Welsh Government

M4 Corridor around Newport

Environmental Statement Volume 3: Appendix 10.22

Great Crested Newt eDNA Survey 2015

M4CaN-DJV-EBD-ZG_GEN-AX-EN-0003

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Summary

S.1 RPS has undertaken a Great Crested Newt Presence/Absence (eDNA Technique) survey of land within the route of the proposed M4 Corridor around Newport (M4CaN) between Castleton and Magor to inform the ecological baseline for environmental impact assessment (EIA) of the Scheme. The EIA is reported in the M4CaN Environmental Statement (ES), of which this document is an appendix to the chapter on Ecology and Nature Conservation. The survey included land generally within 250 metres of the proposed alignment following the methodology set out in the Analytical and Methodological Development for improved Surveillance of the Great Crested Newt (Biggs *et al.*, 2014).

S.2 Previous great crested newt surveys were undertaken along an earlier motorway alignment by Arup in 2014 (Appendix 10.6 of the ES). Initial Habitat Suitability Index (HSI) assessments of those waterbodies within the study area that could be assessed from roads and public rights of way were undertaken by Arup during February 2014.

S.3 The results of the HSI assessments were used to identify waterbodies considered to have average or better than average suitability for great crested newts. These waterbodies were then selected for presence/absence surveys where access allowed. The presence/absence surveys did not identify great crested newt in any of the waterbodies surveyed.

S.4 The South East Wales Biodiversity Records Centre (SEWBReC) provided records of great crested newts within and to the south of the study area: within ponds at Solutia; and in Marshfield (approximately 2 km south of the study area).

S.5 Records of a large population of great crested newts at the eastern end of the new section of motorway were provided by SLR Consulting from surveys undertaken on ponds at Ifton Quarry and within 500 m of the Quarry.

S.6 The previous great crested newt report undertaken by Arup in 2014 states that the surveys only covered a small number of waterbodies within the survey area due to limitations on access at the time. The report recommended that the remaining waterbodies that are considered through HSI assessments to be suitable for great crested newts be surveyed in 2015.

S.7 Due to the large number of waterbodies that were required to be surveyed, it was agreed with Natural Resources Wales (NRW) that the eDNA great crested newt presence/absence technique would be used.

S.8 The great crested newt presence absence (eDNA technique) survey was carried out over the period 18th May to the 30th June 2015, which falls within the optimum period for this type of survey. The survey method was based on that described in Biggs, *et al.* (2014).

S.9 The survey area was based upon the proposed alignment of the new section of motorway, together with an approximate 250 m corridor to either side. A total of seven survey areas were identified from within the survey area which contained the highest concentrations of waterbodies of average or higher than average HSI scores. All waterbodies within each of the survey areas were surveyed, including those with an HSI score of below average or poor due to their close proximity to waterbodies with a higher score. This resulted in a total of 396 waterbodies

being identified for survey. Of these, water samples were collected from 283, with the remaining waterbodies being inaccessible or dry.

S.10 The analysis of the water samples identified great crested newts as being present in four waterbodies. Three of the four waterbodies found to support great crested newt were located in Area 6, at the eastern end of the survey area near Magor. One pond was identified in Area 4 near Nash.

S.11 Great crested newt was incidentally recorded on three occasions during a reptile survey at Tata Steel Works in the centre, east of the survey area.

S.12 The survey results, together with the desk study information and the absence of any records of great crested newt in the 2014 Arup survey, indicate that there is a localised low density of great crested newt within the footprint of the new section of motorway, to the east of the River Usk. The results suggest that at least two different populations are present.

S.13 The possibility of false negative results suggests some additional waterbodies in the survey area could support great crested newts. These are considered most likely to be associated with the existing great crested newt populations that have been identified.

S.14 The new section of motorway would result in disturbance to both aquatic and terrestrial habitats that great crested newts could be using and therefore protection measures would be required to ensure that great crested newts are not directly harmed by the project and to ensure that adequate habitat remains present for them.

S.15 Further surveys will be undertaken in 2016 on the waterbodies found to support great crested newt to determine the population size class. Presence/absence surveys will also be undertaken on all waterbodies within 250 m of those found to support great crested newt, to consider the possibility of false negative eDNA results.

1 Introduction

1.1.1 RPS has undertaken a Great Crested Newt Presence/Absence (eDNA Technique) Survey of land within the route of the proposed M4 Corridor around Newport (M4CaN) between Castleton and Magor to inform the ecological baseline for environmental impact assessment (EIA) of the Scheme. The survey included land generally within 250 metres of the proposed alignment, following the methodology set out in the Analytical and Methodological Development for improved Surveillance of the Great Crested Newt (Biggs *et al.*, 2014). The EIA is reported in the M4CaN Environmental Statement (ES), of which this document is an appendix to the chapter on Ecology and Nature Conservation.

1.1.2 A Habitat Suitability Index (HSI) assessment and Great Crested Newt Presence/Absence survey previously undertaken by Arup on behalf of Welsh Government in 2014 are reported separately in Appendix 10.6 of the ES. A review of that work was undertaken independently by Hyder (see Appendix A of Appendix 9.1 in the ES Scoping Report (ES Appendix 5.1)) and RPS (see ES Chapter 10). The conclusions of that review and the requirements for additional surveys in 2015 were set out in the Scope of Ecology Surveys Report (see Appendix 9.1 of the ES Scoping Report). This was discussed with Natural Resources Wales (NRW) who was further consulted on the scope of the proposed surveys during consultation on the ES Scoping Report.

1.1.3 This document reports the findings of the Great Crested Newt Presence/Absence (eDNA Technique) Survey which was undertaken from the 18th May to the 30th June 2015 using standard Biggs, *et al.* (2014) methods.

1.1.4 This report document outlines the previous survey carried out and the reasons for this additional survey (Section 2), describes the methods used in the survey (Section 3) and the findings of the survey (Section 4). A discussion of the survey findings is provided in Section 5.

2 Previous Surveys

2.1 Introduction

2.1.1 Previous great crested newt surveys were undertaken along an earlier route of the new section of motorway by Arup in 2014 (Appendix 10.6 of the ES). The 2014 presence / absence survey areas are shown on Figure 1.

2.1.2 The objectives of the surveys were:

- to establish whether great crested newts are present in the waterbodies included within the survey;
- to provide information on the existing ecological conditions at the site with regards to great crested newts;
- to identify potential constraints and opportunities that great crested newts may pose to any development; and
- to identify further studies that may be required to ensure that great crested newts are fully considered.

2.1.3 The study area for the purpose of the survey was based on a 500 m buffer around the physical extents of the previous area studied in 2007/2008.

2.2 Survey Method

2.2.1 Initial Habitat Suitability Index (HSI) assessments of those waterbodies within the study area that could be assessed from roads and public rights of way were undertaken by Arup during February 2014, prior to issuing Notices of Intention to Enter Land under the Highways Act 1980. These assessments were made in accordance with the methodology set out in Advice Note 5 published by the Amphibian and Reptile Group UK (ARGUK, 2010).

2.2.2 The HSI methodology has been developed to assess the suitability of ponds for use as breeding sites by great crested newts. It was therefore necessary to adapt the methodology to take into account the reens and ditches which characterise the Gwent Levels, which great crested newts have been found to use. This was achieved by discounting the area, waterfowl and pond density indices from the HSI assessment. NRW was consulted on the adaptation of the methodology prior to the surveys commencing and NRW welcomed the approach.

2.2.3 The results of the HSI assessments were used to identify waterbodies considered to have average or better than average suitability for great crested newts. These waterbodies were then selected for presence/absence surveys where access allowed. The waterbodies to be surveyed were located in three discrete groups, referred to as Areas A, B and C (as indicated in Appendix 10.6).

2.2.4 Presence/absence surveys were carried out according to the guidance provided in the Great Crested Newt Mitigation Guidelines (English Nature, 2001). Appropriate methods were used unless site specific limitations were identified and all surveys were undertaken in correct weather conditions.

2.2.5 The site visits were undertaken between 24 April 2014 and 29 May 2014, which falls within the aquatic phase of the great crested newt life cycle (mid-March to mid-June).

2.2.6 The presence/absence surveys did not identify great crested newt in any of the waterbodies surveyed.

2.3 Desk Study

2.3.1 The South East Wales Biodiversity Records Centre (SEWBReC) provided records of great crested newts within and to the south of the study area: within ponds at Solutia and in Marshfield (approximately 2 km south of the study area).

2.3.2 Records of a large population of great crested newts at the eastern end of the new section of motorway were provided by SLR Consulting from surveys undertaken on ponds at Ifton Quarry and within 500 m of it.

2.3.3 A plan showing the locations where great crested newt have been recorded previously is included in the desk study report at Appendix 10.17 of the ES.

2.4 Requirements for Further Survey

2.4.1 The previous great crested newt report undertaken by Arup in 2014 states that the surveys only covered a small number of waterbodies within the survey area due to limitations regarding access at the time. The report recommended that the remaining waterbodies that were considered through HSI assessments to be suitable for great crested newts be surveyed in 2015.

2.4.2 Due to access constraints, only 55 of the 89 waterbodies identified as being of average or better than average suitability for great crested newts within the HSI exercise could be surveyed. These constraints included areas for which there was no known owner or occupier and areas where notices had yet to be served at the time of the surveys.

2.4.3 At the time of a meeting (30th January 2015) between NRW and Hyder (ecological advisor for the Welsh Government), a minimum of 327 waterbodies had been identified as potentially suitable for great crested newts through HSI assessment. This did not include those areas of the route corridor that were not accessible. Additional HSI assessments carried out by Arup in 2014 as part of the Extended Phase 1 Habitat Survey (Appendix 10.2 of the ES) identified a total of 409 waterbodies as of average or above average suitability for great crested newts.

2.4.4 Carrying out four great crested newt survey visits at each of these locations (and at any additional waterbodies once the areas not previously accessed are included) would be extremely onerous and costly. It was therefore agreed with NRW that a more pragmatic approach could be investigated.

2.4.5 NRW have issued guidance on the use of environmental DNA testing for great crested newt licensing purposes (Natural Resources Wales, 2015) (attached at Annex A), which confirms that NRW will accept eDNA test results as evidence of presence or absence of great crested newts for licencing purposes.

2.4.6 Having considered the 2014 Arup report (Appendix 10.6 of the ES) of the great crested newt survey in the context of the above recommendations and the NRW guidance, Thomson Ecology (on behalf of RPS) undertook a further survey of waterbodies. The further surveys used the eDNA technique in areas which fall within 250 m of the new section of motorway, where the Arup HSI assessments identified waterbodies of average or higher than average suitability to investigate the presence/absence of the species.

