

MONA OFFSHORE WIND PROJECT

Preliminary Environmental Information Report

Volume 7, annex 18.3: Great Crested Newt Interim Survey Technical Report



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FINAL

Image of an offshore wind farm

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Glossary

Term	Meaning
eDNA Analysis	The analysis of water samples using quantitative Polymerase Chain Reaction methods to identify environmental DNA from great crested newt, as a method to confirm presence
HSI Assessment	A standard methodology for assessing the potential value of a waterbody to great crested newt

Acronyms

Acronym	Description
Cofnod	the North Wales Environmental Information Service
eDNA	Environmental Deoxyribonucleic Acid
EIA	Environmental Impact Assessment
ES	Environmental Statement
HSI	Habitat Suitability Index
MAGIC	Multi-Agency Geographic Information for the Countryside
NRW	Natural Resources Wales
OS	Ordnance Survey
PEIR	Preliminary Environmental Information Report
SI	Suitability Index

Units

Unit	Description
m	Metre
km	Kilometre
m	Metre
ml	Millimetre
%	Percentage
m ²	Square metres

1 GREAT CRESTED NEWT INTERIM SURVEY TECHNICAL REPORT

1.1 Introduction

1.1.1.1 This technical report provides the methodology and interim results of a great crested newt (*Triturus cristatus*) survey for the Mona Offshore Wind Project. The survey was undertaken in 2022.

1.1.1.2 The purpose of the survey is to identify the presence/absence of great crested newt and inform the baseline conditions and assessment of volume 3, chapter 18: Onshore ecology of the Preliminary Environmental Information Report (PEIR).

1.1.1.3 The purpose of this technical report is to present the results of the 2022 great crested newt surveys. The surveys comprised Habitat Suitability Index (HSI) assessments and environmental Deoxyribonucleic Acid (eDNA) (presence/absence) surveys of all accessible ponds located within the surveyed area (see section 1.3).

1.1.1.4 This report will inform the scope of the population size class surveys, which are proposed in 2023 to evaluate the importance of great crested newt populations that may be located within the survey area.

Legislation

1.1.1.5 Great crested newt are protected under the Conservation of Habitats and Species (EU Exit) Regulations 2019, which make it an offence to:

- deliberately capture or kill a great crested newt
- deliberately disturb a great crested newt
- deliberately take or destroy the eggs of a great crested newt
- damage or destroy a breeding site or resting place of a great crested newt.

1.1.1.6 In addition, the great crested newt is protected under Schedule 5 of the Wildlife and Countryside Act 1981 (as amended), which makes it an offence to:

- intentionally kill, injure, or take a great crested newt
- possess or control any live or dead specimen or anything derived from a great crested newt
- intentionally or recklessly damage, destroy or obstruct any structure or place used for shelter or protection by a great crested newt
- intentionally or recklessly disturb a great crested newt while it is occupying a structure or place, which it uses for that purpose.

1.2 Consultation

1.2.1.1 Consultation with Natural Resources Wales (NRW) was undertaken in March 2022, to confirm the methodology and extent of the survey. A summary of the key issues raised and responses from NRW are presented in Table 1.1 below.

Table 1.1: Summary of key consultation topics raised during consultation activities undertaken for the Mona Offshore Wind Project relevant to great crested newt.

Date	Consultee	Topic covered/consultee response
10 March 2022 (email)	NRW	<p>A report was prepared for NRW (bp/EnBW, 2022) describing the proposed great crested newt survey methodology, including a map of the survey area, for review and comment.</p> <p>The proposed methodology included:</p> <ul style="list-style-type: none"> • HSI assessment (in accordance with ARGUK, 2010) • eDNA analysis of water samples (in accordance with Biggs <i>et al.</i>, 2014) • Standard presence/absence surveys of waterbodies that eDNA analysis was not undertaken for, or population class size surveys of waterbodies that recorded positive results from eDNA analysis, or located within 250m of a waterbody that positive eDNA results were obtained from (in accordance with English Nature, 2001). <p>The proposed survey area:</p> <ul style="list-style-type: none"> • Included all waterbodies within the Mona Proposed Onshore Development Area and a surrounding 250m wide buffer zone. • Excluded waterbodies that are part of an on-going monitoring programme.
21 March 2022 (email)	NRW	<p>NRW's response to the report was: "<i>NRW Advisory (A) have no objection in principle to the proposed methodology for the Great Crested Newt (GCN) Survey However, NRW (A) would advise inclusion of the following provisions:</i></p> <ul style="list-style-type: none"> • <i>Traditional survey techniques should also be carried out in respect of</i> <ul style="list-style-type: none"> – <i>Any ponds where there has been a previous record of GCNs</i> – <i>All ponds within 250m of a pond where GCN's are or have been confirmed, provided habitats are available for connectivity purposes and that these ponds do not support fish</i> • <i>Where practical, modelling should also be used to inform the site selection process."</i>
29 March 22 (email)	NRW	<p>Confirmation was sought from NRW on the following points:</p> <ul style="list-style-type: none"> • <i>Would NRW accept the use of eDNA to confirm absence – if eDNA results are negative, would NRW agree that no standard methodology survey would be required unless ...</i> <ul style="list-style-type: none"> – <i>there has been a previous record of [great crested newt].</i> – <i>ponds [are] within 250m of a pond where [great crested newt] are or have been confirmed, provided habitats are available for connectivity purposes and that these ponds do not support fish.</i> • <i>Could surveys of the St Asaph, Gwent Y Mor and Burbo Bank ponds, which are already being monitored annually, be excluded.</i>

Date	Consultee	Topic covered/consultee response
30 March 2022 (email)	NRW	<p>NRW's response to RPS's email: "NRW Advisory (A) have no objection to the proposed approach outlined in the email ... dated 29 March 2022.</p> <p>In addition to the points you have outlined, NRW (A) would advise the following:</p> <ul style="list-style-type: none"> • Modelling data that suggests likelihood of occurrence of the species is considered as a material component of surveillance strategies. • A presumption in favour of all ponds with extant records being subject to survey using traditional survey techniques, unless the ponds are known to be subject to ongoing surveys by third parties."

1.3 Survey areas

- 1.3.1.1 Considering NRW responses to consultation summarised in Table 1.1 above, the aim of the great crested newt survey was to cover the areas below:
- Mona Proposed Onshore Development Area
 - Desk study buffer zone: a buffer zone of approximately 2km for a desk-based study, which considers the potential for great crested newt to disperse up to 1.3km from a breeding pond (English Nature, 2001)
 - Field survey buffer zone: a buffer zone of 250m for field surveys, based on feedback received during the consultation (Table 1.1), and the understanding that "... great crested newt commonly move between ponds that are within around 250m of each other" (English Nature, 2001), and that "... by far the most ... [are] recorded within 50m of ponds ... few ... at distances greater than 100m ... [and only a] small number ... up to 150-200m from breeding ponds" (Cresswell and Whitworth, 2004).
- 1.3.1.2 The location and geographic extent of the survey areas, including the 2km desk study buffer zone and 250m field survey buffer zone are shown on Figure 1.1.
- 1.3.1.3 Since the surveys were undertaken in 2022 the Mona Proposed Onshore Development Area has been refined. This has resulted in some of the surveyed ponds now falling outside the Mona Proposed Onshore Development Area. The results from such ponds have been included in this report as they provide useful context information regarding potential great crested newt habitat in the surrounding area.
- 1.3.1.4 Not all ponds located within the field survey buffer zone could be surveyed in 2022 due to access restrictions (these ponds are labelled on Figure 1.6 to Figure 1.10 as ponds 'not surveyed'). Therefore, further surveys will be undertaken in 2023 to target these ponds and undertake population size class surveys, where there is potential for great crested newt to be present.
- 1.3.1.5 The scope of the 2023 great crested newt surveys will seek to address NRW's requirements and comments set out in Table 1.1 above, including any potential subsequent additions or amendments to these.

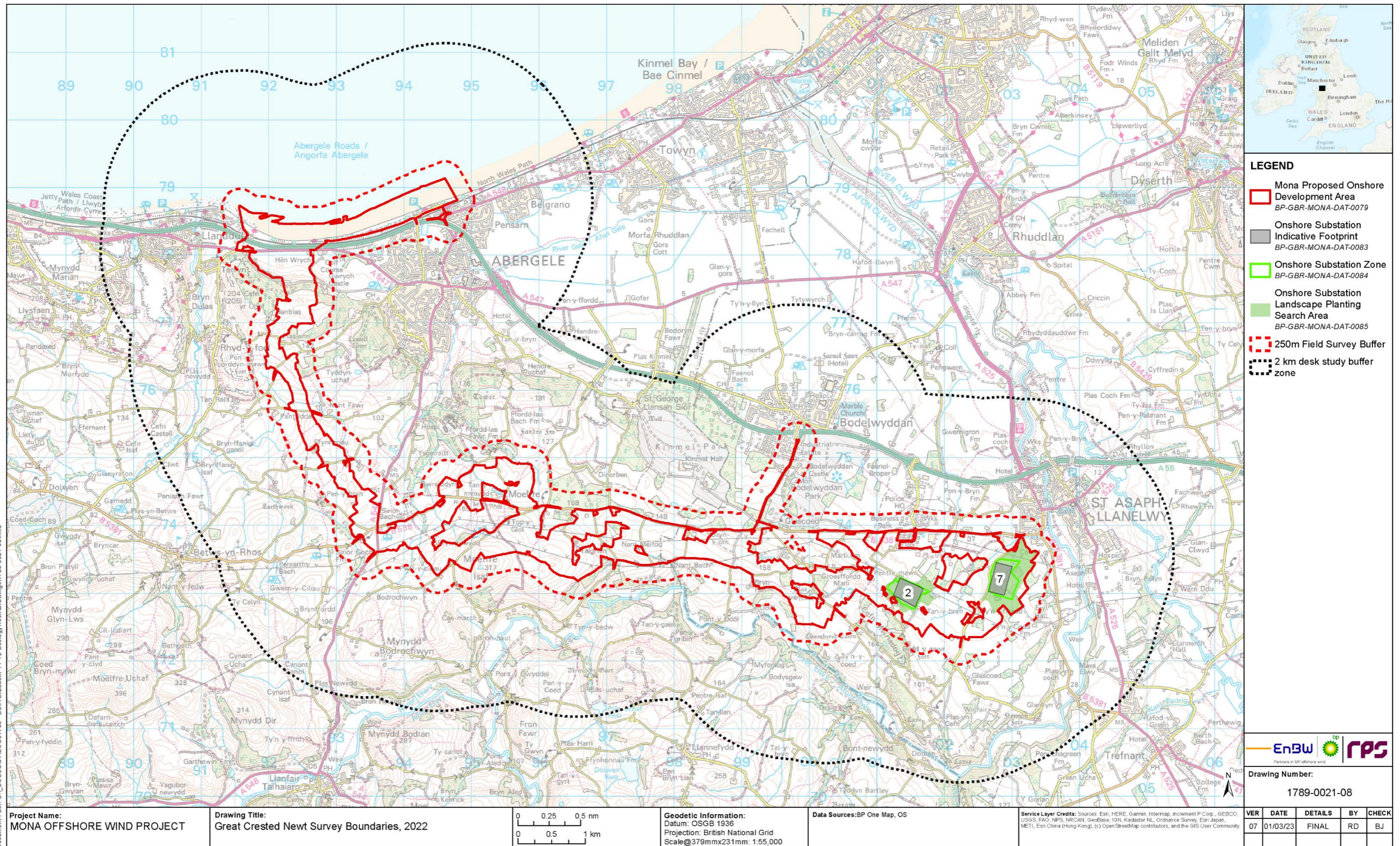


Figure 1.1: Great crested newt survey area.

1.4 Survey methodologies

1.4.1 Desk study

1.4.1.1 Historic records of great crested newt reported since 2010 from within the desk study buffer zone were requested from Cofnod, the North Wales Environmental Information Service, in March 2022. Records collected as part of the great crested newt monitoring coordinated by NRW are included within the Cofnod dataset (dated 20 October 2022).

1.4.1.2 In addition, the Lle Geo-Portal (developed by the Welsh Government and NRW) , and the Multi-Agency Geographic Information for the Countryside (MAGIC) online mapping system were reviewed to identify any potential sites that are designated for great crested newt within the desk study buffer zone.

1.4.2 Field surveys

1.4.2.1 The location of waterbodies within the field survey buffer zone were identified through a review of Ordnance Survey (OS) maps, online satellite imagery and aerial photography. A total of 114 ponds were identified within 250m of the Mona Proposed Onshore Development Boundary.

1.4.2.2 Due to the number of waterbodies that required a survey, the restrictions on land access, and the timescale for refining the Mona Proposed Onshore Development Area (post PEIR), it was considered appropriate to undertake the field surveys in two phases. The first phase of field surveys would be completed in 2022, and the second phase to be completed in 2023. The requirement for 2023 field surveys would be informed by the results of the 2022 surveys.

1.4.2.3 Where landowner consent had been granted for the 2022 field surveys (hereafter referred to as 'accessible waterbodies'), waterbodies were surveyed using at least one of the following methodologies:

- HSI assessment
- eDNA analysis.

1.4.2.4 As agreed with NRW during the scoping stage in the EIA process (Table 1.1), the results of eDNA analysis were used to inform, at least in part, the need for standard presence/absence and population class size surveys to be undertaken (in accordance with English Nature 2001).

1.4.2.5 All field surveyors were suitably trained and experienced in undertaking the HSI assessment and eDNA survey methodologies set out in the following sections of this report.

HSI assessment

1.4.2.6 All accessible waterbodies were assessed for their suitability to support great crested newt following the HSI assessment methodology described in the UK Amphibian and Reptile Group's Advice Note 5 (ARGUK, 2010), which is a modified version of the original HSI methodology described in Oldham *et al.* (2000).

