	Dries annually	Moderate	0	Absent	Absent	>=13	Poor	50 or 100	0.65	Average
519	SD4995527635	2024	Zone A	1077.403m2	Never dries	Poor	0.3	Minor	Possible	>=13	Poor	0.3	0.69	Average
527	SD5024728264	2024	Zone A	362.4866m2	Sometimes dries	Poor	0	Absent	Absent	>=13	Moderate	50 or 100	0.76	Good
533	SD5044927512	2024	Zone A	344.9012m2	Never dries	Moderate	0.15	Minor	Possible	>=13	Poor	60 or 90	0.75	Good
535	SD5055527406	2024	Zone A	464.0466m2	Rarely dries	Moderate	0.6	Absent	Possible	>=13	Poor	50 or 100	0.80	Excellent

Page 52

HSI scores for ponds outside the survey area (contextual)

Pond ID	Grid reference	Year of survey	Geographic location	Pond area	Permanence	Water quality	Shade	Waterflow	Fish	No ponds within 1km	Terrestrial habitat	Macrophytes	HSI score	Suitability for GCN
68	SD3619631516	2022	Zone A	500-700m2	Rarely dries	Good	0-60%	Minor	Absent	>12	Poor	61-65%	0.86	Excellent
113	SD3783030252	2022	Zone A	1300m2	Never dries	Moderate	0.2	Minor	Possible	>15	Moderate	0.3	0.63	Average
137	SD3894729733	2022	Zone A	500-700m2	Never dries	Good	0-60%	Major	Possible	>12	Poor	1-5%	0.48	Poor
144	SD3958630097	2022	Zone A	500-700m2	Rarely dries	Moderate	0-60%	Minor	Absent	>12	Poor	51-55%	0.81	Excellent
153	SD4068630068	2022	Zone A	50-100m2	Dries annually	Poor	91-95%	Absent	Absent	>12	Moderate	<1%	0.43	Poor
162	SD4115730240	2022	Zone A	900m2	Dries annually	Poor	0-60%	Minor	Absent	>12	Poor	36-40%	0.59	Below Average
168	SD4156130347	2022	Zone A	500-700m2	Never dries	Poor	1	Major	Possible	>13	Poor	0.3	0.43	Poor
174	SD4195230340	2022	Zone A	250m2	Never dries	Moderate	0.8	Minor	Absent	>19	Poor	0.3	0.66	Average
175	SD4206230376	2022	Zone A	400m2	Never dries	Poor	0.6	Minor	Possible	>18	Poor	0.3	0.60	Average
182	SD4238830555	2022	Zone A	500-700m2	Dries annually	Bad	96-100%	Absent	Absent	>11	Poor	<1%	0.31	Poor
187	SD4258330499	2022	Zone A	450m2	Rarely dries	Moderate	0-60%	Minor	Absent	>12	Poor	36-40%	0.79	Good
265	SD4400230970	2022	Zone A	450m2	Never dries	Moderate	0.4	Minor	Possible	>12	Moderate	0.3	0.67	Average
267	SD4404630343	2022	Zone A	175m2	Never dries	Moderate	0-60%	Minor	Minor	>12	Moderate	26-30%	0.67	Average
271	SD4413030356	2022	Zone A	225m2	Rarely dries	Poor	0-60%	Minor	Absent	>12	Poor	1-5%	0.64	Average
272	SD4413630688	2022	Zone A	250m2	Never dries	Moderate	1	Major	Possible	>10	Poor	0.3	0.43	Poor
273	SD4414630710	2022	Zone A	175m2	Never dries	Moderate	1	Major	Possible	>9	Poor	0.3	0.41	Poor
277	SD4420030565	2022	Zone A	500-700m2	Never dries	Good	1	Absent	Possible	>8	Poor	0.3	0.75	Good
281	SD4430530788	2022	Zone A	400m2	Never dries	Good	1	Minor	Possible	>7	Poor	0.3	0.71	Good
282	SD4436127202	2022	Zone A	1200m2	Never dries	Poor	1	Absent	Possible	>4	Moderate	0.3	0.72	Good
397	SD4668926844	2022	Zone A	500-700m2	Dries annually	Bad	1	Absent	Absent	>3	Poor	0.3	0.40	Poor
409	SD4691527383	2022	Zone A	1300m2	Never dries	Poor	1	Minor	Possible	>2	Poor	0.3	0.64	Average