2.4.7 A 250 m survey area was chosen instead of the 500 m survey area used by Arup, as the proposed alignment was more closely defined by this time. Although great crested newts can disperse over larger areas, evidence suggests the vast majority will remain within 250 m of a breeding pond (English Nature, 2001) and therefore areas within 250 m of the footprint of the new section of motorway were the focus of the survey.

2.4.8 The methodology used during these surveys is provided in the following section.

3 Survey Methods

3.1 Introduction

3.1.1 The great crested newt presence absence (eDNA technique) survey was carried out over the period 18th May to the 30th June 2015, which falls within the optimum period for this type of survey.

3.1.2 The survey area was based upon the proposed alignment of the new section of motorway, together with an approximate 250 m corridor to either side.

3.2 Methodology

3.2.1 The survey method was based on that described in Biggs, *et al*. (2014).

3.2.2 Thomson Ecology was commissioned by RPS to collect water samples from the relevant waterbodies. Thomson Ecology used the laboratories of two British Universities to analyse the samples for great crested newt presence or absence.

Field protocol

3.2.3 The team of surveyors ranged between graduate ecologist and ecologist level and all team members were experienced in carrying out traditional great crested newt surveys. The team were specifically trained in the eDNA surveying technique, as described by Biggs, *et al.* (2014), by a licensed senior ecologist prior to collecting water samples.

3.2.4 A total of seven survey areas were identified from within the footprint of the new section of motorway and surrounding 250 m buffer, which contained the highest concentrations of waterbodies with average or higher than average HSI scores. The seven survey areas are shown on the plan at Figure 1. All waterbodies within each of the survey areas were surveyed including those with an HSI score of below average or poor due to their close proximity to waterbodies with a higher score. This resulted in a total of 396 waterbodies being identified for survey. Of these, water samples were collected from 283.

3.2.5 A total of 73 waterbodies were found to not provide suitable habitat for great crested newts at the time of the survey because they were dry.

3.2.6 A further 37 waterbodies were not surveyed because they were inaccessible, either due to barriers preventing access or due to health and safety reasons.

3.2.7 A total of three waterbodies were found not to exist where they had been mapped.

3.2.8 Each of the 283 waterbodies were accessed and a water sample collected between the 18th May and 30th June 2015.

3.2.9 Water samples were collected using sampling kits supplied by the laboratories.

3.2.10 Surveyors collected 30 millilitres (ml) water samples from twenty locations along the margins of each reen or ditch surveyed using a sterile ladle. Surveyors collected the sample from the bank edge and did not enter the water. Where access allowed, the samples were collected from points evenly spaced along

each side of the ditch or reen. However, access restraints meant this was not possible in some locations but samples were spread out as much as possible. Further details of the accessibility of each waterbody are provided in the full data set provided in Annex B of this report.

3.2.11 The surveyors used the ladle to gently agitate the water to mix the water column, whilst taking care not to disturb and collect any sediment. The twenty 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.

3.2.12 A pipette was used to collect six 15 ml subsamples of the pond water from the bag into sterile tubes already containing 35 ml of ethanol to preserve the eDNA sample.

3.2.13 The samples were then removed from site and stored in a refrigerator before being sent off in weekly batches to the laboratories.

Laboratory protocol

3.2.14 The water samples were analysed using the quantitative Polymerase Chain Reaction (qPCR) eDNA test, following the protocols provided by Biggs, *et al*. (2014).

3.2.15 The protocol sets out that laboratories undertaking eDNA analysis should be able to demonstrate adequate quality assurance standards which would typically comprise a documented quality management system. This would usually follow, or be equivalent to ISO/IEC 17025 standard.

3.2.16 The ISO system is not applicable to university laboratories. Both of the university laboratories used to assess the samples have been assessed under the equivalent quality assessment; Research Excellence Framework (REF). In 2014, one laboratory received a REF assessment that 92% of its research is internationally excellent or better and the other achieved 91%.

3.2.17 The laboratory methodologies followed those described by Biggs, et al. (2014) and included appropriate precautions to avoid laboratory contamination and minimise the risk of false positive and negative results. Details of the laboratory standards in place at each laboratory are included in Annex C.

3.3 Limitations

Land Access

3.3.1 A total of 37 of the 396 waterbodies identified for survey were not surveyed because they were inaccessible. These were located within four of the seven survey areas, including the two where great crested newts were found to be present.

3.3.2 The majority of the inaccessible waterbodies were located within the two survey areas where great crested newt had been recorded previously. These predominantly comprised relatively short sections of reens surrounded by much larger sections that had predominantly been found not to support great crested newt and therefore it is considered unlikely that the majority of the unsurveyed sections would support them.

3.3.3 However, there is potential for some of the additional waterbodies to support great crested newts, particularly those south west of Magor, which were located near to waterbodies where great crested newt had been recorded.

3.3.4 A low number of the inaccessible waterbodies were located within two of the survey areas where great crested newt had not been recorded and therefore there is considered to be lower potential for great crested newts to be found in these waterbodies as they had been found to be absent from the surrounding waterbodies.

False Positives/Negatives

3.3.5 There is a possibility of false positive or false negative results when using the eDNA technique.

False Positives

3.3.6 False positives can occur when there is contamination between samples. This is considered unlikely to be a constraint due to the strict field and laboratory procedures in place to prevent such contamination. A false positive from cross contamination is also less likely to occur when great crested newt abundance is low.

False Negatives

3.3.7 Three main factors have been found to lead to false positives (Biggs, *et al.*, 2014):

- ponds with very low numbers of newts;
- ponds with wide shallow edges; and
- ponds where sampling was restricted to a small part of the pond.

3.3.8 A number of the waterbodies surveyed were very shallow or heavily vegetated, restricting the collection of samples to certain parts of them. There was also potential for newts to be present in very low numbers in some of the waterbodies surveyed.

3.3.9 Biggs, *et al*. (2014) identify two main actions which may help to reduce false negatives:

- providing face to face training for surveyors; and
- using appropriate survey methods that ensure samples are collected from as much of the pond as possible; avoidance of very shallow water (e.g. less than 5 cm deep); and, avoidance of very densely vegetated areas (such as dense floating mats which newts may not be able to penetrate).

3.3.10 The surveyors collecting the eDNA samples were adequately trained in sample collection methods and informed of the need to avoid shallow and densely vegetated water where practicable and to collect samples from as much of the waterbody as possible. Therefore, the likelihood of false positives was reduced as far as possible.

3.3.11 Biggs, *et al.* (2014) report a 9% occurrence of false negatives from a survey of a similar number of waterbodies. For the survey of waterbodies for the new section

of motorway, a 9% occurrence of false negative results would account for 25 waterbodies supporting great crested newts which were found to have negative results.

3.3.12 The 9% occurrence rate is based on a survey undertaken by volunteers who did not receive face to face training and who were trained prior to the adaption of the survey methods to reduce the occurrence of false negatives. They therefore did not have the experience of the surveyors used in the M4CaN survey. The percentage of false negatives for this survey is therefore considered to be less than 9%.

4 Results

4.1 Introduction

4.1.1 This section of the report provides a description of the survey area and the findings of the great crested newt presence/absence (eDNA technique) survey. A table listing the waterbodies found to support great crested newts is included (Table 1). Incidental sightings of great crested newts during a reptile survey are also described below and are shown on Figure 1.

4.2 Results

4.2.1 The analysis of the water samples identified great crested newts as being present in four waterbodies. A summary of the results from the waterbodies identified as having great crested newts present are provided below in Table 1. The locations of these waterbodies with positive results are shown on the plan at Figure 1. The full set of results for all the waterbodies sampled is provided in Annex B.

Table 1: Waterbodies Supporting Great Crested Newt

Date	Area	Nearest Settlement	Notes	eDNA Score
05/06/2015	6	Magor	Heavily overgrown	1
09/06/2015	6	Magor	N/A	0.08
10/06/2015	6	Magor	Dense vegetation both banks, access difficult. Highly shaded waterbody.	0.08
15/06/2015	4	Nash	N/A	1

4.2.2 Three of the four waterbodies found to support great crested newt were located in Area 6, at the eastern end of the survey area near Magor. One pond was identified in Area 4 near Nash.

4.2.3 An eDNA score of 1 was achieved for two of the ponds and a score of 0.8 was achieved for the other two. The lower score of 0.8 suggests less eDNA was present in the samples collected but is still a positive result. There is a weak relationship between eDNA scores and great crested newt counts. However, at present the relationship between eDNA score and newt abundance is not strong enough to use eDNA as a reliable index of population size (Biggs *et al.*, 2014).

4.2.4 None of the waterbodies found to support great crested newt were located within the footprint of the new section of motorway but were within 250 m of it.

4.3 Reptile Survey 2015

4.3.1 Great crested newt was incidentally recorded on three occasions during a reptile survey at Tata Steel Works in the centre east of the survey area (Appendix 10.27 of the ES). A female great crested newt was recorded once and juveniles were recorded twice. These records are shown on Figure 1.

5 Discussion

5.1 Introduction

5.1.1 This section sets out the main findings of the survey and refers also to the results of the previous surveys. It sets out the key considerations for the new section of motorway and requirements for any further surveys.

5.2 Survey Findings

5.2.1 Out of a total of 283 waterbodies surveyed, four were found to support great crested newt. This, together with the desk study information and the absence of any records of great crested newt in the 2014 Arup survey, indicates that there is a localised low density of great crested newt within the footprint of the new section of motorway, to the east of the River Usk.

5.2.2 Three of the four waterbodies which were positive for great crested newt were located at the eastern end of the survey area, west of Magor and one was located towards the centre of the survey area, east of the River Usk. This suggests that at least two different populations are present.

5.2.3 The possibility of false negative results suggests up to 25 additional waterbodies in the survey area could support great crested newts, although this number is likely to be lower. Given the distribution of great crested newts identified during this survey and from historic records, it is considered likely that any unidentified presence would be associated with known populations and from waterbodies within the vicinity (250 m) of where great crested newts have been recorded.

5.3 Key Considerations

5.3.1 Great crested newt was found to be present within the footprint of the new section of motorway and in habitats within 250 m of it. The new section of motorway would result in disturbance to both aquatic and terrestrial habitats that great crested newts could be using. Therefore, protection measures would be required to ensure that great crested newts are not directly harmed by the new section of motorway and to ensure that adequate habitat remains present for them.

5.3.2 This would require the exclusion of great crested newts from parts of the new section of motorway within 250 m of where they have been found and a translocation exercise to move any newts present out of the limit of temporary and permanent works. Additional nearby habitats would need to be found or created to support any great crested newts displaced from the area of works.