1.4.2.7 The HSI methodology (ARGUK, 2010) involves the assessment of 10 key habitat parameters, which are set out in Table 1.2 below and are typically associated with ponds used by great crested newt. Each parameter is given a score from 0 to 1, based

on the descriptions and HSI scoring system provided in UK Amphibian and Reptile Group's Advice Note 5 (ARGUK, 2010).

Table 1.2: HSI indices.

Suitability Index (SI)	Topic	Description
SI1	Location	Sites scored according to UK zone in which they occur.
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 50m ² a score of 0.05 is used. For ponds larger than 2000m ² this factor is omitted. Index score is measured from a correlation graph.
SI3	Permanence	Local knowledge and personal judgement using the following four category scale: never dries, rarely dries, sometimes dries, dries annually.
SI4	Water quality	Based on invertebrate diversity, presence of submerged plants and knowledge of the water sources. Not to be confused with water clarity. Four point scale: good, moderate, poor, bad.
SI5	Shade	Estimate percentage (%) pond perimeter shaded, to at least 1m 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 located within 1km. Score taken from a correlation graph.
SI9	Terrestrial habitat	Requires understanding of newt requirements. Habitat within 250m of a pond, not separated by a significant barrier to newt movement. Four point scale: good, moderate, poor, none.
SI10	Macrophytes	Estimate of % 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.

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

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

1.4.2.9 The overall HSI score is then translated into a classification of habitat suitability, as listed in Table 1.3 below.

Table 1.3: HSI classification.

HSI Score	Suitability for great crested newt
>0.8	Excellent
0.7 – 0.79	Good
0.6 – 0.69	Average
0.5 – 0.59	Below average
<0.5	Poor

eDNA analysis

- 1.4.2.10 Great crested newt release eDNA into waterbodies, for example on shed skins cells, faeces, and eggs. Therefore, water samples can be analysed for great crested newt eDNA to confirm the presence or absence of the species.
- 1.4.2.11 The eDNA survey technique is set out in the Analytical and Methodological Development for Improved Surveillance of the Great Crested Newt (Biggs *et al.*, 2014). The method has been developed for standing waterbodies, due to the potential in flowing waterbodies/watercourses for eDNA to be washed downstream from a sample location.
- 1.4.2.12 The acceptable survey period for eDNA analysis to be undertaken is between mid-March and June. Water samples were collected from all accessible waterbodies that held sufficient water, between May and June 2022, which is within the optimal survey period (i.e. between mid-April and the end of June).
- 1.4.2.13 As set out above, the water samples were collected by surveyors who had been sufficiently trained in the methodology described in Analytical and Methodological Development for Improved Surveillance of the Great Crested Newt (Biggs *et al.*, 2014).
- 1.4.2.14 Water samples were collected using sampling kits supplied by ADAS Ltd. Surveyors collected 30 millilitres (ml) of water samples from 20 locations along the margins of each waterbody surveyed, 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, whilst taking care not to disturb any sediment, before collecting each sample.
- 1.4.2.15 The 20 samples collected from each waterbody were emptied into a sterile plastic bag and homogenised by gently shaking the bag to ensure eDNA was evenly mixed through the sample. A pipette was then used to transfer six 15ml sub-samples of the water from the bag into sterile tubes containing 35ml of ethanol to preserve the eDNA samples.
- 1.4.2.16 The samples were then removed from site and stored in a refrigerator before being couriered to ADAS for laboratory analysis to confirm presence or absence of great crested newt eDNA.

Laboratory Protocol

- 1.4.2.17 The ADAS laboratory reports that eDNA analysis is undertaken following the protocols for quantitative Polymerase Chain Reaction eDNA testing described in Biggs *et al.* (2014), which has subsequently been accepted as a means of confirming presence/absence of great crested newt by NRW.

1.5 Limitations

1.5.1 Desk study

- 1.5.1.1 The desk study data is third party controlled data, purchased for the purposes of this report only. RPS cannot vouch for its accuracy. Whilst the records show where a

species has been recorded, they do not show where a species is absent. A lack of records in a particular area may be due to no surveys having been undertaken or results of surveys and incidental sightings having been reported. Therefore, the findings of the desk study should not be relied upon alone to determine species presence or absence but should be used to help inform where further detailed surveys are required for a particular species.

1.5.2 Field surveys

- 1.5.2.1 HSI assessments provide an indication of suitability for great crested newt only and are not a substitute for determining the likely presence or absence.
- 1.5.2.2 With regard to eDNA surveys, there is some potential for water samples collected for analysis to be contaminated (e.g. sediment, white precipitate or algae), which may lead to misleading or inconclusive results, and for the water chemistry to inhibit the eDNA extraction process.
- 1.5.2.3 Therefore, the laboratory will carry out an assessment of the quality of the water sample, and undertake control tests, to confirm if there were any limitations to the eDNA analysis and if any results should be considered inconclusive. This reporting has been considered in conclusions drawn from eDNA analysis regarding the presence or absence of great crested newt.

1.6 Results

1.6.1 Desk study

- 1.6.1.1 The historic records of great crested newt reported by Cofnod are provided in Appendix A of this report. A summary of the more recent records are shown in Figure 1.2 to Figure 1.5, which includes records reported since 2010, with a six-figure grid reference or higher, since lower resolutions do not allow accurate calculation of distance from the boundary of the Mona Proposed Onshore Development Area.

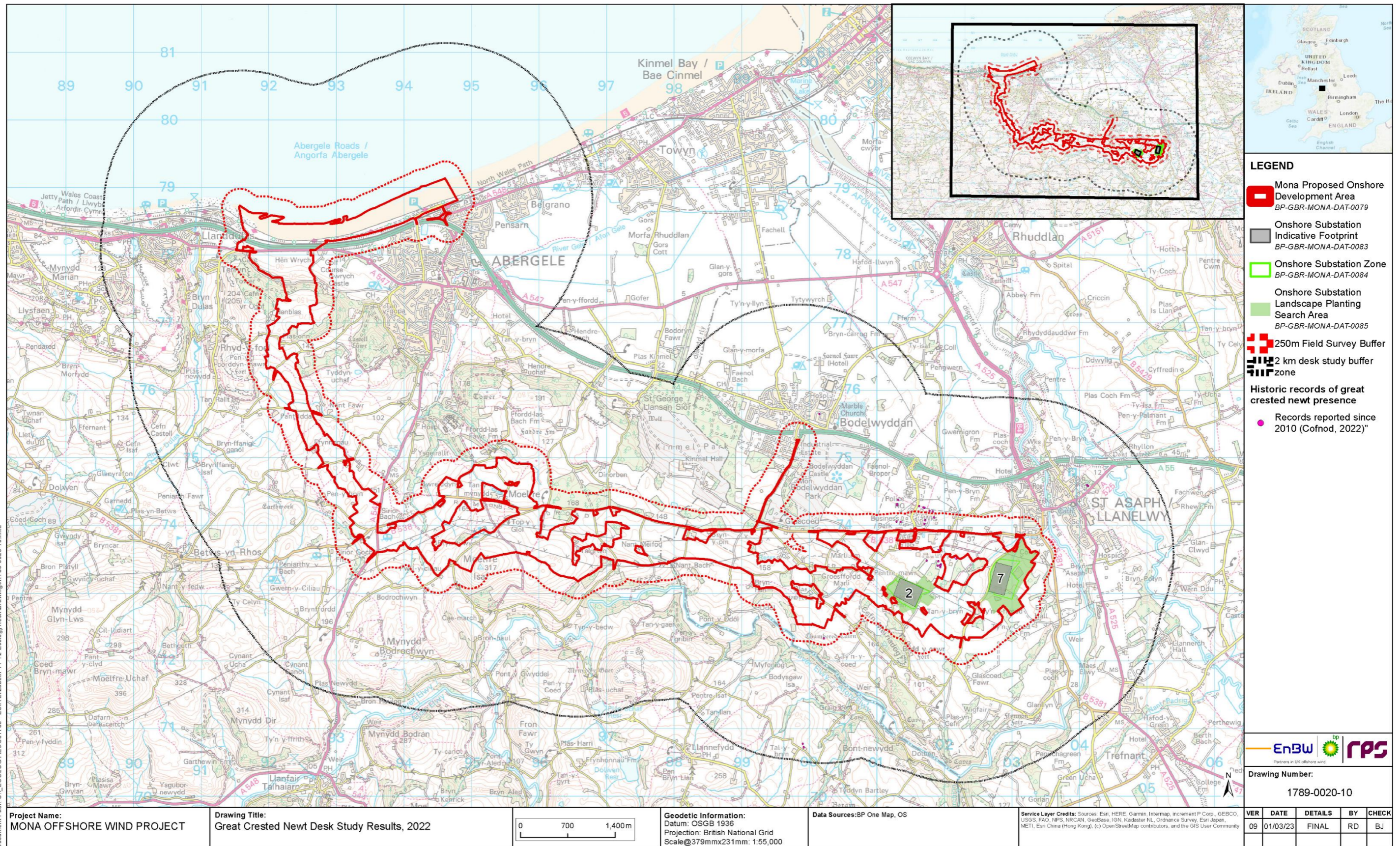


Figure 1.2: Great crested newt desk study results.

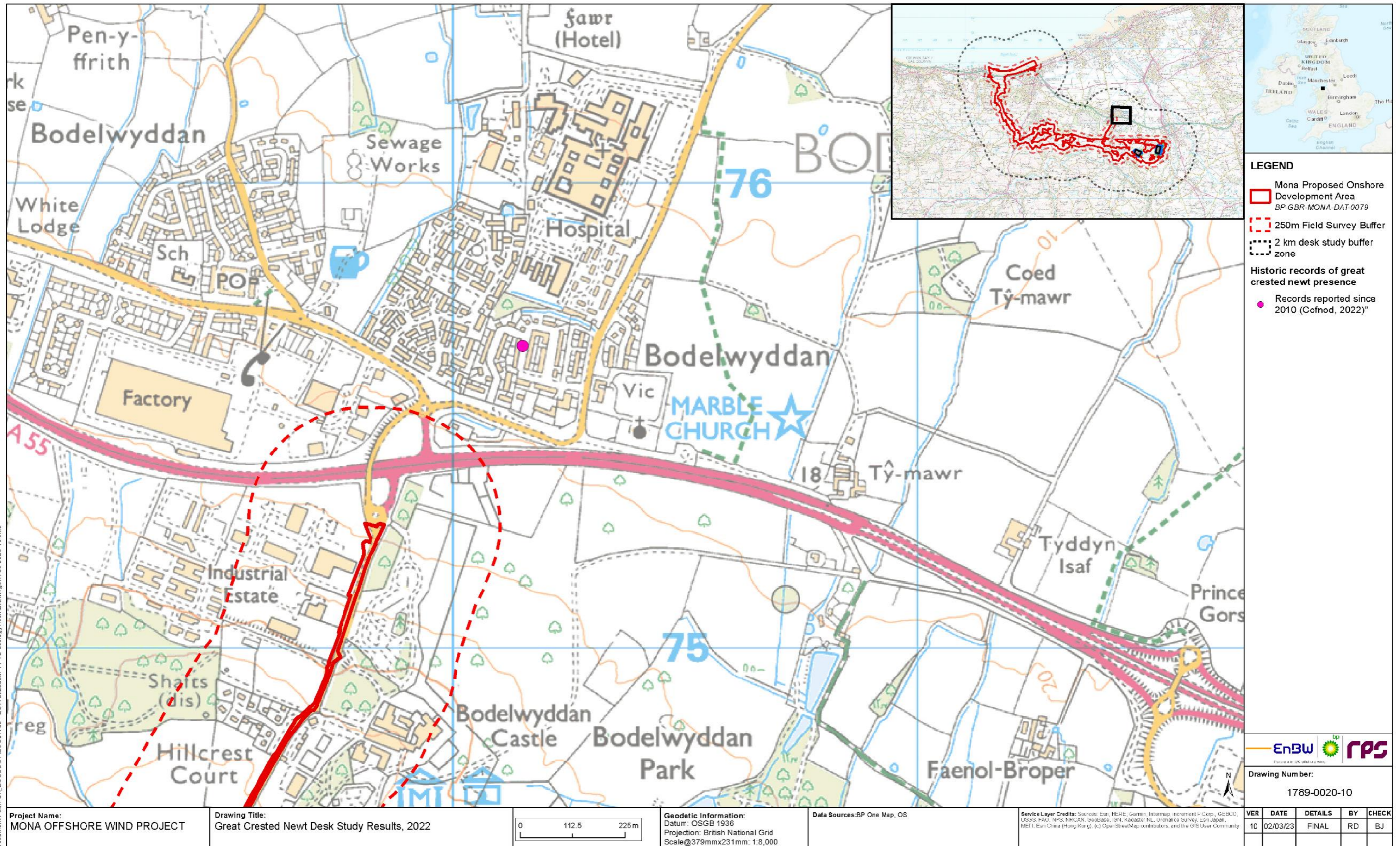


Figure 1.3: Great crested newt desk study results.