Pond ID	Grid reference	Year of survey	Geographic location	Pond area	Permanence	Water quality	Shade	Waterflow	Fish	No ponds within 1km	Terrestrial habitat	Macrophytes	HSI score	Suitability for GCN
410	SD4691929087	2022	Zone A	450m2	Never dries	Moderate	1	Major	Possible	>1	Poor	0.3	0.45	Poor
417	SD4724227200	2022	Zone A	200m2	Never dries	Poor	0.2	Minor	Absent	>0	Poor	0.3	0.52	Below average
420	SD4728627453	2022	Zone A	435m2	Never dries	Moderate	1	Major	Possible	>1	Poor	0.3	0.45	Poor
430	SD4738126985	2022	Zone A	500-700m2	Never dries	Moderate	1	Minor	Major	>2	Poor	0.3	0.65	Average
431	SD4739827474	2022	Zone A	150m2	Rarely dries	Poor	1	Minor	Absent	>3	Poor	0.5	0.61	Average
432	SD4739828949	2022	Zone A	425m2	Never dries	Poor	1	Minor	Possible	>4	Poor	0.3	0.64	Average
434	SD4741726901	2022	Zone A	150m2	Rarely dries	Poor	1	Minor	Possible	>5	Poor	0.3	0.56	Below average
435	SD4741928988	2022	Zone A	450m2	Never dries	Poor	1	Minor	Possible	>6	Poor	0.3	0.64	Average
441	SD4760426716	2022	Zone A	450m2	Never dries	Moderate	1	Absent	Absent	>7	Poor	0.3	0.75	Good
450	SD4778926669	2022	Zone A	50-100m2	Sometimes dries	Moderate	1	Absent	Absent	>8	Moderate	1	0.68	Average
496	SD4903627684	2022	Zone A	500-700m2	Dries annually	Bad	1	Minor	Absent	>17	Poor	0.3	0.38	Poor
497	SD4909727655	2022	Zone A	300m2	Rarely dries	Bad	1	Minor	Possible	>16	Poor	0.3	0.44	Poor
501	SD4921627489	2022	Zone A	225m2	Sometimes dries	Poor	0.2	Absent	Absent	>9	Good	0.3	0.58	Below average
505	SD4941327404	2022	Zone A	450m2	Never dries	Poor	1	Minor	Possible	>20	Poor	0.3	0.64	Average
506	SD4943027224	2022	Zone A	50-100m2	Never dries	Bad	1	Absent	Absent	>10	Poor	0.3	0.39	Poor
522	SD5000529427	2024	Zone A	195.5413m2	Sometimes dries	Poor	0	Absent	Absent	>6	Good	0.25	0.70	Good

Appendix B: eDNA survey sheets

SureScreen Scientifics

Folio No: E12942
Report No: 1
Purchase Order: AMC007
Client: AMC ECOLOGICAL
Contact:

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: 25/04/2022
Date Reported: 04/05/2022
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates	Pond ID
1646	Morgan 853174-2 Prospect Farm	SD 40754 30264 l	Pass	Pass	Pass	Negative	0	558
1648	Morgan LA853174-1	SD 40778 30308	Pass	Pass	Pass	Negative	0	559
1650	Morgan 924775-2	SD 41257 30122	Pass	Pass	Pass	Negative	0	N.A
1651	Morgan LAN211592	SD 40685 30067	Pass	Pass	Pass	Negative	0	153

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

Reported by **Approved by:**

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

SureScreen Scientifics

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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SureScreen Scientifics

Folio No: E13523
Report No: 1
Purchase Order: 3818
Client: AMC ECOLOGICAL
Contact:

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: 12/05/2022
Date Reported: 20/05/2022
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates	Pond ID
3818	Pond 271 3124	SD 41510 29803	Pass	Pass	Pass	Negative	0	166
3819	Pond 261 3124	SD 40911 29883	Pass	Pass	Pass	Negative	0	159
3820	Pond 245 3124	SD 41257 30030	Pass	Pass	Pass	Negative	0	163
3821	Pond 228 3124	SD 41338 30124	Pass	Pass	Pass	Negative	0	164
3822	Pond 214 3124	SD 41177 30236	Pass	Pass	Pass	Negative	0	162
3823	Pond 257 3124	SD 41463 29957	Pass	Pass	Pass	Negative	0	165
3825	Pond 219 3124	SD 41628 30176	Pass	Pass	Pass	Negative	0	170