5.4 Further Surveys

5.4.1 Further surveys will be undertaken in 2016 on the waterbodies found to support great crested newt to determine the population size class. Presence/absence surveys will also be undertaken on all waterbodies within 250 m of those found to support great crested newt to consider the possibility of false negative eDNA results.

5.4.2 The surveys will follow the guidelines provided in the Great Crested Newt Mitigation Guidelines (English Nature, 2001).

References

Amphibian and Reptile Group UK (2010) ARG UK Advice Note 5 Great Crested Newt Habitat Suitability Index.

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.

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Natural Resources Wales (2015) The use of Environmental DNA Test for Great crested newt licensing purposes. NRW: Cardiff

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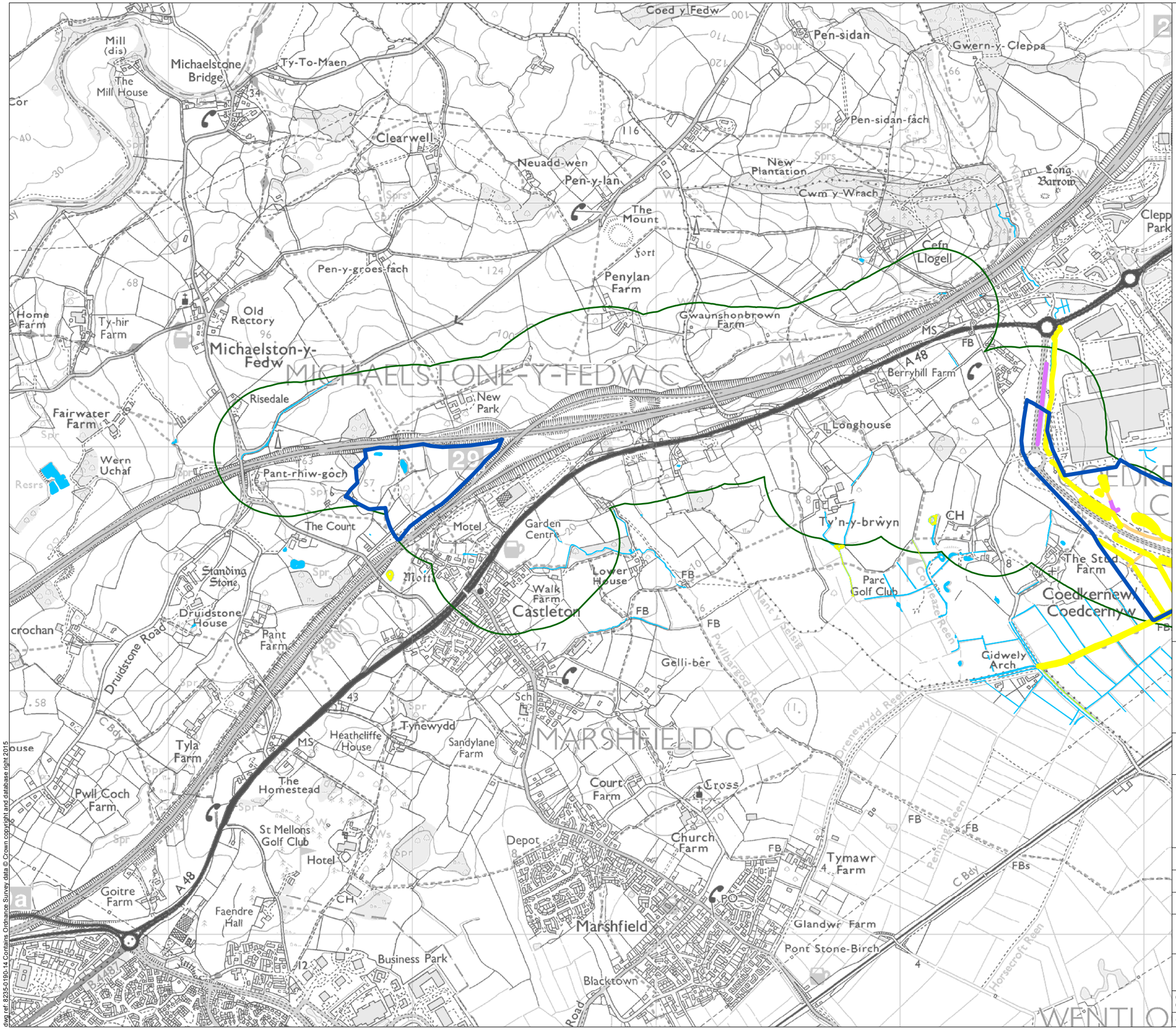
Figures



Legend

- 250m Study Area

2015 eDNA survey

- GCN present
- GCN absent
- Unsurveyed as water-levels too low/dry
- Unsurveyed due to inaccessibility or land owner permission refused/not obtained
- Mapping error, no reen/waterbody
- 2015 eDNA survey areas
- Unsurveyed due to inaccessibility
- Sample location

2015 Incidential Records

- GCN found during 2015 reptile survey

Study area shown reflects the scheme design at the time of the survey

Llywodraeth Cymru
Welsh Government

Appendix 10.22 - Great Crested Newt eDNA Survey

Great Crested Newt Survey of Reens / Waterbodies (2014, 2015)

Figure: 1a	Revision: -
Date: March 2016	Status: AT ISSUE
Drawn: CR	Checked: KJ

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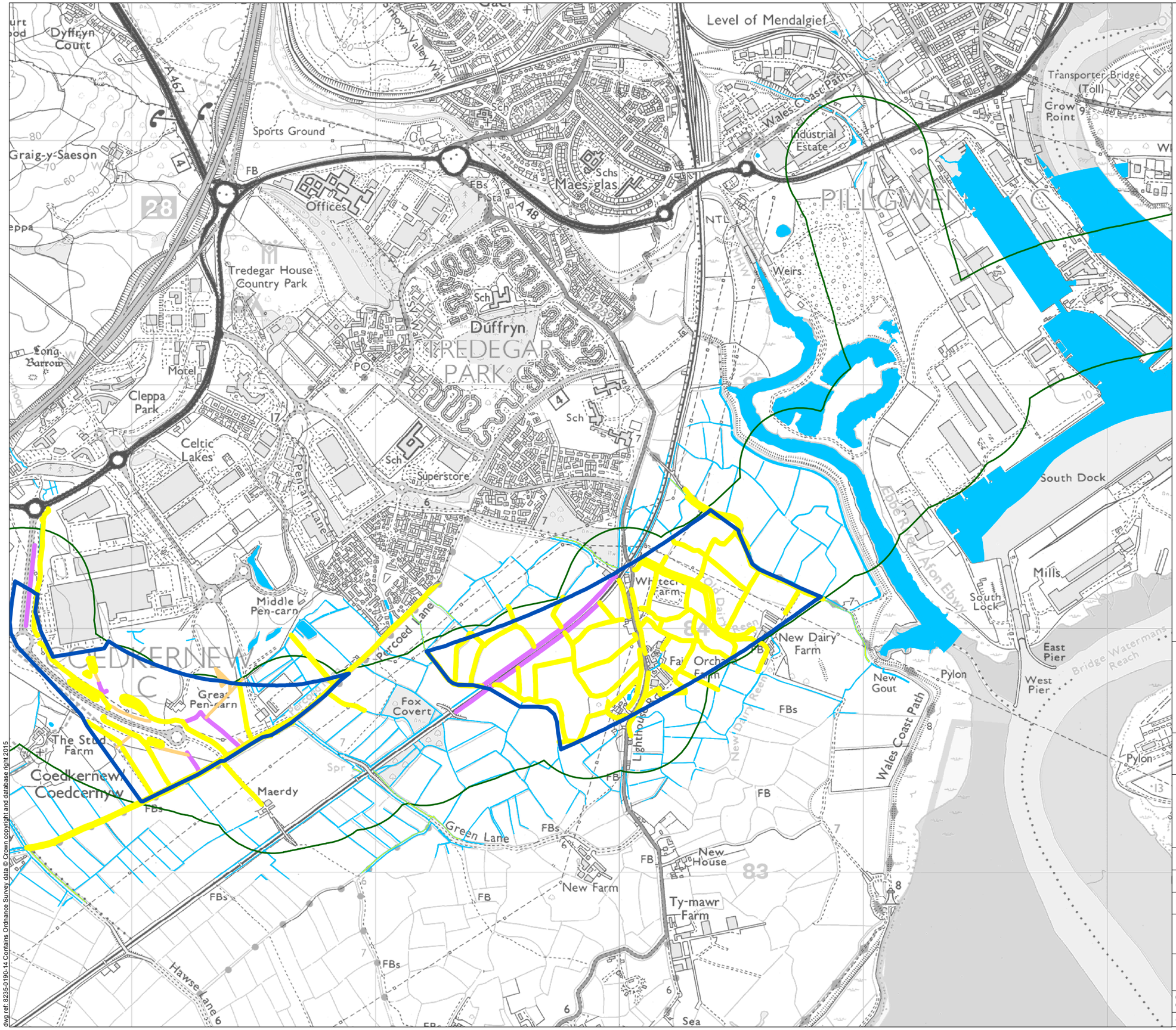

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Welsh Government

Appendix 10.22 - Great Crested Newt eDNA Survey

Great Crested Newt Survey of Reens / Waterbodies (2014, 2015)