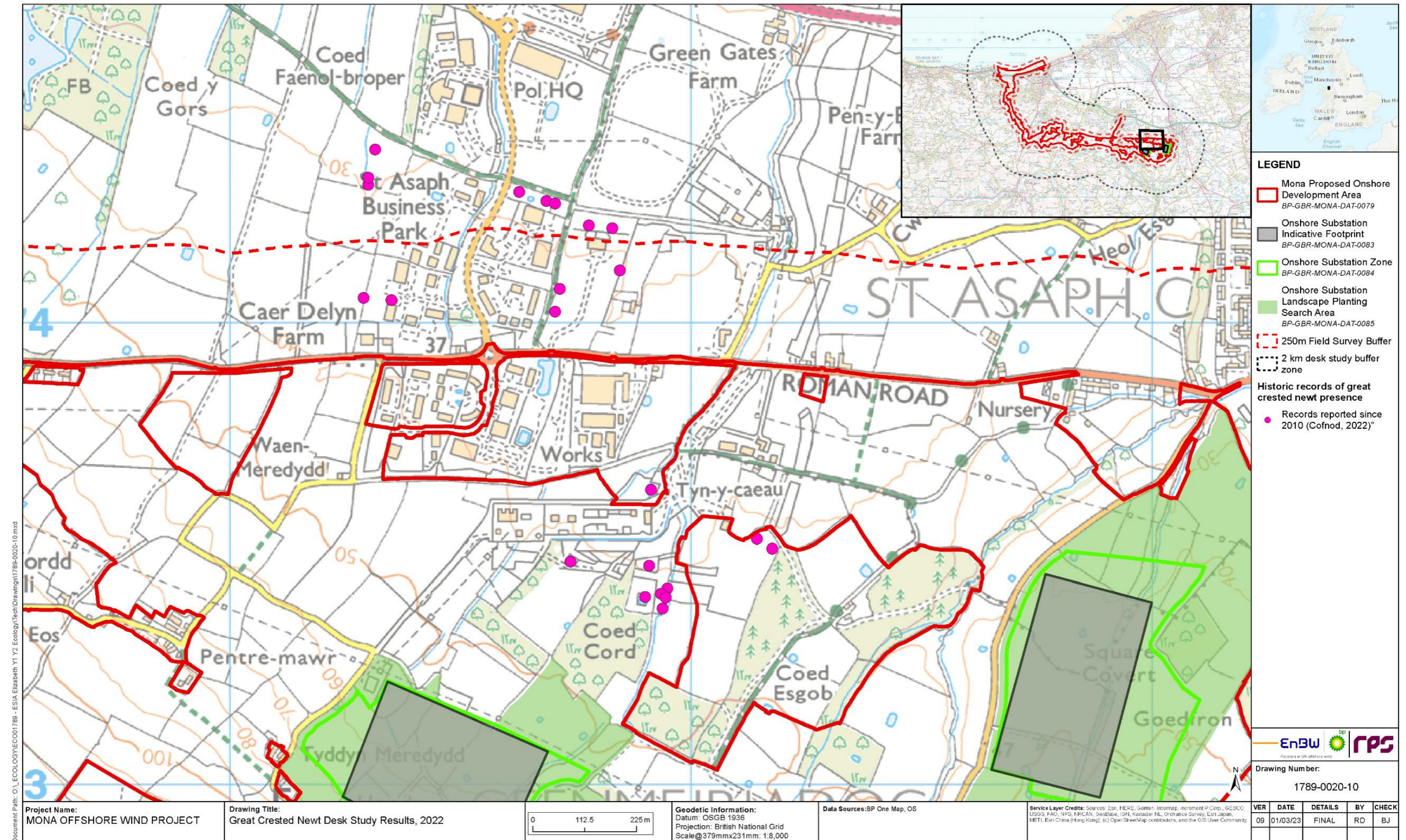


Figure 1.4: Great crested newt desk study results.

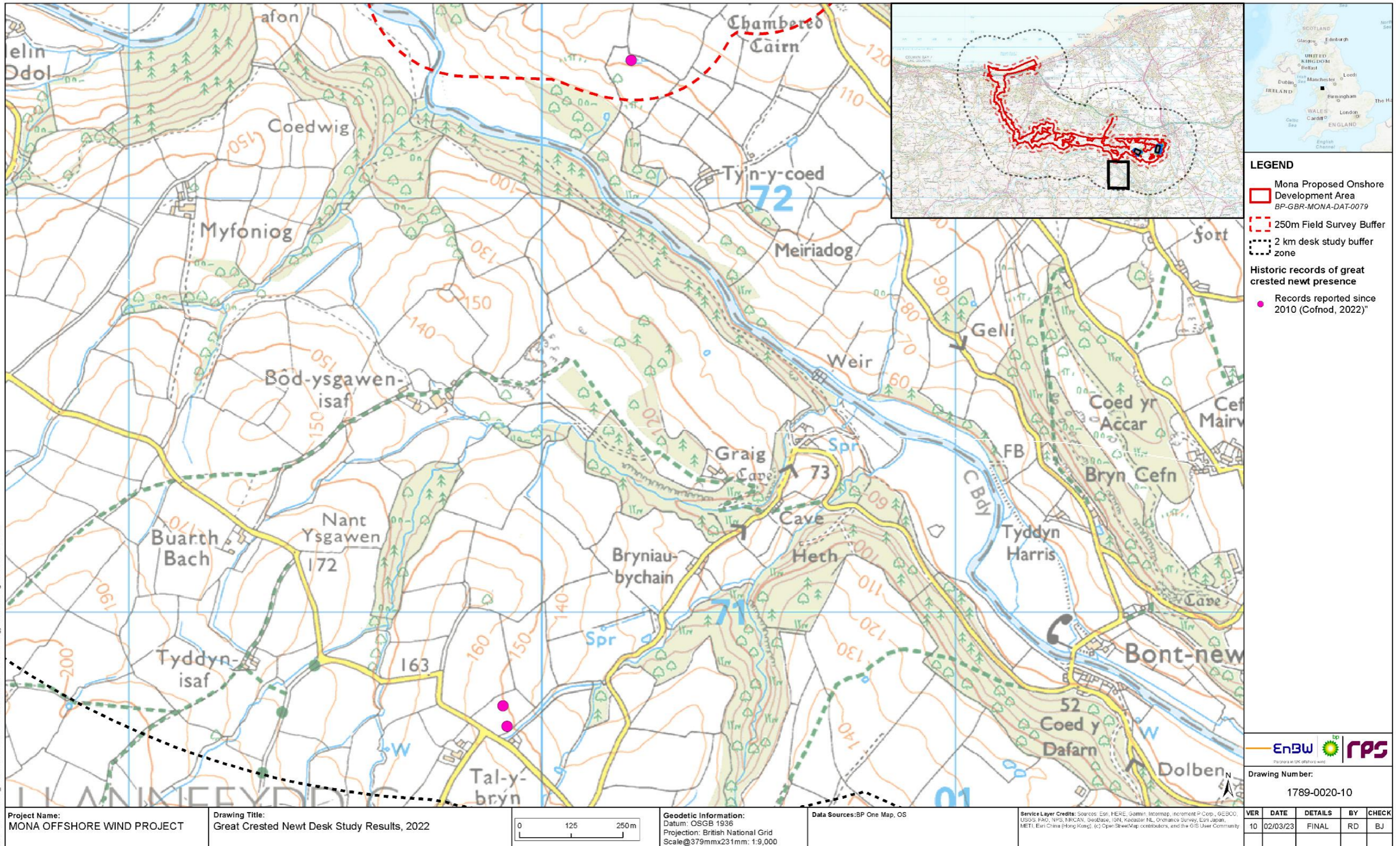


Figure 1.5: Great crested newt desk study results.

1.6.2 HSI assessment

1.6.2.1 Landowner permission to access all waterbodies within the field survey buffer zone was not obtained; however, several waterbodies were accessed and the results of the HSI assessment of these accessible waterbodies are provided at Appendix B and summarised in Table 1.4 below. Pond references are shown on Figure 1.6 to Figure 1.10 of this report.

1.6.2.2 Results from some surveyed ponds located beyond the field survey buffer zone are also presented in Table 1.4 for the reasons presented in paragraph 1.3.1.3 of this report.

Table 1.4: HSI assessment results.

Pond reference	HSI score	Additional note
Ponds located within the field survey buffer zone		
P039	Poor	Medium sized pond located between an area of dense scrub and pasture. Appears to be frequently used by waterfowl and cattle.
P050	Average	Medium sized pond surrounded by steep earth banks, with a small island present on which a single willow tree was in its centre.
P096	Poor	Small reservoir previously provided water to the main estate's house. Fish and waterfowl present.
P097	N/A	Dried out pond in logging area. Overgrown with regen and grasses. No signs that the area has held standing water for a considerable time.
P068	Poor	Very small defunct well, along field boundary hedgerow. Stagnant with near 100% macrophyte cover.
P106	Poor	Medium sized pond surrounded by trees, located within a large pasture field.
P111	Average	Medium, shallow sided pond, with a marshy area surrounding and some scrub/mature willow trees in the immediate area. Short cut grass in the adjacent fields with four hedge lines creating some habitat connectivity.
P113	Good	Long oval pond with an area of approximately 6m x 25m.
P114	Poor	Pond on hillside, very little aquatic vegetation. Fitted with an overflow pipe. Very little standing water during the survey visit.
P116	N/A	Dry ground in area of felled woodland. Overgrown with scrub and grasses.
P121	Poor	Shallow farm pond in centre of large field, surrounded by trees. Very little aquatic vegetation. Potentially impacted by farm pollutants.
P125	N/A	Pond no longer present, areas of slightly damper ground only.
P127	Below Average	Two ponds on map merged into one large pond. Large hawthorn trees surrounding.
P131	N/A	Dry sandy area of field with no pond present.
P210	Poor	Small deep pond at edge of field. Water surface covered in duckweed. Bankside willow and bramble overgrowth.
P211	Average	Small rectangular water body just off field margin. Pond is tightly fenced to pond edge with minor pond edge fauna (mostly dogwood, and bramble). Pond fauna is dominated by Typha, which is dominating the water surface area.

Pond reference	HSI score	Additional note
Ponds located beyond field survey buffer zone		
P009	N/A	Dry pond.
P021	Good	Large kidney bean shaped pond with small, vegetated island on one lobe, marginal macrophyte cover including flag iris, duckweed, and broad leaved pondweed.
P042	Average	Medium sized pond, based at the bottom of a sharp incline. Pond margins are heavily vegetated; however, no obvious floating, submerged or emergent vegetation recorded.
P045	N/A	Area of old earth that could very well have been a pond and may fill in adverse weather, but dry and devoid of water species flora.
P051	Poor	Large pond located at the edge of small woodland. Waterfowl and fish present.
P052	Poor	Large pond located in a small area of deciduous woodland, waterfowl and fish present.
P053	Poor	Small ponds situated on the margin of a field, low levels of water at the time of the survey.
P054	Poor	Large pond located in small area of woodland, waterfowl, and fish present.
P063	Below Average	Temporary attenuation pond, to ease flooding from run-off during high rainfall. Slow-flowing input from ditch and output into ditch. Holding very little water at base during time of survey.
P064	Average	Medium sized pond located in pasture field. Evidence of poaching from livestock.
P073	Poor	Dried out pond, still damp but thick, muddy base, lots of shading from bankside vegetation. Good surrounding terrestrial habitat.
P076	Poor	Standing water in woodland, no aquatic vegetation.
P084	Below Average	Medium sized apparently man-made pond in sheep grazed field. Contained internal ledge. Water-level very low at the time of the survey, so that only the deep central area was holding water.

1.6.3 eDNA analysis

1.6.3.1 The results of the HSI survey were assessed to determine which waterbodies would require eDNA analysis in 2022. The results of this assessment are summarised in Table 1.6 below.

1.6.3.2 As with the HSI assessments, the survey results from some of the ponds located outside the field survey buffer zone have also been included in Table 1.5.

1.6.3.3 Due to the limited access to survey areas, and the restricted time available to collect eDNA samples within the acceptable survey period (i.e. mid-April to June 2022), ponds that were assessed to be of poor potential value to great crested newt during the HSI assessment (i.e. HSI score < 0.5), and those which had water-levels too low to enable a water sample to be collected without contamination from silts, were omitted from the eDNA survey.

Table 1.5: Results of the assessment of waterbodies requiring eDNA analysis.

Pond reference	HSI score	eDNA analysis required in 2022	Reason for no eDNA analysis in 2022
Ponds located within the 250m field survey buffer zone			
P039	Poor	No	Significant numbers of waterfowl present, fish, and livestock disturbance, resulting in the pond being of poor value.
P050	Average	Yes	N/A
P068	Poor	No	Small defunct well unfavourable to newt.
P096	Poor	No	Small reservoir, containing fish and visited by waterfowl, making it less than favourable to newt.
P097	N/A	No	Dry pond
P106	Poor	No	Waterfowl present, fish, and very few macrophytes, made the pond less than favourable to newt.
P111	Average	Yes	N/A
P113	Good	Yes	N/A
P114	Poor	No	Pond fitted with overflow pipe, very little semi-aquatic vegetation and almost dry. No/not enough water to sample.
P116	N/A	No	No pond present
P121	Poor	No	Water-level too low to enable a water sample to be collected.
P125	N/A	No	No pond present
P127	Below Average	Yes	N/A
P131	N/A	No	No pond present
P210	Poor	Yes	N/A – eDNA water sample collected prior to HSI assessment.
P211	Average	Yes	N/A
Ponds located beyond the 250m field survey buffer zone			
P009	N/A	No	Dry pond
P021	Good	Yes	N/A
P042	Average	Yes	N/A
P045	N/A	No	Dry pond
P051	Poor	No	Poor value – waterfowl and fish present, and very limited amount of macrophytes.
P052	Poor	No	Poor value – waterfowl and fish present, and very limited amount of macrophytes.
P053	Poor	No	Water-level too low to enable a water sample to be collected.
P054	Poor	No	Poor value – waterfowl and fish present, and very limited amount of macrophytes.

Pond reference	HSI score	eDNA analysis required in 2022	Reason for no eDNA analysis in 2022
P063	Below Average	No	Temporary/fluctuating waterbody (constructed to ease run-off flooding during heavy rainfall), and water-level too low to enable a sample to be collected.
P064	Average	Yes	N/A
P073	N/A	No	Dry pond
P076	Poor	No	Water-level too low to enable a water sample to be collected.
P084	Below Average	Yes	N/A

1.6.3.4 The results of the eDNA analysis of accessible waterbodies listed as requiring an eDNA survey in Table 1.5 are summarised in Table 1.6 below and presented on Figure 1.6 to Figure 1.10. The laboratory analysis reports provided by ADAS are provided at Appendix C of this report.