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SureScreen Scientifics

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

Reported by: **Approved by:**

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

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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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SureScreen Scientifics

Folio No: E13664
Report No: 1
Purchase Order: AMC08
Client: AMC ECOLOGICAL
Contact: Allan Conlin

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: 18/05/2022
Date Reported: 30/05/2022
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates	Pond ID
3794	Morgan-2037-122/~~Parcel 2037~~	SD 40995 30793	Pass	Pass	Pass	Negative	0	562
3795	Morgan-2955-65	SD 36170 31084	Pass	Pass	Pass	Positive	12	556
3796	Morgan-2037-55	SD 41633 31119	Pass	Pass	Pass	Positive	9	557
3797	Morgan-3375-277	SD 38965 29720	Pass	Pass	Pass	Negative	0	P137
3799	Morgan 3093-231	SD 39601 30094	Pass	Pass	Pass	Negative	0	144
3800	Morgan 2399-194	SD 44147 30351	Pass	Pass	Pass	Negative	0	271
3801	Morgan 2037-48	SD 41373 31162	Pass	Pass	Pass	Negative	0	563

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3802	Morgan 2399-196	SD 44061 30335	Pass	Pass	Pass	Negative	0	267
3804	Morgan 3089-289	SD 59412 29501	Pass	Pass	Pass	Negative	0	143
3805	Morgan 0399-149	SD 44042 30668	Pass	Pass	Pass	Negative	0	266
3808	Morgan 3089-278	SD 39996 29694	Pass	Pass	Pass	Negative	0	146
3809	Morgan 1863-170	SD 40060 30561	Pass	Pass	Pass	Negative	0	561
3810	Morgan 1132-~~65~~-10	SD 36206 31538	Pass	Pass	Pass	Positive	1	68
3811	Morgan 2399-143	SD 43919 30692	Pass	Pass	Pass	Negative	0	261
3812	Morgan 2001-211	SD 40753 30267	Pass	Pass	Pass	Negative	0	558
3813	Morgan 1132-135	SD 36087 30734	Pass	Pass	Pass	Negative	0	67
3815	Morgan 2399-136&142	SD 44141 30693	Pass	Pass	Pass	Negative	0	272 & 273 (one pond)
3816	Morgan 1962-235	SD 40688 30070	Pass	Pass	Pass	Negative	0	153
3817	Morgan 2001-202	SD 40965 30325	Pass	Pass	Pass	Negative	0	560

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.

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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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Folio No: E13546
Report No: 1
Purchase Order: 3798
Client: AMC ECOLOGICAL
Contact: Allan Conlin

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	Pond ID
3798	3357	SD 37570 29722	Pass	Pass	Pass	Negative	0	99

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

Reported by: Esther Strafford **Approved by:** Gabriela Danickova

Forensic Scientists and Consultant Engineers
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SureScreen Scientifics

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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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.

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Folio No: 456-2024
Purchase Order: 2257
Contact: RPS Group
Issue Date: 24.04.2024

GCN Report

Technical Report

SureScreen Scientifics

Folio No: 456-2024
Purchase Order: 2257
Contact: RPS Group
Issue Date: 24.04.2024

SureScreen Scientifics

GCN eDNA Analysis

Summary

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

Results

Lab ID	Site Name	Pond ID	Degradation Check	Inhibition Check	Result	Positive Replicates
1633	Morgan, Pond 311	435	Pass	Pass	Negative	0/12
1634	Morgan, Pond 262	246	Pass	Pass	Negative	0/12
1636	Morgan, Pond 408	89	Pass	Pass	Negative	0/12
1638	Morgan, Pond 522	35	Pass	Pass	Negative	0/12
1639	Morgan, Pond 579	133	Pass	Pass	Negative	0/12
1640	Morgan, Pond 581	125	Pass	Pass	Negative	0/12
1641	Morgan, Pond 648	285	Pass	Pass	Negative	0/12
1642	Morgan, Pond 235	154	Pass	Pass	Negative	0/12
1643	Morgan, Pond 217	179	Pass	Pass	Negative	0/12
1644	Morgan, Pond 16	243	Pass	Pass	Positive	6/12

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1645	Morgan, Pond 355	527	Pass	Pass	Positive	3/12
1646	Morgan, Pond 324	535	Pass	Pass	Negative	0/12
1647	Morgan, Pond 330	533	Pass	Pass	Positive	10/12
1648	Morgan, Pond 327	519	Pass	Pass	Negative	0/12