Figure: 1b	Revision: -
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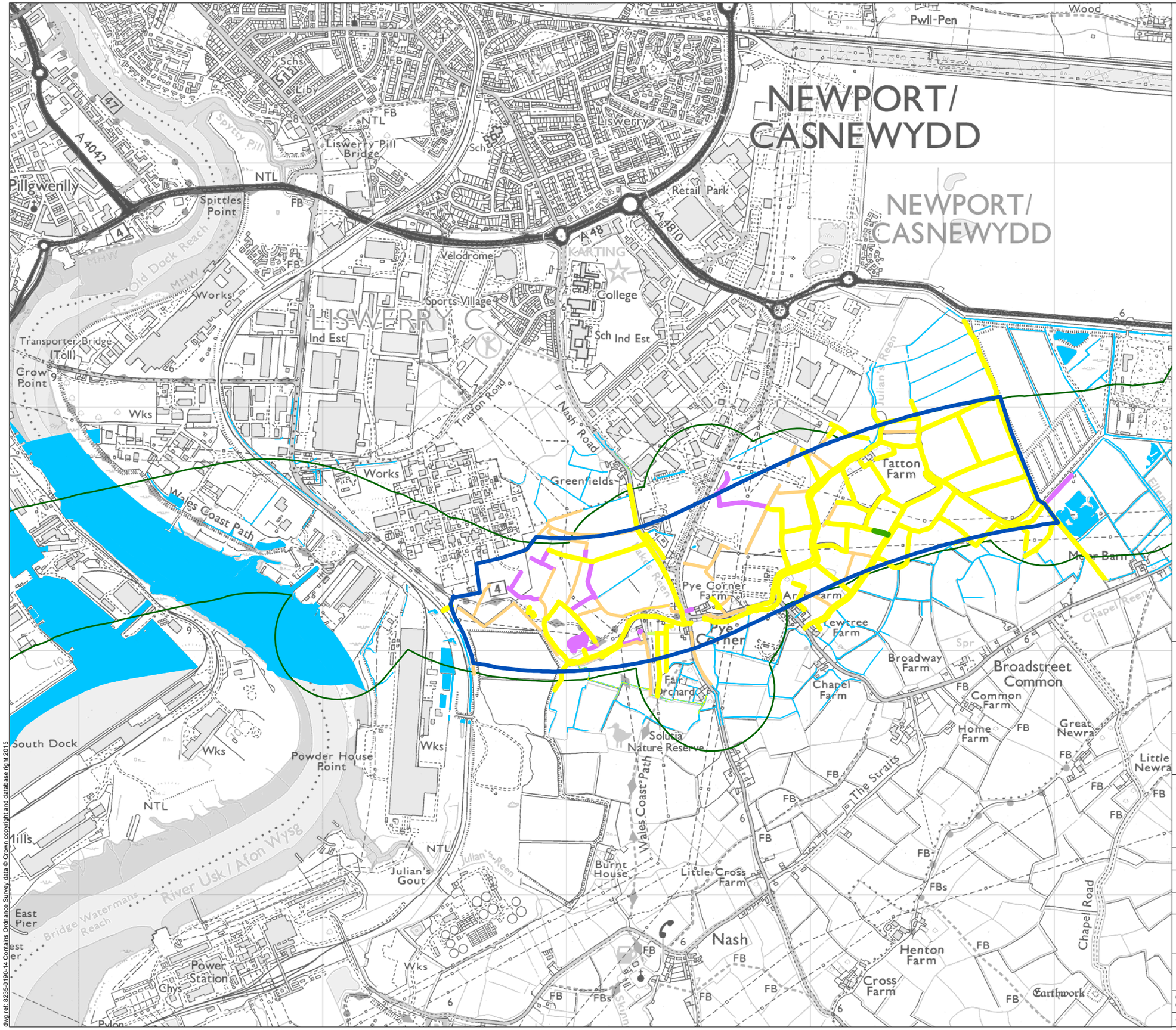

Legend

- 250m Study Area

2015 eDNA survey

- GCN present
- GCN absent
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- Unsurveyed due to inaccessibility or land owner permission refused/not obtained
- Mapping error, no reen/waterbody
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- Unsurveyed due to inaccessibility
- Sample location

2015 Incidential Records

- GCN found during 2015 reptile survey

Study area shown reflects the scheme design at the time of the survey

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Welsh Government

Appendix 10.22 - Great Crested Newt eDNA Survey

Great Crested Newt Survey of Reens / Waterbodies (2014, 2015)

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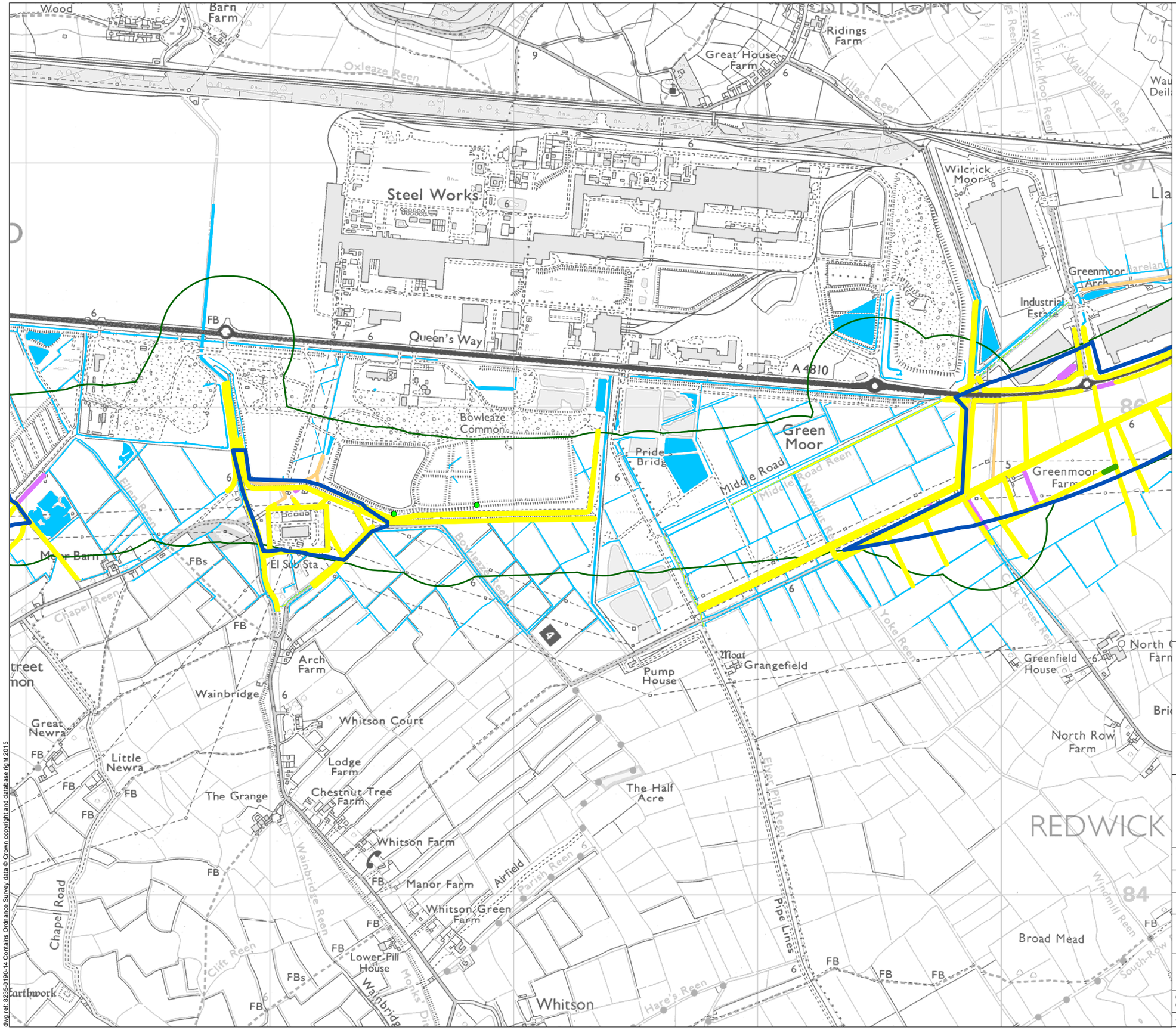

Legend

- 250m Study Area

2015 eDNA survey

- GCN present
- GCN absent
- Unsurveyed as water-levels too low/dry
- Unsurveyed due to inaccessibility or land owner permission refused/not obtained
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Great Crested Newt Survey of Reens / Waterbodies (2014, 2015)

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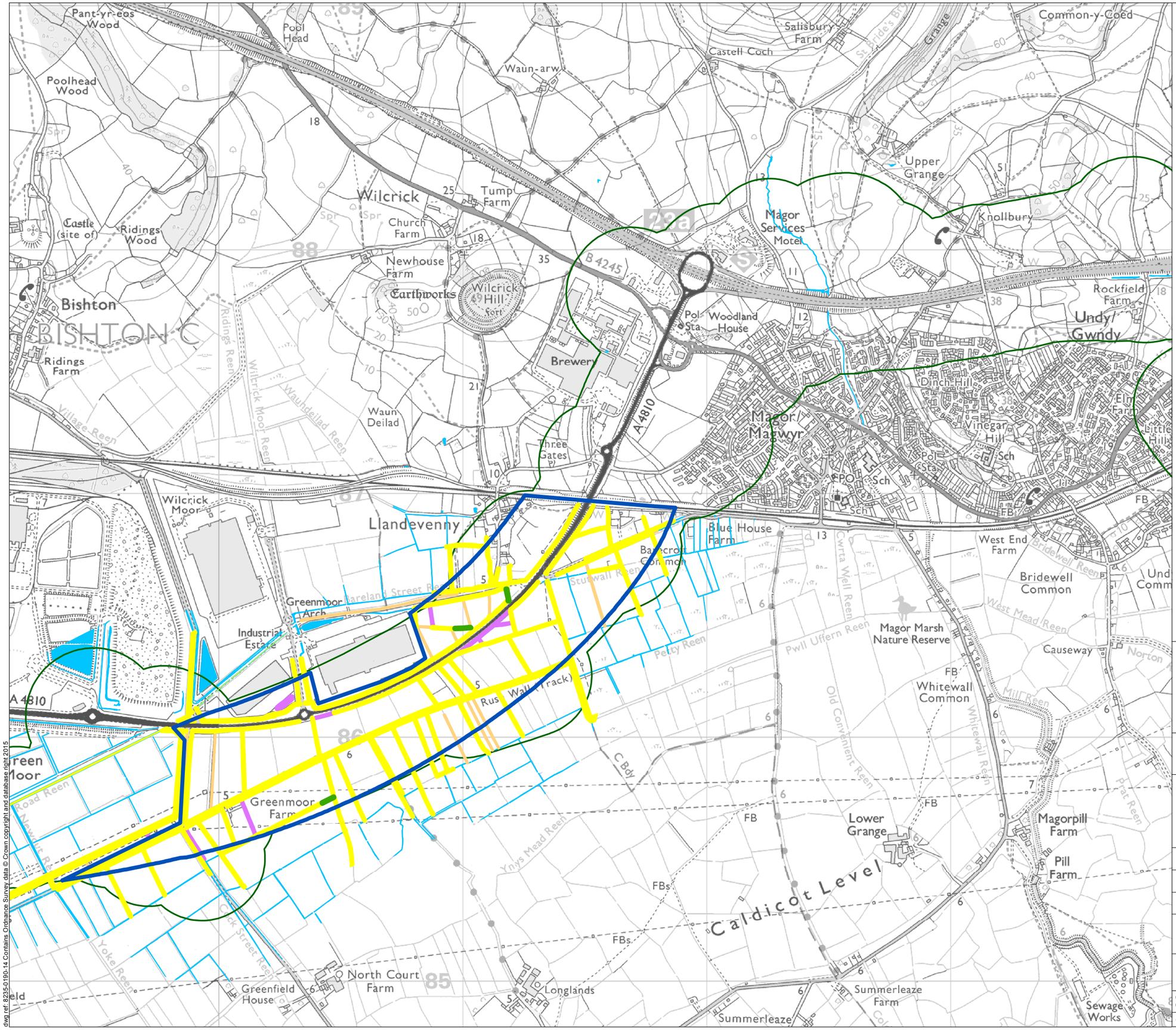