1.6.3.5 The results of the eDNA analysis have confirmed the presence of great crested newt eDNA in one of the surveyed waterbodies within 250m of the Mona Proposed Onshore Development Area (P111), and three waterbodies located beyond 250m (P021, P064, P084). The locations of these waterbodies are presented in Figure 1.6 to Figure 1.10 below.

Table 1.6: eDNA analysis results.

Pond reference	HSI score	eDNA analysis result (positive or negative for great crested newt eDNA)
Ponds located within the field survey buffer zone		
P050	Average	Negative
P111	Average	Positive
P113	Good	Negative
P127	Below average	Negative
P210 (referred to on ADAS sheets as new)	Poor	Negative
P211	Average	Negative
Ponds located beyond the field survey buffer zone		
P021	Good	Positive
P042	Average	Indeterminate – sediment levels too high to provide definitive result.
P064	Average	Positive
P084	Below average	Positive

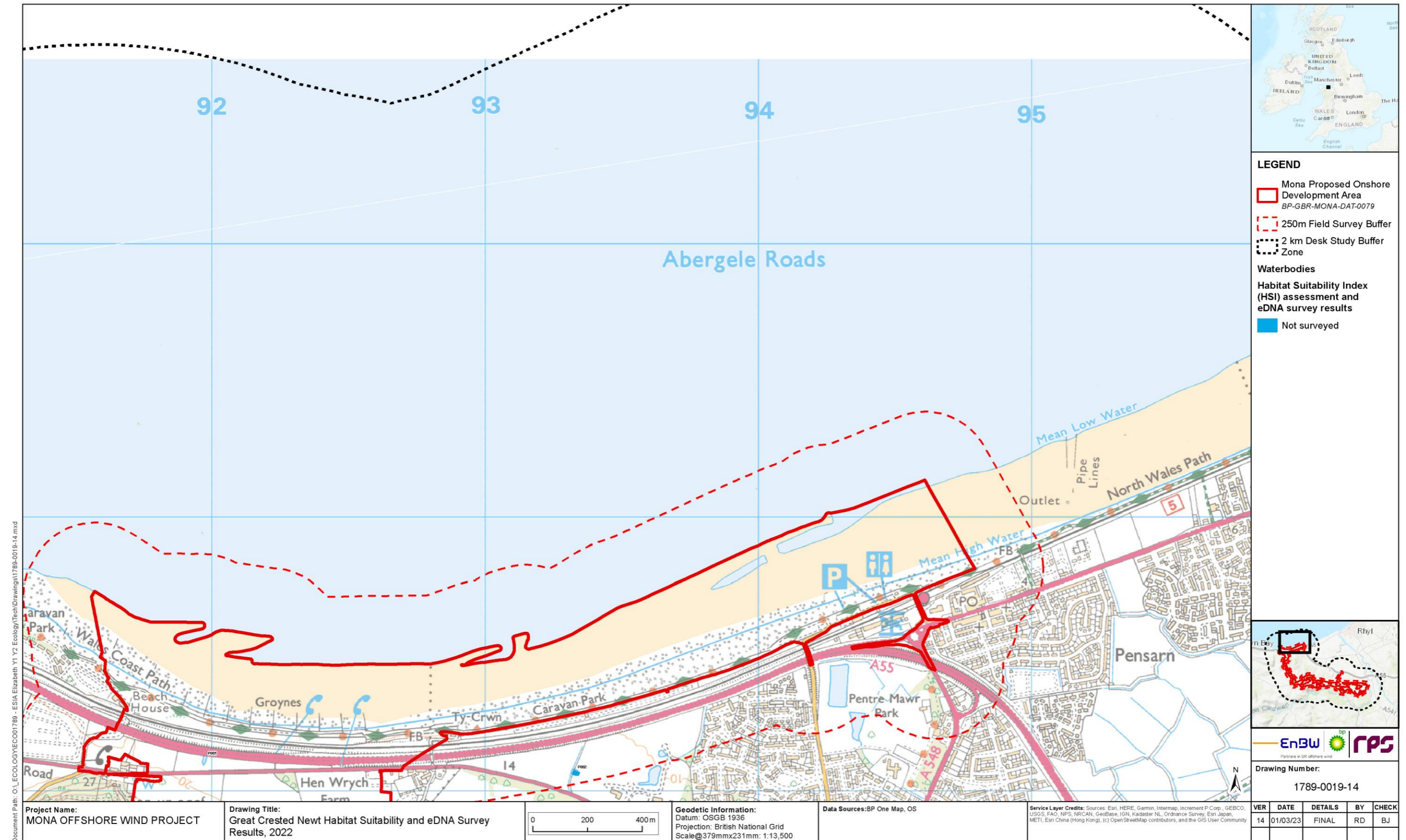


Figure 1.6: Great crested newt field survey locations and results.

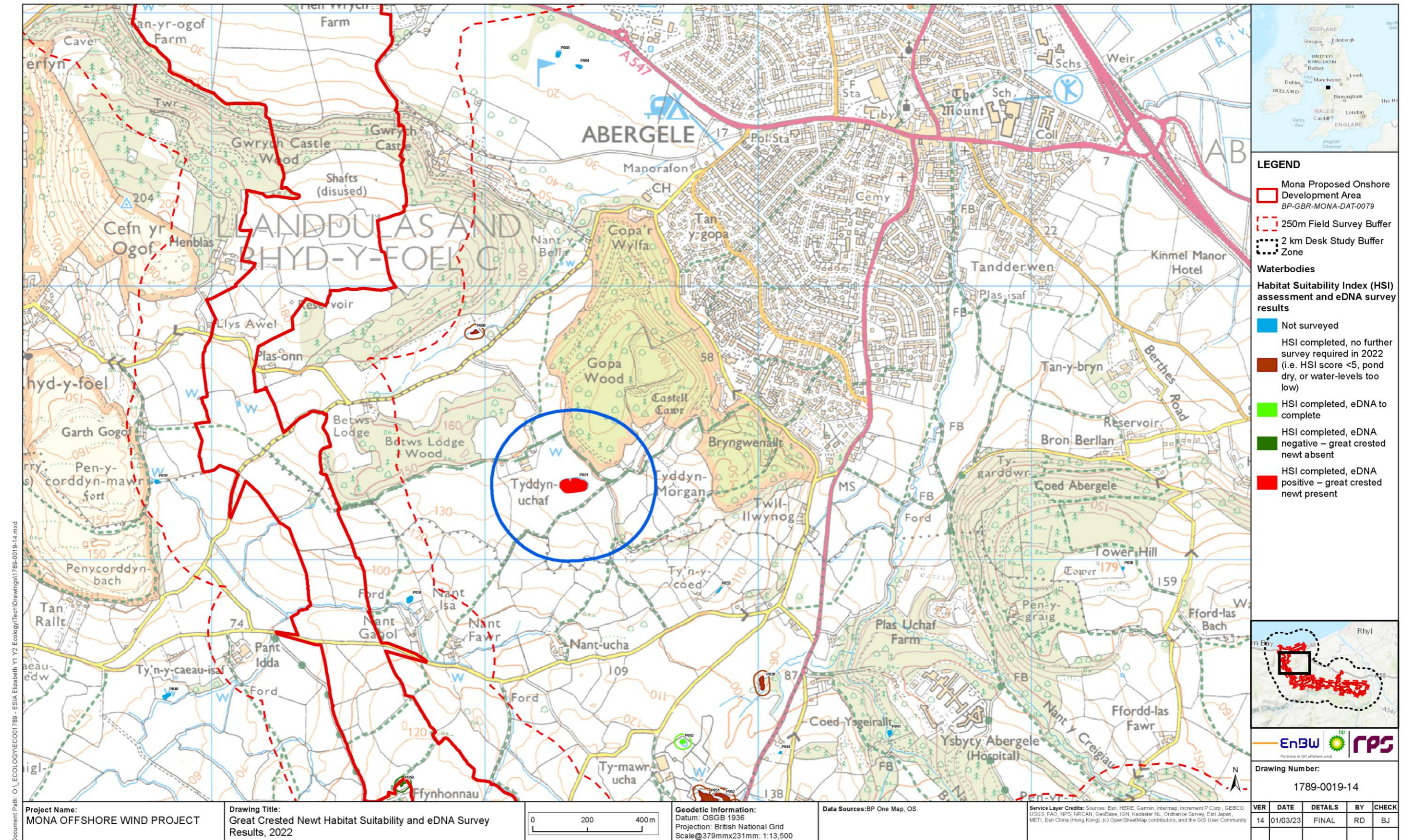


Figure 1.7: Great crested newt field survey locations and results.

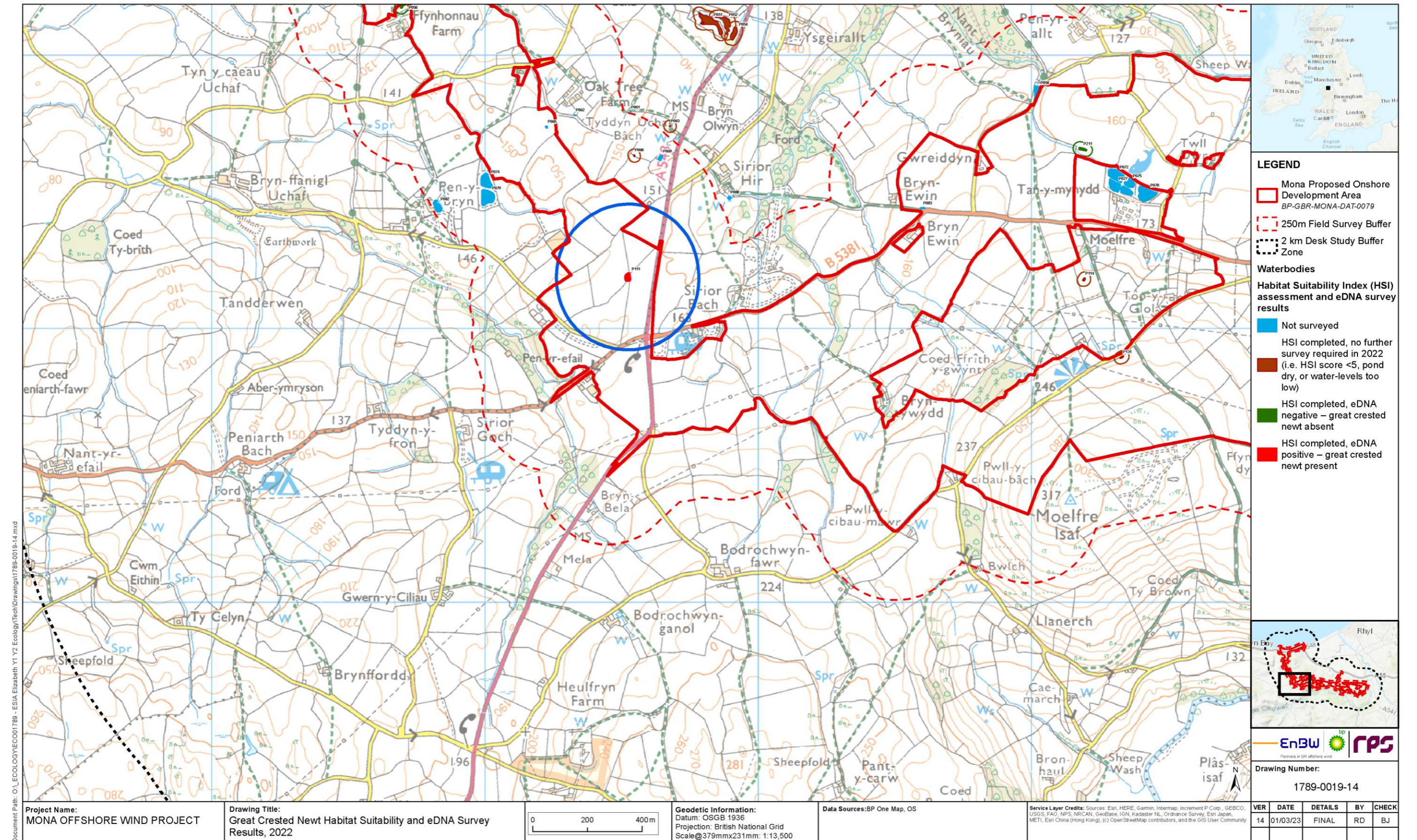


Figure 1.8: Great crested newt field survey locations and results.