Matters affecting result: none

Reported by: Daisy Chambers

Approved by: Chelsea Warner

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Folio No:	456-2024
Purchase Order:	2257
Contact:	RPS Group
Issue Date:	24.04.2024

SureScreen Scientifics

Methodology

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

If GCN DNA is present, the DNA is amplified up to a detectable level, resulting in positive species detection. If GCN DNA is not present then amplification does not occur, and a negative result is recorded. Analysis of eDNA requires attention to detail to prevent the risk of contamination. True positive controls, negative controls, and spiked synthetic DNA are included in every analysis and these have to be correct before any result is declared and reported. Stages of the DNA analysis are also conducted in different buildings at our premises for added analytical security.

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

Interpretation of Results

Sample Integrity Check: When samples are received in the laboratory, they are inspected for any tube leakage, suitability of sample (not too much mud or weed etc.) and absence of any factors that could potentially lead to inconclusive results. Any samples which fail this test are rejected and eliminated before analysis.

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

Inhibition Check: **Pass/Fail.** The presence of inhibitors within a sample is assessed using a DNA marker. If inhibition is detected, samples are purified and re-analyzed. Inhibitors cannot always be removed, if the inhibition check fails, the sample should be re-collected.

Result: **Presence of GCN eDNA (Positive/Negative/Inconclusive)**
Positive: GCN DNA was identified within the sample, indicative of GCN presence within the sampling location at the time the sample was taken or within the recent past at the sampling location.
Positive Replicates: Number of positive qPCR replicates out of a series of 12. If one or more of these are found to be positive the pond is declared positive for GCN presence. It may be assumed that small fractions of positive analyses suggest low level presence, but this cannot currently be used for population studies. In accordance with the WC1067 Natural England protocol, even a score of 1/12 is declared positive. 0/12 indicates negative GCN presence.
Negative: GCN eDNA was not detected or is below the threshold detection level and the test result should be considered as evidence of GCN absence, however, does not exclude the potential for GCN presence below the limit of detection.
Inconclusive: Controls indicate inhibition or degradation of the sample, resulting in the inability to provide conclusive evidence for GCN presence or absence.

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Folio No: 886-2024
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Issue Date: 06.05.2024

GCN Report

Technical Report

SureScreen Scientifics

Folio No: 886-2024
Purchase Order: 2271
Contact: RPS Group
Issue Date: 06.05.2024

SureScreen Scientifics

GCN eDNA Analysis

Summary

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

Results

Lab ID	Site Name	OS Reference	Degradation Check	Inhibition Check	Result	Positive Replicates	Pond ID
GCN2063	1369-320	SD4965027360	Pass	Pass	Negative	0/12	511
GCN2068	2163 Pond 77	SD4348930281	Pass	Pass	Positive	1/12	235
GCN2069	LP687 P232	SD3942129485	Pass	Pass	Negative	0/12	143
GCN2071	2163 44	SD4251630991	Pass	Pass	Negative	0/12	238
GCN2072	2163 483	SD4355630776	Pass	Pass	Negative	0/12	241
GCN2073	2163 27	SD4355630776	Pass	Pass	Positive	1/12	240

Matters affecting result: none

Reported by: Daisy Chambers

Approved by: Lauryn Jewkes

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Folio No: 1046-2024
Purchase Order: 2271
Contact: RPS Group
Issue Date: 13.05.2024

GCN Report

Technical Report

SureScreen Scientifics

Folio No: 1046-2024
Purchase Order: 2271
Contact: RPS Group
Issue Date: 13.05.2024

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GCN eDNA Analysis

Summary

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

Results

Lab ID	Site Name	OS Reference	Degradation Check	Inhibition Check	Result	Positive Replicates	Pond ID
2062	2163 NEW	SD4343131121	Pass	Pass	Negative	0/12	233

Matters affecting result: none

Reported by: Daisy Chambers

Approved by: Chelsea Warner

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SureScreen Scientifics

Methodology

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Folio No: 1499-2024
Purchase Order: 2271
Contact: RPS Group
Issue Date: 31.05.2024

GCN Report

Technical Report

SureScreen Scientifics

Folio No: 1499-2024
Purchase Order: 2271
Contact: RPS Group
Issue Date: 31.05.2024