Legend

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2015 eDNA survey

- GCN present
- GCN absent
- Unsurveyed as water-levels too low/dry
- Unsurveyed due to inaccessibility or land owner permission refused/not obtained
- Mapping error, no reen/waterbody
- 2015 eDNA survey areas
- Unsurveyed due to inaccessibility
- Sample location

2015 Incidential Records

- GCN found during 2015 reptile survey

Study area shown reflects the scheme design at the time of the survey

Llywodraeth Cymru
Welsh Government

Appendix 10.22 - Great Crested Newt eDNA Survey

Great Crested Newt Survey of Reens / Waterbodies (2014, 2015)

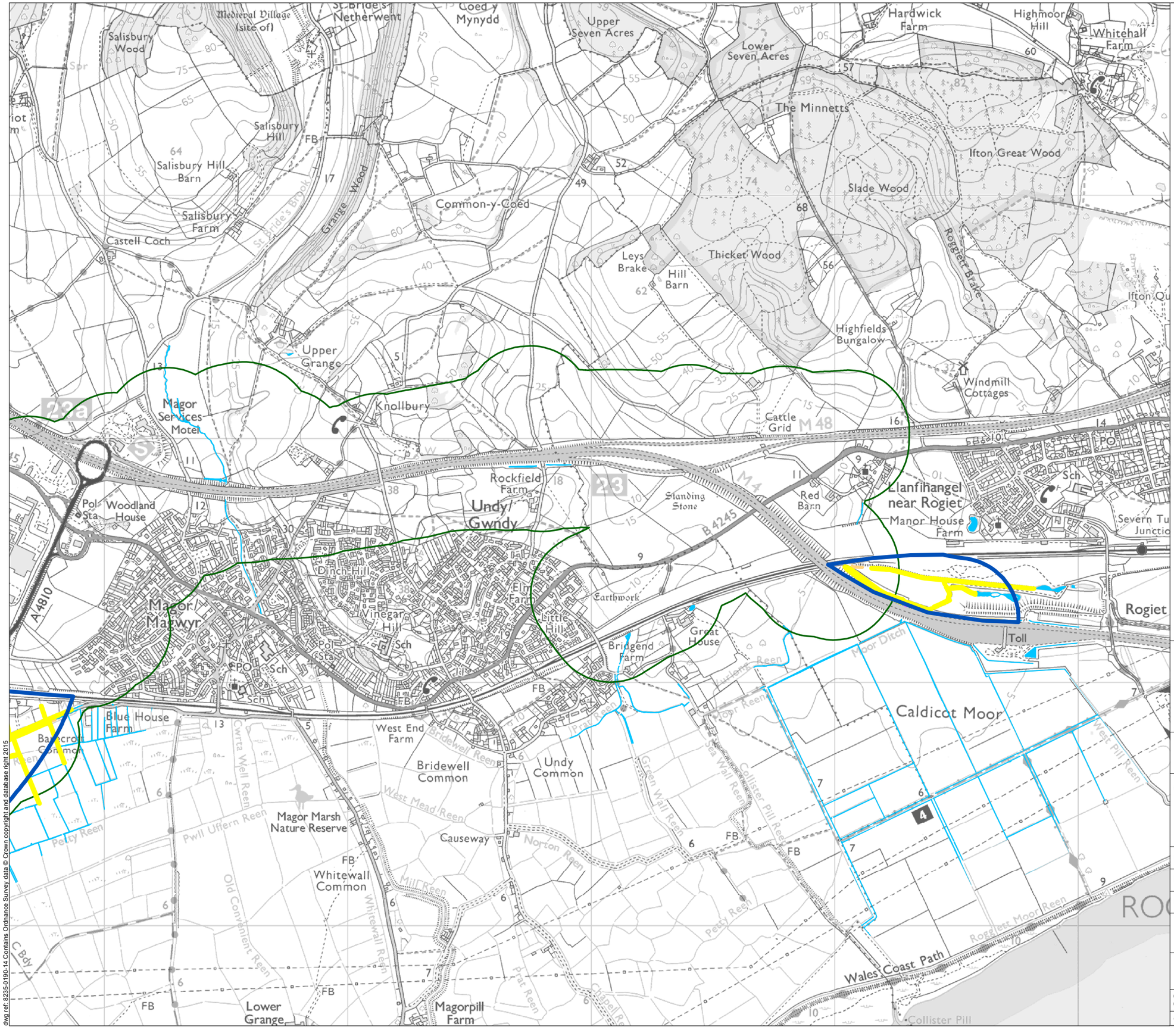
Figure: 1e	Revision: -
Date: March 2016	Status: AT ISSUE
Drawn: CR	Checked: KJ

Scale: A3 @ 1:15,000

0 250 500m

N

dwg ref: 8235-0190-14



Legend

- 250m Study Area

2015 eDNA survey

- GCN present
- GCN absent
- Unsurveyed as water-levels too low/dry
- Unsurveyed due to inaccessibility or land owner permission refused/not obtained
- Mapping error, no reen/waterbody
- 2015 eDNA survey areas
- Unsurveyed due to inaccessibility
- Sample location

2015 Incidential Records

- GCN found during 2015 reptile survey

Study area shown reflects the scheme design at the time of the survey

Llywodraeth Cymru
Welsh Government

Appendix 10.22 - Great Crested Newt eDNA Survey

Great Crested Newt Survey of Reens / Waterbodies (2014, 2015)

Figure: 1f	Revision: -
Date: March 2016	Status: AT ISSUE
Drawn: CR	Checked: KJ

Scale: A3 @ 1:15,000
0 250 500m

N

dwg ref: 8235-0190-14

Annexes

Annex A: The Use of Environmental DNA Test for Great Crested Newt Licensing Purposes

Cyfoeth Naturiol Cymru
Natural Resources Wales

The use of environmental DNA test for Great crested newt licensing purposes

The Department for Environment Food & Rural Affairs (DEFRA) has recently published the results of an investigation into the use of environmental DNA (eDNA) to detect the presence of Great crested newt (GCN) in water bodies together with a technical advice note setting out the field and laboratory methodology DEFRA Science and Research Project WC1067.
http://randd.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&ProjectID=18650&FromSearch=Y&Publisher=1&SearchText=wc1067&SortString=ProjectCode&SortOrder=Asc&Paging=10#Description

On the basis of this study Natural Resources Wales (NRW) will now accept eDNA test results as evidence of presence or absence of GCN for licence

Key Findings of the report (this is not comprehensive so please read the 'final report' and 'technical advice note'):

- eDNA can have a better rate of GCN detection (99%) than a combination of conventional survey techniques ((95%);
- It will detect GCN presence or absence within a water body up to 7 – 21 days after newts utilising it;
- It requires 1 day time visit to each water body but the visit must be targeted when GCN are likely to be present in water bodies in the area (which may change on a yearly basis depending on local / regional conditions); and
- The study is based only on a single year and the 'final report' states samples were taken from late April to late June. (Note for 2014 NRW will accept samples taken between 15th April to 30th June – see below).

For GCN licence applications we anticipate this technique will be useful for developments/projects with a **long lead in time** where it can be used for early stage assessments and screening to determine the water bodies that require population size class assessment surveys, and for temporary and low impact cases which may require presence or absence surveys only. This is because it can take several weeks for results to be available from the analytical process.

When using this technique to support a licence application you should be aware that:

1. Use of eDNA is just another survey technique – it is not a mandatory requirement. We will accept this new technique to determine GCN presence or absence if samples are undertaken in strict accordance with the published technical advice note and they are collected by a suitably trained and experienced licensed GCN surveyor. (Note that a survey licence is not

required to take the water samples, but for licence applications we will require evidence and confirmation that experienced, licensed GCN surveyor/s collected the samples to support the proposals in the method statement).

2. Applicants wishing to submit eDNA test results as evidence of presence or absence of GCN, must declare that:

    a. They have strictly followed the technical advice note;

    b. Only licensed GCN surveyors (provide names and references) have taken the samples to support their licence application; and

    c. Present the field and laboratory results as part of their application, by including a separate WORD document with the application clearly setting out

        i. The referenced water bodies which were tested,

        ii. Dates that samples were taken, and

        iii. The results (presence or absence) in tabular form - which must also be reflected on the survey maps/figures submitted (see point 3).

3. Method statements must also include on the relevant survey figure/s water bodies sampled and surveyed, clearly indicating water body references and results (presence or absence).
4. During 2014 we are only accepting eDNA results from samples collected following the onset of suitable weather conditions for surveying GCN between the 15th April and 30 June.
5. This technique will not provide population size class assessments.
6. Should a population size class assessment be required for the proposed development/project then the applicant will require 6 survey visits using conventional survey methods, in accordance with current recommendations within the "*Great Crested Newt Mitigation Guidelines, 2001*".
7. Our *Information to be provided in a European Protected Species Licence application form* document will be adapted in due course, but untill then this advice on what we will typically expect if an applicant wishes to use this technique is to be followed. Should population size class estimates be required then the survey section of the method statement must be completed as usual.
8. Applicants must ensure they retain or have access to the records set out in the technical advice note, and used to support the licence application, for at least 12 months following the first licence return (date of which will be set out in any licence granted).
9. eDNA can also be used for post development monitoring surveys if presence or absence only surveying is required under licence.

The study undertaken did not include an analysis of potential suppliers or likely level of demand. Therefore should you wish to use this test you will need to make an informed **risk based judgement** about whether to use this test or conventional surveys to detect presence and absence of GCN. In particular applicants should be aware of:

- Factors affecting false negatives when collecting water samples – the need for training and recognising there will be difficult or less suitable sites for this technique.
- Factors causing false positives within handling and laboratory analysis – the results were obtained using a high specification laboratory. There is a risk that other laboratory layouts might generate different results. When commissioning laboratory analysis users should satisfy themselves that they can achieve a satisfactory level of performance.
- The study is based on a single years results and expert judgement should be applied to ensure samples are collected at the optimum time, when GCN are active, bearing in mind geographical location and conditions early in the year.
- Whether their project timetable allows sufficient time to undertake the required number of population size class assessment surveys (i.e. the conventional 6 surveys between March-June). If an eDNA test shows presence of GCN and as such a population assessment is required for the proposed development and impacts. This will require careful forward planning.
- The study looked at one type of eDNA test – quantitative Polymerase Chain Reaction (qPCR). We anticipate that variations in the technique from those studied and new techniques will emerge if industry finds this test useful. Industry will need to demonstrate equivalence for alternative tests.