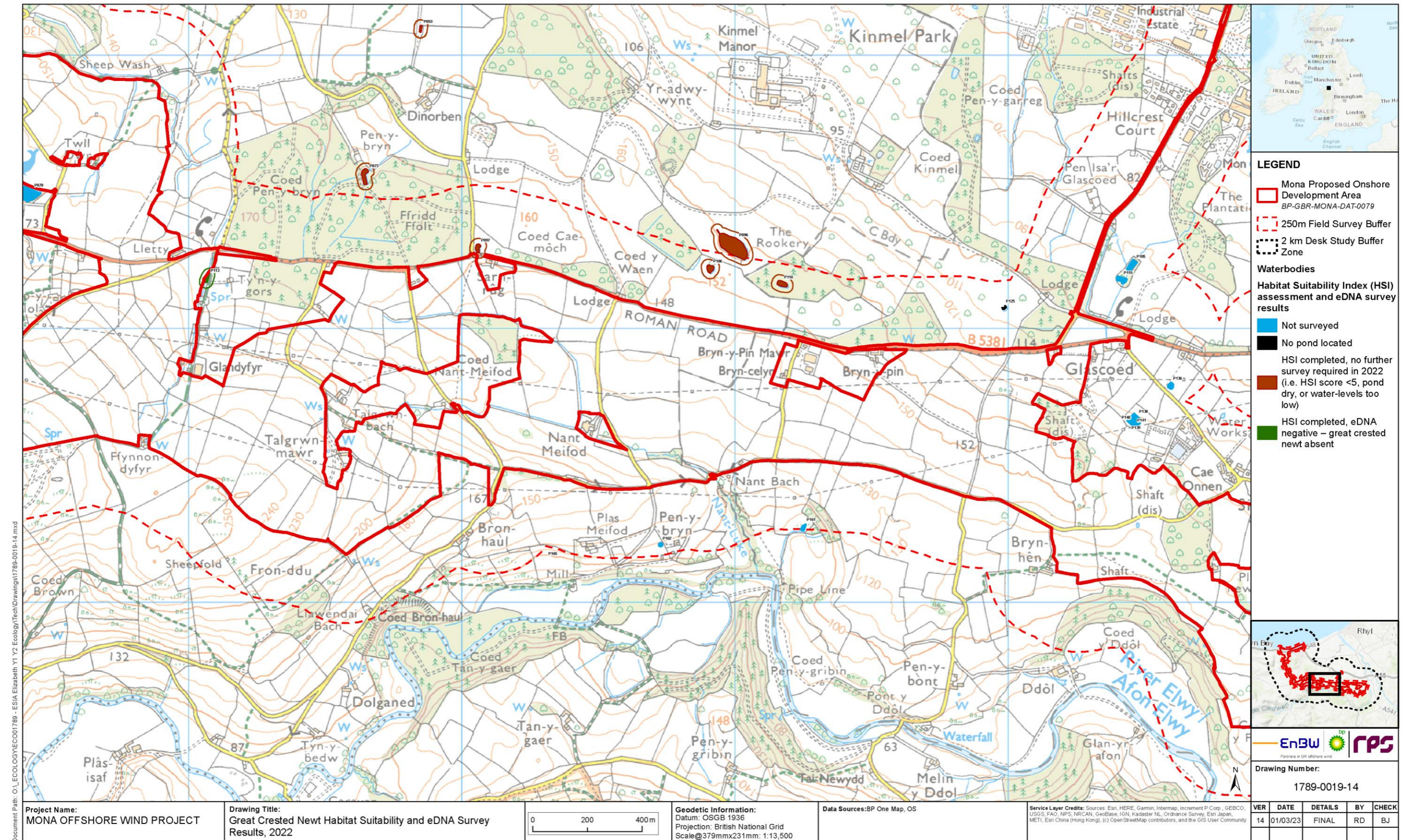


Figure 1.9: Great crested newt field survey locations and results.

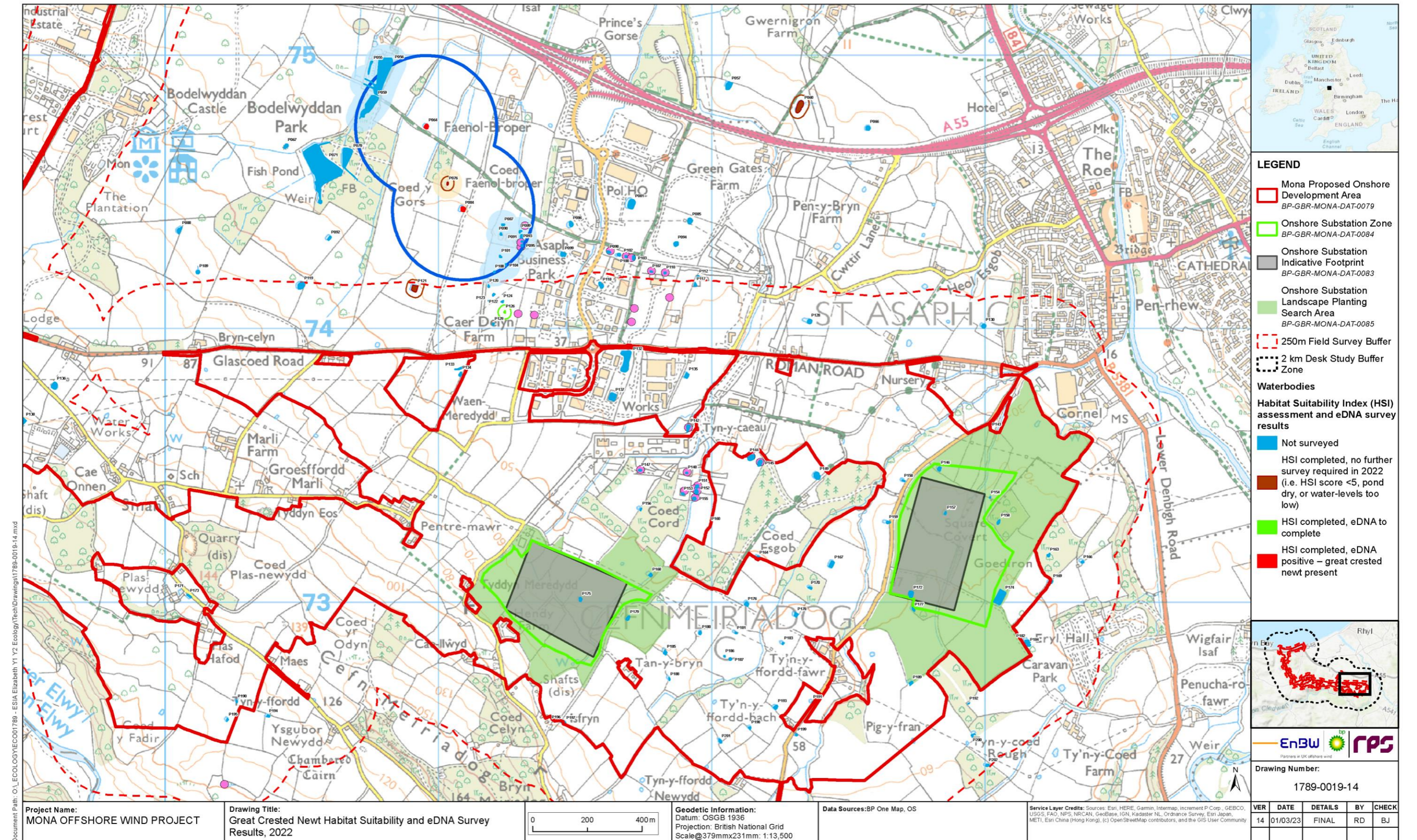


Figure 1.10: Great crested newt field survey locations and results.

1.7 Summary and conclusion

- 1.7.1.1 The presence of great crested newt within and surrounding the Mona Proposed Onshore Development Area is well known. A considerable number of historic records of great crested newt were confirmed by the desk study, primarily associated with the east end of the Mona Proposed Onshore Development Area, around the St Asaph Business Park to the north of the Bodelwyddan National Grid Station and to the east of the National Grid site, associated with the Gwent Y Mor and Burbo Bank sub-stations (as shown on Figure 1.2 to Figure 1.5).
- 1.7.1.2 The results of the eDNA analysis confirmed the presence of great crested newt in an additional two ponds located close to those highlighted in the desk study (i.e. P064 and P084), as well as two ponds in the north half of the Mona Proposed Onshore Development Area, where the desk study reported no historic records of great crested newt (i.e. ponds P021 near Gopa Wood, and P111 to the north of the B5381, shown on Figure 1.6 to Figure 1.10). Only P111 is located within 250m of the Mona Proposed Onshore Development Area.
- 1.7.1.3 Due to restrictions on land access, it was not possible to complete an HSI assessment and eDNA analysis of those waterbodies within the survey area (i.e. those highlighted as 'not surveyed' on Figure 1.6 to Figure 1.10) before the end of the survey season (i.e. before the end of June 2022). However, considering the results of the desk study and field surveys, the potential for great crested newt to be present within the Mona Proposed Onshore Development Area or within the field survey buffer zone, cannot be discounted, and further surveys will be undertaken during 2023 to inform the Environmental Statement (ES) and the mitigation measures required.
- 1.7.1.4 For the purpose of the PEIR, a precautionary approach of assumed presence will be taken for any waterbodies within the field survey buffer zone that were not confirmed to be dry or absent, and for which negative eDNA results were not obtained in 2022.

Further surveys

- 1.7.1.5 HSI, eDNA, standard presence/absence and/or population class size surveys of all un-surveyed waterbodies will be undertaken in 2023, as detailed in the Great Crested Newt Survey Methodology report (bp/EnBW, 2022), and as requested by NRW in their responses to consultation, summarised in Table 1.1. Regarding waterbodies surveyed in 2022, the following is proposed:
- Population class size surveys of waterbody P111, which were confirmed to contain great crested newt eDNA, should be undertaken as described in English Nature 2001.
 - Considering that no desk study data has confirmed the presence of great crested newts in the area associated with P021, a precautionary class size survey of this waterbody will be undertaken. This is to provide further information regarding newt activity in the area. Newt presence is well documented for the areas surrounding P064 and P084; therefore, no precautionary class size surveys of these waterbodies are considered necessary.
 - In recognition that water levels can change significantly over time, waterbodies that were recorded in 2022 to be dry (excluding those confirmed to be no

longer present), or to contain insufficient water to enable a water sample to be collected without significant silt contamination (which would prevent eDNA analysis), should be reassessed in 2023, in case water-levels change sufficiently to enable eDNA analysis or a standard presence/absence survey to be completed. If presence of great crested newt is subsequently confirmed, a population class size survey should be undertaken.

- No further surveys are proposed for waterbodies located beyond the field survey buffer.

1.8 References

- Amphibian and Reptile Groups of the United Kingdom (2010). ARG UK Advice Note 5: Great Crested Newt Habitat Suitability Index.
- bp/EnBW (2022) Mona Offshore Wind Project. Great Crested Newt Survey Methodology.
- Biggs, J., Ewald, N., Valentini, A., Gaboriaud, C., Griffiths, R.A., Foster, J., Wilkinson, J., Arnett, A., Williams, P., Dunn, F. (2014) Analytical and methodological development for improved surveillance of the Great Crested Newt. Defra Project WC1067. Freshwater Habitats Trust: Oxford.
- Cresswell, W. and Whitworth, R. (2004) An assessment of the efficiency of capture techniques and the value of different habitats for the great crested newt *Triturus cristatus*. English Nature Research Reports 576.
- English Nature (2001) Great Crested Newt Mitigation Guidelines. English Nature: Peterborough.
- Oldham R.S., Keeble J., Swan M.J.S. & Jeffcote M. (2000). Evaluating the suitability of habitat for the Great Crested Newt (*Triturus cristatus*). Herpetological Journal 10(4), 143-155.

Appendix A: Historic Records of Great Crested Newt located within 2km

Grid Reference	Easting	Northing	Precision	Site Name	Date
SJ01197401	301195	374015	10	St Asaph Business Park	8 records, between 17/05/2005 and 31/03/2009
SJ01187409	301185	374095	10	St Asaph Business Park	9 records, between 17/05/2005 and 31/03/2009
SJ01207420	301205	374205	10	St Asaph Business Park	5 records, between 10/04/2006 and 31/03/2009
SJ01307433	301305	374335	10	St Asaph Business Park	9 records, between 10/04/2006 and 31/03/2009
SJ01187415	301185	374155	10	St Asaph Business Park	9 records, between 17/05/2005 and 31/03/2009
SJ01247405	301245	374055	10	St Asaph Business Park	9 records, between 17/05/2005 and 31/03/2009
SJ01687388	301685	373885	10	St Asaph Business Park	27 records, between 2002 and 25/04/2008
SJ012742	301250	374250	100	St Asaph Business Park	7 records, between 20/09/2004 and 25/04/2008
SH96467524	296465	375245	10	St Georges/Abergele Quarry	5 records, between 06/04/2004 and 11/04/2006
SJ012743	301250	374350	100	St Asaph Business Park	28/04/2005
SJ01297435	301295	374355	10	St Asaph Business Park	4 records, between 10/04/2008 and 31/03/2009
SJ012741	301250	374150	100	St Asaph Business Park	31/03/2009
SJ01227426	301225	374265	10	St Asaph Business Park	2 records, between 14/04/2007 and 31/03/2009
SJ01227434	301225	374345	10	St Asaph Business Park	3 records, between 13/04/2007 and 31/03/2009
SJ01637374	301635	373745	10	St Asaph Business Park	7 records, between 27/03/2006 and 25/04/2008
SJ01207412	301205	374125	10	St Asaph Business Park	8 records, between 30/03/2007 and 31/03/2009
SJ01237437	301235	374375	10	St Asaph Business Park	5 records, between 13/04/2007 and 25/04/2008
SJ01247422	301245	374225	10	St Asaph Business Park	3 records, between 13/04/2007 and 25/04/2008
SJ01237409	301235	374095	10	St Asaph Business Park	3 records, between 13/04/2007 and 31/03/2009
SJ01307437	301305	374375	10	St Asaph Business Park	3 records, between 10/04/2008 and 25/04/2008
SJ01277429	301275	374295	10	St Asaph Business Park	10/04/2008 to 12/04/2008
SJ00907414	300905	374145	10	St Asaph Business Park	08/08/2006
SJ01527386	301525	373865	10	St Asaph Business Park	08/08/2006
SJ01907382	301905	373825	10	St Asaph Business Park	April 2008 to May 2008
SJ01697378	301695	373785	10	St Asaph Business Park	08/08/2006
SJ018738	301850	373850	100	St Asaph Business Park	2 records, between 29/03/2002 and 26/01/2012
SJ01607373	301605	373735	10	St Asaph Business Park	19/08/1999 to 20/08/1999
SJ01657391	301655	373915	10	St Asaph Business Park	19/08/1999 to 20/08/1999
SH98707626	298705	376265	10	Faenol Bach	2 records, between 1993 and 2004
SH98837670	298835	376705	10	Faenol Bach	2 records, between 1993 and 2004
SH99097643	299095	376435	10	Faenol Bach	3 records, between 1993 and 2004