SureScreen Scientifics

GCN eDNA Analysis

Summary

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

Results

Lab ID	Site Name	OS Reference	Degradation Check	Inhibition Check	Result	Positive Replicates	Pond ID
GCN2066	1314 Pond 1	SD4843829652	Pass	Pass	Negative	0/12	472

Matters affecting result: none

Reported by: Daisy Chambers

Approved by: Lauryn Jewkes

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Folio No:	1499-2024
Purchase Order:	2271
Contact:	RPS Group
Issue Date:	31.05.2024

SureScreen Scientifics

Methodology

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Appendix C: Reptile raw survey data

C	Completed
CP	Partially Completed

Area (group name, refer to figure in report for locations)	Date	Refugia deployment	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Weather/notes
Area 1	13/03/2024	C									N/A - Deployment Day
	04/04/2024		C								Temp (C) = 9 Rain= 3 Heavy Showers Notes: Weather extremely poor. Drizzle consistent on 10/04 and 11/04 with scattered showers throughout both days.
	10/04/2024			C							Temp (C) = 11 Rain= 2 Light Showers Notes: Weather extremely poor. Drizzle consistent on 10/04 and 11/04 with scattered showers throughout both days.
	16/04/2024				C						Temp (C) = 11 Rain= 2 Light Showers
	23/04/2024					C					Temp (C) = 10 Rain= 0 None
	01/05/2024						C				Temp (C) = 13 Rain= 0 None Notes: Highs of 16°and Lows of 10° during surveying hours over 3 days. No rain.
	08/05/2024							C			Temp (C) = 10 Rain= 0 None
	15/05/2024								C		Temp (C) = 17 Rain= 2 Light Showers
	05/06/2024									C	Temp (C) = 12 Rain= 2 Light Showers
	29/05/2024									CP	Temp (C) = 14 Rain= 2 Light Showers

Area (group name, refer to figure in report for locations)	Date	Refugia deployment	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Weather/notes
Area 2	13/03/2024	C									N/A - Deployment Day
	04/04/2024		C								Temp (C) = 9 Rain= 3 Heavy Showers Notes: Weather extremely poor. Drizzle consistent on 10/04 and 11/04 with scattered showers throughout both days.
	10/04/2024			C							Temp (C) = 11 Rain= 2 Light Showers Notes: Weather extremely poor. Drizzle consistent on 10/04 and 11/04 with scattered showers throughout both days.
	16/04/2024				C						Temp (C) = 11 Rain= 2 Light Showers
	24/04/2024					C					Temp (C) = 18 Rain= 0 None
	01/05/2024						C				Temp (C) = 13 Rain= 0 None Notes: Highs of 16°and Lows of 10° during surveying hours over 3 days. No rain.
	08/05/2024							C			Temp (C) = 10 Rain= 0 None
	15/05/2024								C		Temp (C) = 17 Rain= 2 Light Showers Notes:
	05/06/2024									C	Temp (C) = 12 Rain= 2 Light Showers
	29/05/2024									CP	Temp (C) = 14 Rain= 2 Light Showers
Area 3	20/03/2024	C									N/A - Deployment Day

Area (group name, refer to figure in report for locations)	Date	Refugia deployment	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Weather/notes
	04/04/2024		C								Temp (C) = 9 Rain= 3 Heavy Showers Notes: Weather extremely poor. Drizzle consistent on 10/04 and 11/04 with scattered showers throughout both days.
	11/04/2024			C							Temp (C) = 11 Rain= 1 Drizzle/Mist Notes: Weather extremely poor. Drizzle consistent on 10/04 and 11/04 with scattered showers throughout both days.
	16/04/2024				C						Temp (C) = 11 Rain= 2 Light Showers
	24/04/2024					C					Temp (C) = 18 Rain= 0 None
	02/05/2024						C				Temp (C) = 13 Rain= 0 None Notes: Highs of 16°and Lows of 10° during surveying hours over 3 days. No rain.
	09/05/2024							C			Temp (C) = 10 Rain= 0 None
	16/05/2024								C		Temp (C) = 17 Rain= 2 Light Showers
	12/06/2024									C	Temp (C) = 12 Rain= 0 None
	06/06/2024									CP	Temp (C) = 13 Rain= 2 Light Showers
Area 4	15/03/2024	C									N/A - Deployment Day
	05/04/2024		C								Temp (C) = 11 Rain= 1 Drizzle/Mist