For the immediate future we will therefore only accept eDNA evidence using the specific qPCR test set out in the technical advice note.

We will monitor how much uptake there is of this technique and seek feedback from industry on their experience.

Annex B: Full eDNA Survey Results

Index	WB	Date	Area	OS Grid X	OS Grid Y	Number of inflows	% of perimeter accessible	Number of GCN on site during sampling	Flow	Notes	Unsampled	eDNA Result	eDNA Score
1	246	18/05/2015	3	329905.65	183646.43	1	60	0		N/A		Negative	
2	247	18/05/2015	3	330014.02	183637.41	2	50	0		N/A		Negative	
3	261	18/05/2015	3	329862.51	133609.91	1	95	0		N/A		Negative	
4	262	18/05/2015	3	329797.60	183551.78	1	75	0		N/A		Negative	
5	228	19/05/2015	3	330020.49	184137.43	1	50	0		N/A		Negative	
6	229	19/05/2015	3	329932.20	184080.00	0	100	0		N/A		Negative	
7	230	19/05/2015	3	329918.57	184033.20	1	60	0		N/A		Negative	
8	231	19/05/2015	3	329948.06	183867.70	1	50	0		N/A		Negative	
9	248	19/05/2015	3	329852.00	183777.00	2	40	0		N/A		Negative	
10	249	19/05/2015	3	329806.00	183878.00	3	60	0		N/A		Negative	
11	250	19/05/2015	3	329730.07	183875.20	2	55	0		N/A		Negative	
12	251	19/05/2015	3	329782.37	183959.31	2	60	0		N/A		Negative	
13	253	19/05/2015	3	329864.40	184051.89					No sample: Access not possible from field side as the ditch is beside the railway with access restricted by barbed fence and thick hedge. 95% of ditch appeared dry at time of survey and where there was water it was heavily shaded. Due to this access questionable due to vegetation even if had permission from railway. Ditch labelled 683 on Mobile mapper	Inaccessible		
14	257	19/05/2015	3	329655.00	183781.00	2	50	0		N/A		Negative	
15	258	19/05/2015	3	329660.77	183701.10	5	95	0		N/A		Negative	
16	259	19/05/2015	3	329576.71	183782.23	2	30	0		N/A		Negative	
17	260	19/05/2015	3	329536.77	183753.74	2	100	0		N/A		Negative	

Index	WB	Date	Area	OS Grid X	OS Grid Y	Number of inflows	% of perimeter accessible	Number of GCN on site during sampling	Flow	Notes	Unsampled	eDNA Result	eDNA Score
18	218	20/05/2015	3	330150.99	184000.00	3	50	0		Internal perimeter in field fenced off. Accessed through farmyard		Negative	
19	219	20/05/2015	3	330095.30	18395.70	1	80	0		N/A		Negative	
20	220	20/05/2015	3	330057.94	184098.43	0	70	0		N/A		Negative	
21	222	20/05/2015	3	330149.98	184178.66	0	100	0		N/A		Negative	
22	226	20/05/2015	3	330120.94	184256.85	2	80	0		N/A		Negative	
23	227	20/05/2015	3	330085.24	184263.21	3	80	0		N/A		Negative	
24	232	20/05/2015	3	330000.18	183796.65	1	65	0		35% dry, majority very shallow <10cm deep		Negative	
25	233	20/05/2015	3	329997.30	183730.41	3	45	0		Accessible one side only due to garden		Negative	
26	235	20/05/2015	3	330076.65	183763.71	2	50	0		No access one side due to residents, partially dry		Negative	
27	236	20/05/2015	3	330120.30	183796.79	3	5	0		Heavily vegetated along edge, access restricted as a result to area from road side		Negative	
28	252	20/05/2015	3	329538.45	183810.64					No sample: Access not possible from field side as the ditch is beside the railway with access restricted by barbed wire fence and thick hedge. 75% of ditch appeared dry at time of survey and where there was water it was heavily shaded. Due to this access questionable due to vegetation even if had permission from railway	Inaccessible		
29	992	20/05/2015	3	330162.63	184032.31	1	50	0		Only one edge of ditch accessible		Negative	
30	1000	20/05/2015	3	330062.68	183966.69	0	50	0		N/A		Negative	

Index	WB	Date	Area	OS Grid X	OS Grid Y	Number of inflows	% of perimeter accessible	Number of GCN on site during sampling	Flow	Notes	Unsampled	eDNA Result	eDNA Score
31	186	21/05/2015	3	330311.15	184350.80	1	70	0		N/A		Negative	
32	189	21/05/2015	3	330424.00	184239.00	2	60	0		N/A		Negative	
33	194	21/05/2015	3	330181.64	184278.15	1	100	0		N/A		Negative	
34	195	21/05/2015	3	330241.33	184217.85	3	100	0		N/A		Negative	
35	196	21/05/2015	3	330349.75	184268.85	2	50	0		N/A		Negative	
36	197	21/05/2015	3	330380.89	184118.00	3	100	0		N/A		Negative	
37	198	21/05/2015	3	330471.49	184058.52	2	30	0		N/A		Negative	
38	199	21/05/2015	3	330480.54	183922.29	2	50	0		N/A		Negative	
39	203	21/05/2015	3	330305.46	184000.07	4	40	0		N/A		Negative	
40	204	21/05/2015	3	330304.86	184019.09	1	55	0		Very dry along much of its length. Limited areas to take water for sample		Negative	
41	205	21/05/2015	3	330390.20	183902.90	2	80	0		N/A		Negative	
42	215	21/05/2015	3	330252.50	183050.60	4	95	0		N/A		Negative	
43	221	21/05/2015	3	330174.20	184038.49	2	55	0		N/A		Negative	
44	223	21/05/2015	3	330278.19	184142.30	2	45	0		N/A		Negative	
45	224	21/05/2015	3	330346.84	184154.98	2	40	0		N/A		Negative	
46	225	21/05/2015	3	330225.24	184251.09	4	55	0		N/A		Negative	
47	805	22/05/2015	3	330513.08	183968.07	2	50	0	OBVIOUS	Is actually WB 805. Mapper called this 127 near the east end of the site. It is a small section of ditch at the east edge of 199, where it joins 201. could possibly be 805. Hard copy of map unclear. There is a second 127 on hard copy in another area of the site not yet surveyed north of the train line		Negative	
48	187	22/05/2015	3	330430.52	184338.75	3	60	0	No	N/A		Negative	

Index	WB	Date	Area	OS Grid X	OS Grid Y	Number of inflows	% of perimeter accessible	Number of GCN on site during sampling	Flow	Notes	Unsampled	eDNA Result	eDNA Score
49	188	22/05/2015	3	330417.30	184456.40	2	100	0	No	Nightshade along top of ditch		Negative	
50	190	22/05/2015	3	330549.62	184196.93	3	80	0	SLIGHT FLOW	N/A		Negative	
51	191	22/05/2015	3	330527.37	184314.88	3	100	0		N/A		Negative	
52	192	22/05/2015	3	330624.08	184221.95	1	65	0	No	Steep side to one side of bank		Negative	
53	193	22/05/2015	3	330667.33	184035.11	1	40	0	No	Couldn't access whole ditch as Nightshade along banks		Negative	
54	209	22/05/2015	3	330336.19	183793.21	3	85	0	No	Deep, evidence of dredging		Negative	
55	212	22/05/2015	3	330223.76	183780.40	2	35	0		Mainly dry, sludge and mud with one Very deep dangerous pond		Negative	
56	213	22/05/2015	3	330203.26	183381.00	2	100	0	No	N/A		Negative	
57	214	22/05/2015	3	330294.49	183911.70	2	100	0	No	N/A		Negative	
58	216	22/05/2015	3	330186.11	183950.39	1	70	0		N/A		Negative	
59	237	22/05/2015	3	330137.21	183742.21	1	50	0	No	N/A		Negative	
60	238	22/05/2015	3	330200.06	183735.27	2	50	0	SLIGHT FLOW	N/A		Negative	
61	320	22/05/2015	3	330778.51	184139.57	2	100	0	SLIGHT FLOW	Slow flow		Negative	
62	140	01/06/2015	3	329745.00	184032.00	1	50	0	No	N/A		Negative	
63	120	02/06/2015	3	329384.00	183771.00	4	50	0	No	N/A		Negative	
64	121	02/06/2015	3	329380.00	183965.00	2	50	0	No	N/A		Negative	
65	123	02/06/2015	3	329389.00	183792.00	4	50	0	No	N/A		Negative	
66	124	02/06/2015	3	329423.00	183954.00	2	50	0	No	N/A		Negative	
67	125	02/06/2015	3	329370.00	184011.00	3	50	0	No	N/A		Negative	
68	126	02/06/2015	3	329556.00	184057.00	3	50	0	No	N/A		Negative	
69	127	02/06/2015	3	329624.00	183960.00	1	70	0	No	Marked as WB 814 on mapper, 127 on paper maps		Negative	

Index	WB	Date	Area	OS Grid X	OS Grid Y	Number of inflows	% of perimeter accessible	Number of GCN on site during sampling	Flow	Notes	Unsampled	eDNA Result	eDNA Score
70	201	02/06/2015	3	330553.00	183951.00	3	40	0	No	N/A		Negative	
71	254	02/06/2015	3							No sample: No access - Railway and dense vegetation	Inaccessible		
72	255	02/06/2015	3							No sample: No access - Railway and dense vegetation	Inaccessible		
73	256	02/06/2015	3							No sample: Inaccessible - Railway and hedge	Inaccessible		
74	626	02/06/2015	6							No sample: Inaccessible - Dense hedge both sides and kennels on one side	Inaccessible		
75	631	02/06/2015	6	340978.00	186143.00	1	100	0	No	Marked as 738 on mapper and 631 on paper maps. North end of land parcel 413, at north end of WB961, parallel to track		Negative	
76	961	02/06/2015	6	341019.00	186060.00	2	30	0	No	50% of ditch dry at time of survey		Negative	
77	976	02/06/2015	6	340855.00	186093.00	2	15	0	No	N/A		Negative	
78	977	02/06/2015	6							No sample: Dry ditch	Dry		
79	1388	02/06/2015	3	329743.00	184086.00	3	70	0	No	N/A		Negative	
80	604	05/06/2015	6	339819.00	185609.00	3	70	0		N/A		Negative	
81	605	05/06/2015	6	339828.00	185626.00	5	15	0		N/A		Negative	
82	608	05/06/2015	6			1	0			No sample: Dry ditch	Dry		
83	609	05/06/2015	6	340058.94	185378.70	1	90	0		Marked as WB618 on Mapper		Negative	
84	610	05/06/2015	6	339975.46	185507.35	1	100	0		Marked as WB615 on Mapper		Negative	
85	611	05/06/2015	6	339865.00	185589.00	2	75	0		N/A		Negative	
86	612	05/06/2015	6			1				No sample: Inaccessible	Inaccessible		
87	620	05/06/2015	6	335982.00	185642.00	2	10	0		Only small area accessible		Negative	
88	621	05/06/2015	6	340103.00	185750.00	5	90	0		N/A		Negative	