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Grid Reference	Easting	Northing	Precision	Site Name	Date
SH99007710	299005	377105	10	Faenol Bach	2 records, between 1993 and 2004
SJ01607370	301605	373705	10	Thales	Before 2004
SJ02307296	302305	372965	10	Ty'n-y-ffordd-fawr	3 records, between 1993 and 2004
SJ02737302	302735	373025	10	Ty'n-y-ffordd-fawr	2 records, between 11/06/1993 and 2004
SH96647455	296645	374555	10	Pen-y-bryn	3 records, between 1993 and 2004
SH98707627	298705	376275	10	Faenol Bach	03/06/1993
SH98837668	298835	376685	10	Faenol Bach	03/06/1993
SH9900077100	299000	377100	1	Faenol Bach	03/06/1993
SJ02727302	302725	373025	10	Ty'n-y-ffordd-fawr	1993
SH9646275253	296462	375253	1	Pwll Fardre	13/05/2001
SJ0125574337	301255	374337	1	St. Asaph Business Park	Before 23/03/2007
SJ0168473884	301684	373884	1	St. Asaph Business Park	Before 23/03/2007
SJ016737	301650	373750	100	St. Asaph Business Park	Before 23/03/2007
SJ0169173786	301691	373786	1	St. Asaph Business Park	Before 23/03/2007
SJ0119974017	301199	374017	1	St Asaph Business Park - Glas Coed: SA001	19 records, between 17/05/2005 and 16/04/2021
SJ0124274062	301242	374062	1	St Asaph Business Park - Glas Coed: SA002	17 records, between 17/05/2005 and 09/05/2018
SJ0123474099	301234	374099	1	St Asaph Business Park - Glas Coed: SA003	22 records, between 15/05/2006 and 09/04/2021
SJ0118874099	301188	374099	1	St Asaph Business Park - Glas Coed: SA004	27 records, between 28/04/2005 and 14/05/2021
SJ0120174125	301201	374125	1	St Asaph Business Park - Glas Coed: SA005	25 records, between 30/03/2007 and 14/05/2021
SJ0118274156	301182	374156	1	St Asaph Business Park - Glas Coed: SA006	15 records, between 17/05/2005 and 24/04/2019
SJ0120374208	301203	374208	1	St Asaph Business Park - Glas Coed: SA007	25 records, between 10/04/2006 and 14/05/2021
SJ0124674232	301246	374232	1	St Asaph Business Park - Glas Coed: SA008	12 records, between 13/04/2007 and 08/05/2019
SJ0122274266	301222	374266	1	St Asaph Business Park - Glas Coed: SA009	25 records, between 13/04/2007 and 14/05/2021
SJ0122774346	301227	374346	1	St Asaph Business Park - Glas Coed: SA011	16 records, between 15/05/2006 and 24/04/2019
SJ0123674381	301236	374381	1	St Asaph Business Park - Glas Coed: SA012	21 records, between 13/04/2007 and 14/05/2021
SJ0130474371	301304	374371	1	St Asaph Business Park - Glas Coed: SA014	11 records, between 08/04/2008 and 08/05/2019
SJ0130074355	301300	374355	1	St Asaph Business Park - Glas Coed: SA013	10 records, between 08/04/2008 and 11/05/2016
SJ0130074300	301300	374300	1	St Asaph Business Park - Glas Coed: SA015	4 records, between 09/04/2015 and 11/05/2016
SJ0130074316	301300	374316	1	St Asaph Business Park - Glas Coed: SA016	4 records, between 01/04/2011 and 11/05/2016
SJ0163673750	301636	373750	1	St Asaph Business Park - WG Land Holdings: P01	52 records, between 19/08/1999 and 13/05/2021
SJ0173873484	301738	373484	1	St Asaph Business Park - Bodelwyddan Substation: P1	7 records, between 10/05/2015 and 15/05/2019
SJ0190973475	301909	373475	1	St Asaph Business Park - Bodelwyddan Substation: P3	7 records, between 07/05/2015 and 15/05/2019
SJ0191373639	301913	373639	1	St Asaph Business Park - Bodelwyddan Substation: P4	6 records, between 07/05/2015 and 13/05/2016

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Grid Reference	Easting	Northing	Precision	Site Name	Date
SJ0130674338	301306	374338	1	St Asaph Business Park - Glas Coed: SA010	15 records, between 10/04/2006 and 24/04/2019
SJ0168673883	301686	373883	1	St Asaph Business Park - WG Land Holdings: P02	37 records, between 19/08/1999 and 15/04/2009
SJ0103374531	301033	374531	1	St Asaph Business Park - WG Land Holdings: P13	6 records, between 29/03/2009 and 16/04/2009
SJ0170673790	301706	373790	1	St Asaph Business Park - WG Land Holdings: P08	2 records, between 02/05/2002 and 15/04/2009
SJ0108974438	301089	374438	1	St Asaph Business Park - WG Land Holdings: P12	2 records, between 15/04/2009 and 16/04/2009
SJ0151373873	301513	373873	1	St Asaph Business Park - WG Land Holdings: P14	2 records, between 15/04/2009 and 16/04/2009
SJ0214373533	302143	373533	1	St Asaph Business Park - Bodelwyddan Substation: Pond BB8	7 records, between 19/06/2018 and 14/05/2021
SJ0190073408	301900	373408	1	St Asaph Business Park - Bodelwyddan Substation: P2	5 records, between 23/05/2018 and 14/05/2020
SH99537665	299535	376655	10	Gwynt y Mor Offshore Wind Farm	2008
SH99957627	299955	376275	10	Gwynt y Mor Offshore Wind Farm	2008
SJ00907633	300905	376335	10	Gwynt y Mor Offshore Wind Farm	29/07/2009
SJ02347402	302345	374025	10	Gwynt y Mor Offshore Wind Farm	2008
SH98997709	298995	377095	10	Gwynt y Mor Offshore Wind Farm	2 records, between 13/05/2009 and 29/05/2009
SJ0194873426	301948	373426	1	St Asaph Business Park - Bodelwyddan Substation: Pond BB7	5 records, between 24/04/2019 and 14/05/2020
SJ01937341	301935	373415	10	St Asaph, Bodelwyddan substation	12/08/2019
SJ013743	301350	374350	100		2 records, between 12/10/2007 and 14/10/2007
SH965753	296550	375350	100		2 records, between 10/04/2005 and 10/04/2006
SJ0193873383	301938	373383	1	St Asaph Business Park - Bodelwyddan Substation: Pond BB5	4 records, between 16/04/2020 and 14/05/2021
SJ0194573407	301945	373407	1	St Asaph Business Park - Bodelwyddan Substation: Pond BB6	16/04/2020
SJ0217673512	302176	373512	1	St Asaph Business Park - Bodelwyddan Substation: Pond BB9	2 records, between 14/05/2020 and 06/05/2021
SJ0129074055	301290	374055	1	Glascoed	23/09/2020
SJ0131574377	301315	374377	1	Glascoed	03/11/2020
SJ015739	301550	373950	100		3 records, all on 03/05/2002
SJ013740	301350	374050	100	Glascoed	7 records, all on 15/04/2016
SJ014746	301450	374650	100	St Asaph Business Park	4 records, between 04/03/2002 and 10/04/2002
SJ014745	301450	374550	100	St Asaph Business Park	28/08/1999
SH97907422	297905	374225	10	Plas Kinmel	3 records, between 1993 and 2004
SJ014743	301450	374350	100		22/03/2004 to 14/05/2004
SJ01707402	301705	374025	10	St Asaph Business Park - K&C: Swale 1	03/05/2017
SJ01457427	301455	374275	10	St Asaph Business Park	8 records, between 1993 and 15/05/2006
SJ0145674272	301456	374272	1	St Asaph Business Park - WG Land Holdings: P07	10 records, between 09/06/1993 and 13/05/2021
SJ006743	300650	374350	100		05/04/2004
SJ016740	301650	374050	100	St Asaph Business Park	23/02/2003 to 05/05/2003

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Grid Reference	Easting	Northing	Precision	Site Name	Date
SJ0148174383	301481	374383	1	Police Site Pond	Before 23/03/2007
SJ017740	301750	374050	100		2 records, between 12/10/2007 and 14/10/2007
SJ01487438	301485	374385	10	St Asaph Business Park	34 records, between 19/08/1999 and 25/04/2008
SJ0148474381	301484	374381	1	St Asaph Business Park - WG Land Holdings: P04	93 records, between 19/08/1999 and 05/05/2021
SJ015747	301550	374750	100		3 records, between 07/05/2005 and 26/05/2005
SJ01717407	301715	374075	10	St Asaph Business Park - K&C: Swale 2	2 records, between 03/05/2017 and 01/05/2019
SJ01507430	301505	374305	10	Police Site Pond	Before 2004
SJ015745	301550	374550	100	St Asaph Business Park	4 records, between 05/03/2002 and 09/03/2002
SJ01507410	301505	374105	10	Phase V Pond	Before 2004
SJ015744	301550	374450	100	St Asaph Business Park	3 records, between 27/03/2003 and 02/06/2003
SJ01847411	301845	374115	10	St Asaph K&C	20/05/2019
SJ015743	301550	374350	100	St Asaph Business Park	2 records, both on 15/05/2006
SJ017750	301750	375050	100		2 records, between 29/04/2005 and 18/05/2005
SJ0158274136	301582	374136	1	Phase V Pond	Before 23/03/2007
SJ015742	301550	374250	100	St Asaph Business Park - WG Land Holdings: General	2 records, between 21/04/2004 and 19/05/2004
SJ016741	301650	374150	100	St Asaph Business Park	4 records, between 07/03/2002 and 14/05/2004
SJ0159474159	301594	374159	1	St Asaph Business Park - WG Land Holdings: P09	26 records, between 09/06/1993 and 13/05/2021
SJ00217233	300215	372335	10	Near St Asaph	2013
SJ001756	300150	375650	100	Bodelwyddan	26/04/2020
SJ01607420	301605	374205	10	Link Pond	Before 2004
SJ016745	301650	374550	100	St Asaph Business Park	4 records, between 07/03/2002 and 08/03/2002
SJ018750	301850	375050	100		22/03/2004 to 14/05/2004
SJ0162774285	301627	374285	1	St Asaph Business Park - WG Land Holdings: P03c	9 records, between 16/04/2020 and 13/05/2021
SJ01627426	301625	374265	10	St Asaph Business Park	4 records, between 10/04/2008 and 25/04/2008
SJ0162774258	301627	374258	1	St Asaph Business Park - WG Land Holdings: P20	31 records, between 10/04/2008 and 13/05/2021
SJ0182974206	301829	374206	1	St Asaph Business Park - K&C: Pond 2	4 records, between 03/05/2017 and 01/05/2019
SJ0177874212	301778	374212	1	St Asaph Business Park - K&C: Pond 1	4 records, between 03/05/2017 and 01/05/2019
SJ01807490	301805	374905	10	A55 N St Asaph BP	Before 2004
SJ016742	301650	374250	100	St Asaph Business Park	4 records, between 05/03/2002 and 25/04/2002
SJ0165474267	301654	374267	1	Link Pond	Before 23/03/2007
SJ01657426	301655	374265	10	St Asaph Business Park	12 records, between 2002 and 25/04/2008
SJ024744	302450	374450	100		01/09/2004
SJ0165874266	301658	374266	1	St Asaph Business Park - WG Land Holdings: P03	57 records, between 11/04/2002 and 07/05/2020

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Grid Reference	Easting	Northing	Precision	Site Name	Date
SJ01667427	301665	374275	10	St Asaph Business Park	2 records, between 02/05/2001 and 01/08/2003
SJ01847495	301845	374955	10	St Asaph Business Park	16 records, between 2002 and 25/04/2008
SJ0186774999	301867	374999	1	A55 N St Asaph BP	Before 23/03/2007
SJ01857497	301855	374975	10	St Asaph Business Park	10 records, between 02/05/2001 and 27/07/2004
SJ0185674960	301856	374960	1	St Asaph Business Park - WG Land Holdings: P05	37 records, between 15/04/2002 and 28/04/2011
SJ0170574260	301705	374260	1	St Asaph Business Park - WG Land Holdings: P03b	7 records, between 27/04/2016 and 13/05/2021
SJ0185774960	301857	374960	1	Gwynt y Mor Offshore Wind Farm	2008
SJ0170374456	301703	374456	1	St Asaph Business Park - WG Land Holdings: P06	38 records, between 12/04/2002 and 31/05/2013
SJ01707446	301705	374465	10	St Asaph Business Park	22 records, between 2002 and 25/04/2008
SJ0170774460	301707	374460	1	Optic Pond	Before 23/03/2007
SJ0168674265	301686	374265	1	St Asaph Business Park - WG Land Holdings: P03a	13 records, between 08/05/2011 and 13/05/2021
SJ01707440	301705	374405	10	Optic Pond	Before 2004
SJ017746	301750	374650	100	St Asaph Business Park	4 records, between 06/03/2002 and 25/04/2002
SJ018748	301850	374850	100	Bodelwyddan, Faenol-Broper, pond	May 2001
SJ017745	301750	374550	100		7 records, between 26/03/2005 and 18/05/2005
SJ01877430	301875	374305	10	Green Gates Farm	2 records, between 1993 and 2004
SJ01867430	301865	374305	10	Gwynt y Mor Offshore Wind Farm	2008
SJ0186874310	301868	374310	1	St Asaph Business Park - WG Land Holdings: P10	17 records, between 09/06/1993 and 24/04/2019
SJ01877431	301875	374315	10	St Asaph Business Park	4 records, between 09/06/1993 and 25/04/2008
SJ01907439	301905	374395	10	Green Gates Farm	3 records, between 1993 and 2004
SJ01917439	301915	374395	10	St Asaph Business Park	2 records, between 15/05/2006 and 08/08/2006
SJ0192074397	301920	374397	1	St Asaph Business Park - WG Land Holdings: P11	6 records, between 09/06/1993 and 09/05/2018
SJ02057488	302055	374885	10	Gwynt y Mor Offshore Wind Farm	2008
SJ02567473	302565	374735	10	Gwynt y Mor Offshore Wind Farm	May 2009
SJ03417433	303415	374335	10	The Roe, St Asaph	2 records, between 1993 and 2004
SJ02327482	302325	374825	10	St. Asaph	3 records, between 2008 and 09/05/2008
SJ0379674687	303796	374687	1	The Roe, St. Asaph	Before 23/03/2007
SJ0421872843	304218	372843	1	Bryn-polyn Nurseries	Before 23/03/2007
SH98237856	298235	378565	10	Gwynt y Mor Offshore Wind Farm	2008
SJ041744	304150	374450	100		4 records, between 16/03/2004 and 15/05/2004
SJ04117458	304115	374585	10	St. Asaph	4 records, between 14/04/2003 and 21/05/2003
SJ0411274598	304112	374598	1	Bron Wylfa: Pond 1	8 records, between 05/05/2017 and 14/05/2019
SJ0412774583	304127	374583	1	Bron Wylfa: Pond 2	5 records, between 05/05/2017 and 26/04/2018