Area (group name, refer to figure in report for locations)	Date	Refugia deployment	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Weather/notes
	12/04/2024			C							Temp (C) = 10 Rain= 1 Drizzle/Mist Notes: Raining consistently throughout most of survey, from 10.30am onwards.
	16/04/2024				C						Temp (C) = 11 Rain= 2 Light Showers
	26/04/2024					C					Temp (C) = 13 Rain= 0 None
	02/05/2024						C				Temp (C) = 13 Rain= 0 None Notes: Highs of 16°and Lows of 10° during surveying hours over 3 days. No rain.
	18/07/2024							C			Temp (C) = 17 Rain= 0 None
	25/07/2024								C		Temp (C) = 16 Rain= 2 Light showers
Area 5	14/03/2024	C									N/A - Deployment Day
	04/04/2024		C								Temp (C) = 9 Rain= 3 Heavy Showers Notes: Weather extremely poor. Drizzle consistent on 10/04 and 11/04 with scattered showers throughout both days.
	11/04/2024			C							Temp (C) = 11 Rain= 1 Drizzle/Mist Notes: Weather extremely poor. Drizzle consistent on 10/04 and 11/04 with scattered showers throughout both days.
	16/04/2024				C						Temp (C) = 11 Rain= 2 Light Showers
	26/04/2024					C					Temp (C) = 13 Rain= 0 None

Area (group name, refer to figure in report for locations)	Date	Refugia deployment	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Weather/notes
	02/05/2024						C				Temp (C) = 13 Rain= 0 None Notes: Highs of 16°and Lows of 10° during surveying hours over 3 days. No rain.
	09/05/2024							C			Temp (C) = 10 Rain= 0 None
	16/05/2024								C		Temp (C) = 17 Rain= 2 Light Showers
	12/06/2024									C	Temp (C) = 12 Rain= 0 None
	06/06/2024									CP	Temp (C) = 13 Rain= 2 Light Showers
Area 6	23/05/2024	C									N/A - Deployment Day
Area 6 Lea Marsh	06/06/2024		C								Temp (C) = 13 Rain= 2 Light Showers
	11/06/2024			CP							Temp (C) = 12 Rain= 0 None
	28/08/2024				Matts destroyed by livestock						N/A
Area 6 Fields east of Lea Marsh	20/03/2024	C									N/A - Deployment Day
	05/04/2024		C								Temp (C) = 11 Rain= 1 Drizzle/Mist
	10/04/2024			C							Temp (C) = 11 Rain= 2 Light Showers Notes: Weather extremely poor. Drizzle consistent on 10/04 and 11/04 with scattered showers throughout both days.
	16/04/2024				C						Temp (C) = 11 Rain= 2 Light Showers
	26/04/2024					C					Temp (C) = 13 Rain= 0 None

Area (group name, refer to figure in report for locations)	Date	Refugia deployment	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Weather/notes
	03/05/2024						C				Temp (C) = 13 Rain= 0 None Notes: Highs of 16°and Lows of 10° during surveying hours over 3 days. No rain.
	10/05/2024							C			Temp (C) = 10 Rain= 0 None
	17/05/2024								C		Temp (C) = 16 Rain= 0 None
	13/06/2024									C	Temp (C) = 12 Rain= 0 None
	06/06/2024									CP	Temp (C) = 13 Rain= 2 Light Showers
Area 7	20/03/2024	C									N/A - Deployment Day
	03/04/2024		C								Temp (C) = 8 Rain= 3 Heavy Showers Notes: 2 days into survey, checks require 4 days. Additional delays due to adverse weather
	11/04/2024			C							Temp (C) = 11 Rain= 1 Drizzle/Mist Notes: Weather extremely poor. Drizzle consistent on 10/04 and 11/04 with scattered showers throughout both days.
	16/04/2024				C						Temp (C) = 11 Rain= 2 Light Showers
	26/04/2024					C					Temp (C) = 13 Rain= 0 None
	03/05/2024						C				Temp (C) = 13 Rain= 0 None Notes: Highs of 16°and Lows of 10° during surveying hours over 3 days. No rain.

Area (group name, refer to figure in report for locations)	Date	Refugia deployment	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Weather/notes
	10/05/2024							C			Temp (C) = 10 Rain= 0 None
	17/05/2024								C		Temp (C) = 16 Rain= 0 None
	13/06/2024									C	Temp (C) = 12 Rain= 0 None
	06/06/2024									CP	Temp (C) = 13 Rain= 2 Light Showers