Index	WB	Date	Area	OS Grid X	OS Grid Y	Number of inflows	% of perimeter accessible	Number of GCN on site during sampling	Flow	Notes	Unsampled	eDNA Result	eDNA Score
89	622	05/06/2015	6	339931.00	185637.00	3	75	0		N/A		Negative	
90	623	05/06/2015	6	339872.00	185654.00	3	40	0		Half of ditch dry between 625 and 627		Negative	
91	624	05/06/2015	6	339958.00	185753.00			0		No sample: Dry ditch	Dry		
92	625	05/06/2015	6	340097.00	185773.00	2	50	0		N/A		Negative	
93	630	05/06/2015	6	340515.05	185846.92	2	10	0	No	Very overgrown and difficult to access		Negative	
94	677	05/06/2015	6	339867.00	185740.00	1	30	0	No	Overgrown + shaded		Negative	
95	678	05/06/2015	6	339798.00	186027.00	5	30	0	OBVIOUS	Marked as WB832 on Mapper. 678 on paper maps		Negative	
96	760	05/06/2015	6	339857.00	185770.00	2	10	0	No	The majority of the ditch was dry (about 95%). We took a sample from a small area at the southern end.		Negative	
97	810	05/06/2015	6	340669.56	185920.49	2	10	0	No	Heavily overgrown and difficult to access; replicated on Imperial kit		Negative	
98	827	05/06/2015	6	340417.00	185731.00	1	20	0	No	Heavily overgrown		Positive	1
99	853	05/06/2015	6	339865.00	185956.00	2		0		No sample: Dry ditch	Dry		
100	985	05/06/2015	6	340663.83	185907.35	2	25	0	No	Heavily shaded and overgrown		Negative	
101	1066	05/06/2015	6	340010.32	185364.67	1	0			No sample: Not accessible	Inaccessible		
102	627	08/06/2015	6	340400.00	185978.00	2	100	0	No	N/A		Negative	
103	629	08/06/2015	6	340678.00	186038.00	2	100	0	No	N/A		Negative	
104	676	08/06/2015	6	340089.00	186033.00	2	50	0	No	N/A		Negative	
105	744	08/06/2015	6	340801.49	186073.05	1	70	0		N/A		Negative	
106	789	08/06/2015	6	339845.00	185322.00					No sample: Not surveyed. Mapping error. This is a 1m section marked as WB789 between 2 other ditches	Mapping error		
107	810	08/06/2015	6	340611.31	185989.69	1	30	0	No	Replicate: 90% of ditch		Negative	

Index	WB	Date	Area	OS Grid X	OS Grid Y	Number of inflows	% of perimeter accessible	Number of GCN on site during sampling	Flow	Notes	Unsampled	eDNA Result	eDNA Score
										was dry - sample taken from end of pitch			
108	875	08/06/2015	6	341191.83	186225.45	1	100	0		N/A		Negative	
109	890	08/06/2015	6	341081.91	186178.11		100	0		N/A		Negative	
110	898	08/06/2015	6	340115.00	185570.00	3	20	0	No	Heavily overgrown. Bullfinch singing nearby.		Negative	
111	912	08/06/2015	6	341041.48	186138.24	1	10	0		No sample: Ditch dry and dense vegetation difficult to access	Dry		
112	950	08/06/2015	6	341356.47	186259.63	1	100	0		N/A		Negative	
113	953	08/06/2015	6	341027.66	186133.72	1	5	0		No sample: Ditch dry and dense vegetation difficult to access	Dry		
114	973	08/06/2015	6							No sample: Inaccessible	Inaccessible		
115	984	08/06/2015	6	340721.79	186014.82	1	50	0	No	Water shallow, dry in some areas and continuous dense vegetation		Negative	
116	1351	08/06/2015	6	340031.69	185647.76	2	20	0	No	90% dry and heavily shaded		Negative	
117	628	09/06/2015	6	340465.00	185772.00	3	50	0	No	Dense vegetation on both sides. Waterbody heavily shaded		Negative	
118	637	09/06/2015	6	341184.00	186590.00	3	50	0	No	N/A		Positive	0.08
119	675	09/06/2015	6	341201.00	186476.00	2	60	0	No	Dense vegetation on one bank		Negative	
120	745	09/06/2015	6	340924.00	186139.00	1	100	0		N/A		Negative	
121	787	09/06/2015	6	340901.00	186130.00	1	100	0		WB 445 on mapper		Negative	
122	843	09/06/2015	6							No sample: Inaccessible. Not surveyed	Inaccessible		
123	845	09/06/2015	6							No sample: Inaccessible. Not surveyed	Inaccessible		
124	867	09/06/2015	6	341342.00	186266.00	1	100	0	No	N/A		Negative	
125	874	09/06/2015	6							No sample: Inaccessible. Not surveyed	Inaccessible		

Index	WB	Date	Area	OS Grid X	OS Grid Y	Number of inflows	% of perimeter accessible	Number of GCN on site during sampling	Flow	Notes	Unsampled	eDNA Result	eDNA Score
126	888	09/06/2015	6	340995.00	186164.00	1	100	0		N/A		Negative	
127	895	09/06/2015	6	341159.00	186155.00		20	0	No	Partially dry ditch overgrown with vegetation (hedge). Reed buntings & swans in ditch 882		Negative	
128	951	09/06/2015	6	341037.00	186451.00	5	50	0	No	N/A		Negative	
129	955	09/06/2015	6	340925.00	186542.00	8	30	0		Western half very overgrown and inaccessible. Fifteen samples, collected from eastern half where there is better access and great crested newt habitat		Negative	
130	962	09/06/2015	6	341137.00	186195.00	1	100	0		N/A		Negative	
131	968	09/06/2015	6	340892.93	186141.75	2	50	0		90% dry ditch. Wet either end of ditch. Abundant vegetation		Negative	
132	632	10/06/2015	6	341110.00	186717.00	2	50	0	No	N/A		Negative	
133	633	10/06/2015	6	341136.74	186665.01	1	50	0	No	N/A		Negative	
134	634	10/06/2015	6	341157.00	186629.00	2	100	0	No	N/A		Negative	
135	635	10/06/2015	6	341191.00	186670.00	1	70	0	No	30% overgrown and dry		Negative	
136	636	10/06/2015	6	341361.00	186767.00	2	90	0	No	10% of ditch dry at far end		Negative	
137	638	10/06/2015	6	341142.00	186605.00	1	80	0	No	Far end of ditch o/g with vegetation & not accessible		Negative	
138	639	10/06/2015	6	341068.00	186457.00	4	70	0	No	Steep banks, plenty of hawthorn, adjacent to busy highway		Negative	
139	640	10/06/2015	6	340989.00	186595.00	3	75	0	No	Some samples taken from opposite side of bank due to narrow width but most not available		Negative	
140	641	10/06/2015	6			O	0	0	No	No sample: Ditch completely dry and covered with vegetation	Dry		

Index	WB	Date	Area	OS Grid X	OS Grid Y	Number of inflows	% of perimeter accessible	Number of GCN on site during sampling	Flow	Notes	Unsampled	eDNA Result	eDNA Score
141	642	10/06/2015	6			1	80	0	No	No sample: Ditch dry and inaccessible. Overgrown with vegetation	Dry		
142	643	10/06/2015	6	340948.00	186695.00	2	20	0	No	N/A		Negative	
143	644	10/06/2015	6			0	0	0	No	No sample: Dry ditch	Dry		
144	645	10/06/2015	6							No sample: Inaccessible. Not surveyed	Inaccessible		
145	660	10/06/2015	6	341826.00	186868.00	2	95	0	No	Water very shallow only able to sample 30% of entire ditch. muddy water.		Negative	
146	662	10/06/2015	6	341791.00	186854.00	2	100	0	No	Sample labelled 642.		Negative	
147	665	10/06/2015	6	341749.00	186872.00	1	40	0	No	livestock on site.		Negative	
148	891	10/06/2015	6	341000.00	186448.00	2	15	0	No	Dense vegetation both banks, access difficult. Highly shaded waterbody.		Positive	0.08
149	892	10/06/2015	6	340966.00	186410.00	2	30	0	No	No sample: Site access difficult, dense vegetation. Dry ditch, can't be sampled	Dry		
150	963	10/06/2015	6	340913.00	186447.00	2	40	0	No	High levels of shade over waterbody.		Negative	
151	659	11/06/2015	6			3	40	0	No	No sample: Dry ditch	Dry		
152	663	11/06/2015	6	341787.05	186804.06	2	45	0	No	Very low water level		Negative	
153	664	11/06/2015	6	341567.00	186751.00	4	50	0	No	Majority (90%) of ditch was dry. Shallow water. High level of shade over waterbody.		Negative	
154	666	11/06/2015	6	345173.00	186765.00	1	80	0	No	90% of ditch dry. Samples taken at far end.		Negative	
155	667	11/06/2015	6	341672.13	186680.36	2	80	0		20% of ditch dry		Negative	
156	668	11/06/2015	6	341597.00	186859.00	2	70	0	No	Heavily shaded waterbody		Negative	
157	669	11/06/2015	6			3	0	0	No	No sample: Ditch dry with very limited access	Dry		
158	674	11/06/2015	6	341439.00	186810.00	1	30	0	No	Steep banks on one side and dense vegetation on the other – approximately		Negative	