MONA OFFSHORE WIND PROJECT

Grid Reference	Easting	Northing	Precision	Site Name	Date
SJ041745	304150	374550	100	Bron Wylfa: General	3 records, between 14/04/2003 and 30/04/2013
SJ0435572432	304355	372432	1	Gwynant	Before 23/03/2007
SJ0437472427	304374	372427	1	Gwynant	Before 23/03/2007
SJ041746	304150	374650	100		2 records, between 14/04/2003 and 21/05/2003
SJ04517313	304515	373135	10	Bryn-Polyn-Mawr	10/06/1993
SJ04517314	304515	373145	10	Bryn-Polyn-Mawr	2 records, between 1993 and 2004
SJ04597294	304595	372945	10	Bryn-Polyn-Mawr	2 records, between 1993 and 2004
SJ04627294	304625	372945	10	Bryn-Polyn-Mawr	10/06/1993
SJ03217700	303215	377005	10	Criccin Farm	Before 2004
SJ03227700	303225	377005	10	Criccin Farm	2 records, between 1993 and 28/05/1993
SH999787	299950	378750	100	Tyddyn Gwynfa, Kimnel Bay	2 records, between ~ 2002 and ~ 2010
SJ03337715	303335	377155	10	Criccin Farm	Before 2004
SJ03307722	303305	377225	10	Criccin Farm	3 records, between 1993 and 2004
SJ03357718	303355	377185	10	Criccin Farm	3 records, between 1993 and 2004
SH99907077	299905	370775	10	Near Tal y Bryn	2013
SH99917072	299915	370725	10	Near Tal y Bryn	2013
SH999706	299950	370650	100	Cefn Berain	2008
SJ03747674	303745	376745	10	Criccin Farm	2 records, between 1993 and 2004
SJ03757673	303755	376735	10	Criccin Farm	27/05/1993
SJ03607716	303605	377165	10	Criccin Farm	27/05/1993
SJ03607717	303605	377175	10	Criccin Farm	2 records, between 1993 and 2004

Appendix B: HSI Results

Date of Survey	Pond location (eastings and northings)	Description of Waterbody	Pond ref.	Zone	Pond Area	Permanence	Water Quality	Shade	Waterfowl	Fish	No. of Ponds within 1km	Terrestrial Habitat	Macrophytes	Product	HSI score	Suitability for great crested newt
26/04/2022	297314, 373155	Dry pond.	P009	Zone A		Dries annually	N/A	96-100%	N/A	N/A	1	Good	N/A			
		HSI score		1		0.1		0.2			0.45	1		0.0000	N/A	Dry pond
18/05/2022	293315 376282	Large kidney bean shaped pond with small, vegetated island on one lobe, marginal macrophyte cover including flag iris, duckweed, and broad leaved pondweed.	P021	Zone A	>2000 m ²	Never dries	Moderate	0-60%	Minor	Possible	3	Moderate	6-10%			
		HSI score		1	0.45	0.9	0.67	1	0.67	0.67	0.65	0.67	0.4	0.0377	0.72	Good
14/06/2022	294013 375554	Medium sized pond located between an area of dense scrub and pasture. Appears to be frequently used by waterfowl and cattle.	P039	Zone A	150m ²	Never dries	Moderate	0-60%	Major	Minor	8	Poor	6-10%			
		HSI score		1	0.3	0.9	0.67	1	0.01	0.33	0.89	0.33	0.4	0.0001	0.38	Poor
14/06/2022	293723 375339	Medium sized pond, based at the bottom of the a sharp incline. Pond margins are heavily vegetated; however, no floating or submerged or emergent vegetation recorded.	P042	Zone A	100m ²	Never dries	Moderate	0-60%	Minor	Minor	8	Good	<1%			
		HSI score		1	0.2	0.9	0.67	1	0.67	0.33	0.89	1	0.3	0.007	0.61	Average
30/06/2022	296463 375247	Area of old earth that could very well have been a pond and may fill in adverse weather, but dry and devoid of water species flora.	P045	Zone A				0-60%			8	None				
		HSI score		1				1			0.89	0.01	0	0.0000	N/A	Dry pond
17/05/2022	292709 375182	Medium sized pond surrounded by steep earth banks, with a small island present on which a single willow tree located in its centre.	P050	Zone A	<50m ²	Rarely Dries	Good	0-60%	Minor	Possible	6	Good	1-5%			
		HSI score		1	0.05	1	1	1	0.67	0.67	0.84	1	0.35	0.0066	0.61	Average
14/06/2022	293833 375121	Large pond located at the edge of small woodland. Waterfowl and fish present.	P051	Zone A	300m ²	Never dries	Good	0-60%	Major	Major	8	Good	<1%			
		HSI score		1	0.6	0.9	1	1	0.01	0.01	0.89	1	0.3	0.0000	0.33	Poor

MONA OFFSHORE WIND PROJECT

Date of Survey	Pond location (eastings and northings)	Description of Waterbody	Pond ref.	Zone	Pond Area	Permanence	Water Quality	Shade	Waterfowl	Fish	No. of Ponds within 1km	Terrestrial Habitat	Macrophytes	Product	HSI score	Suitability for great crested newt
14/06/2022	293887 375122	Large pond located in a small area of deciduous woodland, waterfowl and fish present.	P052	Zone A	250m ²	Never dries	Good	0-60%	Major	Major	8	Good	<1%			
		HSI score		1	0.5	0.9	1	1	0.01	0.01	0.89	1	0.3	0.0000	0.32	Poor
30/06/2022	296846 375095	Small ponds situated on the margin of a field, low levels of water at time of the survey.	P053	Zone A	<50m ²	Sometimes Dries	Poor	0-60%	Minor	Possible	5	Poor	<1%			
		HSI score		1	0.05	0.5	0.33	1	0.67	0.67	0.75	0.33	0.3	0.0003	0.44	Poor
14/06/2022	293914 375084	Large pond in small area of woodland, waterfowl and fish present.	P054	Zone A	150m ²	Never dries	Good	0-60%	Major	Major	8	Good	<1%			
		HSI score		1	0.3	0.9	1	1	0.01	0.01	0.89	1	0.3	0.0000	0.31	Poor
03/05/2022	293714 374711	Temporary attenuation pond, to ease flooding from run-off during high rainfall. Slow-flowing input from ditch and output into ditch. Water-level very low during the survey.	P063	Zone A	100m ²	Dries annually	Moderate	0-60%	Minor	Absent	2	Poor	46-50%			
		HSI score		1	0.2	0.1	0.67	1	0.67	1	0.6	0.33	0.8	0.0014	0.52	Below Average
27/06/2022	300956 374734	Medium sized pond in pasture field. Evidence of poaching.	P064	Zone A	100m ²	Never dries	Poor	0-60%	Minor	Possible	>12	Moderate	1-5%			
		HSI score		1	0.2	0.9	0.33	1	0.67	0.67	1	0.67	0.35	0.0063	0.60	Average
03/05/2022	293545 374631	Very small defunct well, along field boundary hedgerow. Stagnant with near 100% macrophyte cover.	P068	Zone A	<50m ²	Rarely Dries	Bad	96-100%	Absent	Absent	2	None	96-100%			

MONA OFFSHORE WIND PROJECT

Date of Survey	Pond location (eastings and northings)	Description of Waterbody	Pond ref.	Zone	Pond Area	Permanence	Water Quality	Shade	Waterfowl	Fish	No. of Ponds within 1km	Terrestrial Habitat	Macrophytes	Product	HSI score	Suitability for great crested newt
		HSI score		1	0.05	1	0.01	0.2	1	1	0.6	0.01	0.8	0.0000	0.23	Poor
30/06/2022	296653 374542	Dried out pond, still damp but thick muddy base, lots of shading from bankside vegetation. Good surrounding terrestrial habitat.	P073	Zone A		Dries annually		90			6	Moderate				
		HSI score		1		0.1					0.84	0.67			N/A	Dry pond
27/06/2022	301034 374532	Standing water in woodland, no aquatic vegetation.	P076	Zone A	<50m ²	Never dries	Poor	96-100%	Absent	Absent	>12	Good	<1%			
		HSI score		1	0.05	0.9	0.33	0.2	1	1	1	1	0.3	0.0009	0.50	Poor
27/06/2022	301094 374444	Medium sized pond in sheep grazed field. Appears man-made. Contains internal ledge. Water-level very low at the time of the survey, so that only the deep centre was holding water.	P084	Zone A	<50m ²	Never dries	Poor	0-60%	Absent	Possible	>12	Poor	66-80%			
		HSI score		1	0.05	0.9	0.33	1	1	0.67	1	0.33	1	0.0033	0.56	Below Average
30/06/2022	297949 374349	Small reservoir used to provide water to the main estate's house, now contains fish and waterfowl recorded during the survey visit.	P096	Zone A	350m ²	Never dries	Good	0-60%	Major	Major	5	Moderate	5			
		HSI score		1	0.7	0.9	1	1	0.01	0.01	0.75	0.67	0	0.0000	0	Poor
30/06/2022	297073 374312	Dried out pond in Logging area. Lots of overgrown overgrowth and grass growing, not held water in a long time.	P097	Zone A				5			6	Poor				
		HSI score		1							0.84	0.33			N/A	Dry pond
30/06/2022	297912 374204	Medium sized pond surrounded by trees, located within a large pasture field.	P106	Zone A	<50m ²	Never dries	Good	86-90%	Major	Minor	8	Moderate	<1%			
		HSI score		1	0.05	0.9	1	0.4	0.01	0.33	0.89	0.67	0.3	0.0000	0.32	Poor
31/05/2022	293510, 374192	Medium, shallow sided pond, with a marshy area surrounding and some scrub/mature willow trees in the immediate area. Short cut grass in the adjacent fields with 4 hedge lines creating some habitat connectivity.	P111	Zone A	100m ²	Never dries	Good	0-60%	Minor	Minor	5	Moderate	36-40%			

MONA OFFSHORE WIND PROJECT

Date of Survey	Pond location (eastings and northings)	Description of Waterbody	Pond ref.	Zone	Pond Area	Permanence	Water Quality	Shade	Waterfowl	Fish	No. of Ponds within 1km	Terrestrial Habitat	Macrophytes	Product	HSI score	Suitability for great crested newt
		HSI score		1	0.2	0.9	1	1	0.67	0.33	0.75	0.67	0.7	0.014	0.65	Average
27/04/2022	296059, 374167	Long oval pond approx. 6m x 25m = 150m ²	P113	Zone A	150m ²	Never dries	Good	0-60%	Absent	Possible	7	Moderate	36-40%			
		HSI score		1	0.3	0.9	1	1	1	0.67	0.85	0.67	0.7	0.072	0.77	Good
20/04/2022	295193 374179	Pond on hillside, with overflow pipe. Very little aquatic vegetation and standing water during the survey visit.	P114	Zone A	<50m ²	Dries annually	Bad	0-60%	Absent	Absent	>12	Poor	81-85%			
		HSI score		1	0.05	0.1	0.01	1	1	1	1	0.33	0.95	0.0000	0.33	Poor
30/06/2022	298097 374183	Dry ground in area of felled woodland. Overgrown with scrub and grasses.	P116	Zone A							6					
		HSI score		1							0.84				N/A	Dry pond
27/06/2022	300921 374143	Shallow pond in large field, surrounded by trees. Very little aquatic vegetation. Potential impact by farm pollutants.	P121	Zone A	100m ²	Rarely dries	Poor	96-100%	Minor	Possible	>12	Poor	1-5%			
		HSI score		1	0.2	1	0.33	0.2	0.67	0.67	1	0.33	0.35	0.0007	0.48	Poor
30/06/2022	299202 374150	Pond no longer present, areas of slightly damper ground only.	P125													
		HSI score													N/A	Pond absent
30/05/2022	302374 374022	Two ponds on map merged into one. Large hawthorn trees surrounding.	P127	Zone A	100m ²	Never dries	Moderate	96-100%	Minor	Possible	7	Poor	21-25%			
		HSI score		1	0.2	0.9	0.67	0.2	0.67	0.67	0.85	0.33	0.55	0.0017	0.53	Below Average
30/06/2022	295338 373903	Dry sandy area of field - no pond present.	P131													
		HSI score													N/A	Pond absent
21/04/2022	295925 3764	Small deep pond at edge of field. Water surface covered in duckweed. Bankside willow and bramble overgrowth.	P210	Zone A	<50m ²	Never dries	Good	0-60%	Absent	Possible	5	Moderate	96-100%			
		HSI score		1	0.05	0.9	1	1	1	0.67	0.05	0.67	0.8	0.0008	0.49	Poor
24/05/2022	295198 374657	Small rectangular water body just off field margin. Pond is tightly fenced to pond edge	P211	Zone A	<50m ²	Rarely dries	Moderate	0-60%	Minor	Possible	>12	Moderate	91-95%			

MONA OFFSHORE WIND PROJECT

Date of Survey	Pond location (eastings and northings)	Description of Waterbody	Pond ref.	Zone	Pond Area	Permanence	Water Quality	Shade	Waterfowl	Fish	No. of Ponds within 1km	Terrestrial Habitat	Macrophytes	Product	HSI score	Suitability for great crested newt
		with minor pond edge fauna (mostly dogwood, and bramble). Pond fauna is dominated by Typha, which is dominating the water surface area.														
		HSI score		1	0.05	1	0.67	1	0.67	0.67	1	0.67	0.85	0.0086	0.62	Average

Appendix C: Laboratory eDNA Analysis Results

Client: Rebecca Sambrook,
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Sample ID: ADAS-4445 Condition on Receipt: Good Volume: Passed
Client Identifier: P21, BP Mona Description: pond water samples in preservative
Date of Receipt: 24/05/2022 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control ¹	2 of 2	Real Time PCR	24/05/2022
Degradation Control ⁵	Evidence of degradation	Real Time PCR	24/05/2022
Great Crested Newt*	7 of 12 (GCN positive)	Real Time PCR	24/05/2022
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) ⁶	4 of 4	Real Time PCR	As above for GCN

Report Prepared by: Dr Helen Rees Report Issued by: Dr Ben Maddison

Signed:  Signed: 

Position: Director: Biotechnology Position: MD: Biotechnology

Date of preparation: 26/05/2022 Date of issue: 26/05/2022

eDNA analysis was carried out in accordance with the stipulated methodology found in the Technical Advice Note (WC1067 Appendix 5 Technical Advice Note) published by DEFRA and adopted by Natural England.

** If all PCR controls and extraction blanks give the expected results a sample is considered: negative for great crested newt if all of the replicates are negative; positive for great crested newt if one or more of the replicates are positive.*

¹ Recorded as the number of positive replicate reactions at expected C_t value. If the expected C_t value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.

⁵ No degradation is expected within time frame of kit preparation, sample collection and analysis.

⁶ Additional positive controls (10⁻¹, 10⁻², 10⁻³ ng/μL) are also routinely run, results not shown here.

Client: Rebecca Sambrook,
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Sample ID: ADAS-4556 Condition on Receipt: High Sediment Volume: Passed
Client Identifier: P42, MONA Description: pond water samples in preservative
Date of Receipt: 16/06/2022 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control ¹	2 of 2	Real Time PCR	21/06/2022
Degradation Control ⁵	Evidence of degradation	Real Time PCR	21/06/2022
Great Crested Newt*	Indeterminate	Real Time PCR	21/06/2022
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) ⁶	4 of 4	Real Time PCR	As above for GCN

Report Prepared by: Dr Helen Rees Report Issued by: Dr Ben Maddison

Signed:  Signed: 

Position: Director: Biotechnology Position: MD: Biotechnology

Date of preparation: 22/06/2022 Date of issue: 22/06/2022

eDNA analysis was carried out in accordance with the stipulated methodology found in the Technical Advice Note (WC1067 Appendix 5 Technical Advice Note) published by DEFRA and adopted by Natural England.

** If all PCR controls and extraction blanks give the expected results a sample is considered: negative for great crested newt if all of the replicates are negative; positive for great crested newt if one or more of the replicates are positive.*

¹ Recorded as the number of positive replicate reactions at expected C_t value. If the expected C_t value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.

⁵ No degradation is expected within time frame of kit preparation, sample collection and analysis.

⁶ Additional positive controls (10⁻¹, 10⁻², 10⁻³ ng/μL) are also routinely run, results not shown here.

Client: Rebecca Sambrook,
Sambrook Associates


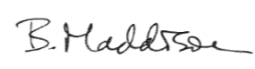


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Sample ID: ADAS-4443 Condition on Receipt: Good Volume: Passed
Client Identifier: P50 Mona Description: pond water samples in preservative
Date of Receipt: 24/05/2022 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	24/05/2022
Degradation Control [§]	Within Limits	Real Time PCR	24/05/2022
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	24/05/2022
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by: Dr Helen Rees Report Issued by: Dr Ben Maddison

Signed:  Signed: 

Position: Director: Biotechnology Position: MD: Biotechnology

Date of preparation: 26/05/2022 Date of issue: 26/05/2022

eDNA analysis was carried out in accordance with the stipulated methodology found in the Technical Advice Note (WC1067 Appendix 5 Technical Advice Note) published by DEFRA and adopted by Natural England.

** If all PCR controls and extraction blanks give the expected results a sample is considered: negative for great crested newt if all of the replicates are negative; positive for great crested newt if one or more of the replicates are positive.*

[†] Recorded as the number of positive replicate reactions at expected C_t value. If the expected C_t value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.

[§] No degradation is expected within time frame of kit preparation, sample collection and analysis.

[#] Additional positive controls (10⁻¹, 10⁻², 10⁻³ ng/μL) are also routinely run, results not shown here.

Client: Mick Smith,
Wildbanks


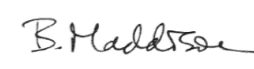


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Sample ID: ADAS-4566 Condition on Receipt: Good Volume: Passed
Client Identifier: 64, Mona 2995 Description: pond water samples in preservative
Date of Receipt: 05/07/2022 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	11/07/2022
Degradation Control [§]	Within Limits	Real Time PCR	11/07/2022
Great Crested Newt*	12 of 12 (GCN positive)	Real Time PCR	11/07/2022
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by: Dr Helen Rees Report Issued by: Dr Ben Maddison

Signed:  Signed: 

Position: Director: Biotechnology Position: MD: Biotechnology

Date of preparation: 12/07/2022 Date of issue: 12/07/2022

eDNA analysis was carried out in accordance with the stipulated methodology found in the Technical Advice Note (WC1067 Appendix 5 Technical Advice Note) published by DEFRA and adopted by Natural England.

** If all PCR controls and extraction blanks give the expected results a sample is considered: negative for great crested newt if all of the replicates are negative; positive for great crested newt if one or more of the replicates are positive.*

[†] Recorded as the number of positive replicate reactions at expected C_t value. If the expected C_t value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.

[§] No degradation is expected within time frame of kit preparation, sample collection and analysis.

[#] Additional positive controls (10⁻¹, 10⁻², 10⁻³ ng/μL) are also routinely run, results not shown here.

Client: Mick Smith,
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Sample ID: ADAS-4561 Condition on Receipt: Good Volume: Passed

Client Identifier: 84, Mona 2987 Description: pond water samples in preservative

Date of Receipt: 05/07/2022 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	11/07/2022
Degradation Control [§]	Within Limits	Real Time PCR	11/07/2022
Great Crested Newt*	12 of 12 (GCN positive)	Real Time PCR	11/07/2022
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by: Dr Helen Rees Report Issued by: Dr Ben Maddison

Signed:  Signed: 

Position: Director: Biotechnology Position: MD: Biotechnology

Date of preparation: 12/07/2022 Date of issue: 12/07/2022

eDNA analysis was carried out in accordance with the stipulated methodology found in the Technical Advice Note (WC1067 Appendix 5 Technical Advice Note) published by DEFRA and adopted by Natural England.

* If all PCR controls and extraction blanks give the expected results a sample is considered: negative for great crested newt if all of the replicates are negative; positive for great crested newt if one or more of the replicates are positive.

[†] Recorded as the number of positive replicate reactions at expected C_t value. If the expected C_t value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.

[§] No degradation is expected within time frame of kit preparation, sample collection and analysis.

[#] Additional positive controls (10⁻¹, 10⁻², 10⁻³ ng/μL) are also routinely run, results not shown here.

Client: Rebecca Sambrook,
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Sample ID: ADAS-4568 Condition on Receipt: Low Sediment Volume: Passed

Client Identifier: P111 (LP2818) MONA Description: pond water samples in preservative

Date of Receipt: 06/06/2022 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	08/06/2022
Degradation Control [§]	Within Limits	Real Time PCR	08/06/2022
Great Crested Newt*	7 of 12 (GCN positive)	Real Time PCR	08/06/2022
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by: Dr Helen Rees Report Issued by: Dr Ben Maddison

Signed:  Signed: 

Position: Director: Biotechnology Position: MD: Biotechnology

Date of preparation: 09/06/2022 Date of issue: 09/06/2022

eDNA analysis was carried out in accordance with the stipulated methodology found in the Technical Advice Note (WC1067 Appendix 5 Technical Advice Note) published by DEFRA and adopted by Natural England.

* If all PCR controls and extraction blanks give the expected results a sample is considered: negative for great crested newt if all of the replicates are negative; positive for great crested newt if one or more of the replicates are positive.

[†] Recorded as the number of positive replicate reactions at expected C_t value. If the expected C_t value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.

[§] No degradation is expected within time frame of kit preparation, sample collection and analysis.

[#] Additional positive controls (10⁻¹, 10⁻², 10⁻³ ng/μL) are also routinely run, results not shown here.

Client: Rebecca Sambrook,
Sambrook Associates/RPS Group


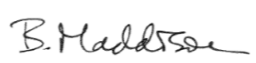


ADAS
Spring Lodge
172 Chester Road
Helsby
WA6 0AR
Tel: 01159 229249
Email: Helen.Rees@adas.co.uk
www.adas.uk

Sample ID: ADAS-4565 Condition on Receipt: Good Volume: Passed
Client Identifier: P113, BP Mona Description: pond water samples in preservative
Date of Receipt: 03/05/2022 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	05/05/2022
Degradation Control [§]	Within Limits	Real Time PCR	05/05/2022
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	05/05/2022
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by: Dr Helen Rees Report Issued by: Dr Ben Maddison

Signed:  Signed: 

Position: Director: Biotechnology Position: MD: Biotechnology

Date of preparation: 10/05/2022 Date of issue: 10/05/2022

eDNA analysis was carried out in accordance with the stipulated methodology found in the Technical Advice Note (WC1067 Appendix 5 Technical Advice Note) published by DEFRA and adopted by Natural England.

* If all PCR controls and extraction blanks give the expected results a sample is considered: negative for great crested newt if all of the replicates are negative; positive for great crested newt if one or more of the replicates are positive.

[†] Recorded as the number of positive replicate reactions at expected C_i value. If the expected C_i value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.

[§] No degradation is expected within time frame of kit preparation, sample collection and analysis.

[#] Additional positive controls (10⁻¹, 10⁻², 10⁻³ ng/μL) are also routinely run, results not shown here.

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Sample ID: ADAS-4563 Condition on Receipt: Low Sediment Volume: Passed
Client Identifier: 127 (LPA65) MONA Description: pond water samples in preservative
Date of Receipt: 06/06/2022 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	07/06/2022
Degradation Control [§]	Within Limits	Real Time PCR	07/06/2022
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	07/06/2022
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by: Dr Helen Rees Report Issued by: Dr Ben Maddison

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Position: Director: Biotechnology Position: MD: Biotechnology

Date of preparation: 09/06/2022 Date of issue: 09/06/2022

eDNA analysis was carried out in accordance with the stipulated methodology found in the Technical Advice Note (WC1067 Appendix 5 Technical Advice Note) published by DEFRA and adopted by Natural England.

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[†] Recorded as the number of positive replicate reactions at expected C_i value. If the expected C_i value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.

[§] No degradation is expected within time frame of kit preparation, sample collection and analysis.

[#] Additional positive controls (10⁻¹, 10⁻², 10⁻³ ng/μL) are also routinely run, results not shown here.

NOTE: ref New – pond P0210

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Sample ID: ADAS-4449 Condition on Receipt: Good Volume: Passed

Client Identifier: new, BP Mona Description: pond water samples in preservative

Date of Receipt: 03/05/2022 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	06/05/2022
Degradation Control [§]	Within Limits	Real Time PCR	06/05/2022
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	06/05/2022
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by: Dr Helen Rees Report Issued by: Dr Ben Maddison

Signed:  Signed: 

Position: Director: Biotechnology Position: MD: Biotechnology

Date of preparation: 10/05/2022 Date of issue: 10/05/2022

eDNA analysis was carried out in accordance with the stipulated methodology found in the Technical Advice Note (WC1067 Appendix 5 Technical Advice Note) published by DEFRA and adopted by Natural England.

* If all PCR controls and extraction blanks give the expected results a sample is considered: negative for great crested newt if all of the replicates are negative; positive for great crested newt if one or more of the replicates are positive.

[†] Recorded as the number of positive replicate reactions at expected C_t value. If the expected C_t value is not achieved, the sample is considered inhibited and is diluted as per the technical advice note prior to amplification with great crested newt primer and probes.

[§] No degradation is expected within time frame of kit preparation, sample collection and analysis.

[#] Additional positive controls (10⁻¹, 10⁻², 10⁻³ ng/μL) are also routinely run, results not shown here.

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Sample ID: ADAS-4440 Condition on Receipt: Low Sediment Volume: Passed

Client Identifier: 211 (new) Description: pond water samples in preservative

Date of Receipt: 06/06/2022 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	07/06/2022
Degradation Control [§]	Within Limits	Real Time PCR	07/06/2022
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	07/06/2022
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [#]	4 of 4	Real Time PCR	As above for GCN

Report Prepared by: Dr Helen Rees Report Issued by: Dr Ben Maddison

Signed:  Signed: 

Position: Director: Biotechnology Position: MD: Biotechnology

Date of preparation: 09/06/2022 Date of issue: 09/06/2022

eDNA analysis was carried out in accordance with the stipulated methodology found in the Technical Advice Note (WC1067 Appendix 5 Technical Advice Note) published by DEFRA and adopted by Natural England.

* If all PCR controls and extraction blanks give the expected results a sample is considered: negative for great crested newt if all of the replicates are negative; positive for great crested newt if one or more of the replicates are positive.

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