Index	WB	Date	Area	OS Grid X	OS Grid Y	Number of inflows	% of perimeter accessible	Number of GCN on site during sampling	Flow	Notes	Unsampled	eDNA Result	eDNA Score
										50% not accessible due to vegetation			
159	830	11/06/2015	6	340907.00	186288.00	1	70	0	No	Only two small pools of water, most of ditch dry. Samples taken from two locations. 10x2 samples.		Negative	
160	873	11/06/2015	6	340938.00	186313.00	2	50	0	No	N/A		Negative	
161	887	11/06/2015	6	341618.48	186696.37	2	100	0	No	N/A		Negative	
162	894	11/06/2015	6	341551.73	186682.34	2	95	0	No	N/A		Negative	
163	914	11/06/2015	6	341460.00	186827.00	1	30	0	No	Dense vegetation (mainly hawthorn) on both banks		Negative	
164	919	11/06/2015	6	341526.00	186848.00	1	90	0	No	10% inaccessible due to hawthorn		Negative	
165	929	11/06/2015	6	340877.00	186587.00	3	0	0	No	No sample: Dry ditch and inaccessible	Dry		
166	966	11/06/2015	6	341085.00	186777.00	3	10	0	No	Only one side accessible		Negative	
167	981	11/06/2015	6	340416.00	185735.00	1	50	0	No	Sampled from one side only		Negative	
168	565	12/06/2015	5	337348.50	185438.70	3	100	0	No	N/A		Negative	
169	566	12/06/2015	5	337369.39	185438.19	2	90	0	No	N/A		Negative	
170	568	12/06/2015	5	337337.80	185533.10	1	0	0	No	N/A		Negative	
171	569	12/06/2015	5	337272.51	185429.07	2	80	0	No	N/A		Negative	
172	573	12/06/2015	5	336977.00	185357.00	2	60	0	OBVIOUS	N/A		Negative	
173	578	12/06/2015	5	336881.00	185676.00	2	50	0	YES, OBVIOUS	N/A		Negative	
174	1064	12/06/2015	5	337192.00	185722.00	0	0	0	No	No sample: Dry ditch	Dry		
175	1081	12/06/2015	5	337173.00	185586.00	4	5	0	No	Very shallow – 5 cm		Negative	
176	1083	12/06/2015	5	336997.00	185484.00	0	30	0	No	One bank inaccessible. Only 30% of other accessible. Very dense vegetation. Hawthorn.		Negative	

Index	WB	Date	Area	OS Grid X	OS Grid Y	Number of inflows	% of perimeter accessible	Number of GCN on site during sampling	Flow	Notes	Unsampled	eDNA Result	eDNA Score
										Water very turbid. Lots of sludge.			
177	1085	12/06/2015	5	337213.00	185604.00	1	20	0	No	Mostly overgrown with yellow flag iris so difficult to sample plus very shallow. 10 cm.		Negative	
178	1153	12/06/2015	5	337107.00	185604.00	1	10	0	No	50% dry and most inaccessible.		Negative	
179	1250	12/06/2015	5	337455.00	185574.00	0	15	0	No	N/A		Negative	
180	1253	12/06/2015	5	337109.00	185657.00	0	0	0	UNKNOWN	No sample: Inaccessible. Not possible to get close to due to overgrown shrubs/brambles. Reeds seen in vicinity	Inaccessible		
181	1254	12/06/2015	5	337058.00	185627.00	0	30	0	No	Dry ditch	Dry		
182	1257	12/06/2015	5	337374.00	185575.00	1	60	0	No	N/A		Negative	
183	1262	12/06/2015	5	337095.00	185625.00	0	10	0	No	No sample: Dry and overgrown	Dry		
184	1263	12/06/2015	5	337123.00	185412.00	2	45	0	No	Sluice opens which makes ditch flow.		Negative	
185	1288	12/06/2015	5	337033.00	185665.00	0	30	0	No	Only one side accessible.		Negative	
186	1293	12/06/2015	5	336990.00	185581.00	1	30	0	No	N/A		Negative	
187	1298	12/06/2015	5	336866.00	185783.00	2	50	0	SLIGHT FLOW	N/A		Negative	
188	1301	12/06/2015	5			0	0	0	No	No sample: Dry and overgrown	Dry		
189	4000	12/06/2015	5	337228.37	185522.71	3	30	0	No	Sedge warbler present.		Negative	
190	454	15/06/2015	4	335042.00	185431.00	2	50	0	No	50% of ditch dry at time of survey.		Negative	
191	455	15/06/2015	4			0	0	0	No	No sample: Dry ditch	Dry		
192	457	15/06/2015	4	335143.00	185605.00	1	35	0	No	60% dry ditch		Negative	
193	458	15/06/2015	4	335164.00	185655.00	2	50	0	No	Cuckoo		Negative	
194	460	15/06/2015	4	335032.00	185666.00	2	90	0	No	Several dead sheep in		Negative	

Index	WB	Date	Area	OS Grid X	OS Grid Y	Number of inflows	% of perimeter accessible	Number of GCN on site during sampling	Flow	Notes	Unsampled	eDNA Result	eDNA Score
										ditch at varying stages of decomposition			
195	491	15/06/2015	4	335309.00	185462.00	4	40	0	No	N/A		Negative	
196	1089	15/06/2015	4	335181.00	185509.00	1	70	0	No	N/A		Negative	
197	1100	15/06/2015	4	333197.00	185396.00	2	0	0	No	No sample: Dry ditch	Dry		
198	1102	15/06/2015	4	335393.00	185442.00	3	30	0	No	Sections were dry.		Negative	
199	1106	15/06/2015	4	335596.00	185506.00	1	80	0	No	Shallow water in certain areas.		Negative	
200	1107	15/06/2015	4	335887.00	185478.00	1	100	0	No	Water level very low.		Negative	
201	1111	15/06/2015	4	335294.00	185485.00	1	60	0	No	N/A		Positive	1
202	1117	15/06/2015	4	335960.00	185458.00	2	60	0	No	N/A		Negative	
203	1118	15/06/2015	4	335764.00	185522.00	1	100	0	No	N/A		Negative	
204	1123	15/06/2015	4	335321.00	185362.00	3	90	0	No	N/A		Negative	
205	1160	15/06/2015	4	335427.00	185492.00	4	85	0	No	Some dry sections		Negative	
206	1164	15/06/2015	4	335728.00	185564.00	3	60	0	No	Connects with 1118		Negative	
207	1338	15/06/2015	4							No sample: inaccessible	Inaccessible		
208	4001	15/06/2015	4	336066.00	185466.00	5	20	0	No	N/A		Negative	
209	452	16/06/2015	4	334912.00	185318.00	2	100	0	No	N/A		Negative	
210	453	16/06/2015	4	335817.00	185363.00	1	100	0		N/A		Negative	
211	459	16/06/2015	4	335135.00	185733.00	2	50	0	No	N/A		Negative	
212	464	16/06/2015	4	334865.00	185226.00	1	10	0	No	50% dry ditch. 2 adult treecreepers and 4 fledgelings flushed from nest in tree in ditch		Negative	
213	764	16/06/2015	4	935106.00	185440.00	2	15	0	No	Accessed through fencing. Very shallow water with lots of sludge (<5cm)		Negative	
214	796	16/06/2015	4	335532.00	185942.00	3	50	0		Long tailed tits. Access to only permitted to one side		Negative	
215	838	16/06/2015	4	335133.00	185474.00	2	15	0	No	Sampling taken through fence. Some areas <1cm		Negative	

Index	WB	Date	Area	OS Grid X	OS Grid Y	Number of inflows	% of perimeter accessible	Number of GCN on site during sampling	Flow	Notes	Unsampled	eDNA Result	eDNA Score
										deep.			
216	1101	16/06/2015	4	335531.00	185564.00	2	60	0	No	N/A		Negative	
217	1103	16/06/2015	4	335466.00	185780.00	3	40	0	No	Access to only one side of ditch		Negative	
218	1119	16/06/2015	4	335314.00	185799.00	1	80	0		50% dry		Negative	
219	1161	16/06/2015	4	335393.00	185835.00	2	90	0	No	15% of ditch dry		Negative	
220	1168	16/06/2015	4							No sample: Ditch dry. Veteran tree along ditch line	Dry		
221	1169	16/06/2015	4	335297.00	185695.00	1	100	0	No	N/A		Negative	
222	1179	16/06/2015	4	335432.00	185616.00	6	30	0	No	Large areas inaccessible		Negative	
223	1183	16/06/2015	4	118335.00	185858.00	3	90	0		N/A		Negative	
224	1325	16/06/2015	4	335219.00	185763.00	0	50	0	No	Half dry		Negative	
225	1335	16/06/2015	4	335295.00	185921.00	2	90	0	No	N/A		Negative	
226	1339	16/06/2015	4	NA	NA					Non-existent	Mapping error		
227	1340	16/06/2015	4	335247.00	185755.00	1	100	0	No	N/A		Negative	
228	42	17/06/2015	2	328377.00	183480.00	2	70	0	No	Sections of ditch dry ~50%		Negative	
229	43	17/06/2015	2	328311.00	183440.00	6	50	0	SLIGHT FLOW	Far bank inaccessible due to hedgerows		Negative	
230	50	17/06/2015	2	328025.00	183411.00	1	30	0	No	Ditch dry in 70%. Not easily accessible		Negative	
231	51	17/06/2015	2	328104.00	183440.00	2	30	0	No	N/A		Negative	
232	53	17/06/2015	2	328252.00	183451.00	2	0	0	No	No Sample: No access	Inaccessible		
233	54	17/06/2015	2	328529.00	183281.00	1	50	0	No	95% of ditch dry. Small section at end where sample taken		Negative	
234	56	17/06/2015	2	327997.00	183565.00	2	100	0	No	N/A		Negative	
235	80	17/06/2015	2	328758.00	183849.00	1	100	0	No	Very short		Negative	
236	90	17/06/2015	2	328746.00	183693.00	4	100	0	No	N/A		Negative	

Index	WB	Date	Area	OS Grid X	OS Grid Y	Number of inflows	% of perimeter accessible	Number of GCN on site during sampling	Flow	Notes	Unsampled	eDNA Result	eDNA Score
237	91	17/06/2015	2	328882.00	183810.00	1	25	0	No	No Sample: Ditch dry	Dry		
238	94	17/06/2015	2	328525.00	183551.00	2	50	0	No	N/A		Negative	
239	95	17/06/2015	2	328629.00	183591.00	1	100	0	No	3 x lapwing including juvenile		Negative	
240	98	17/06/2015	2	328782.00	183713.00	2	5	0	No	No Sample: Ditch dry. 2 pairs of lapwings defending territory in field adjacent to reen	Dry		
241	315	17/06/2015	2	328564.00	183575.00	3	50	0	No	N/A		Negative	
242	812	17/06/2015	2	328757.00	183722.00	1	100	0	No	Very short ditch. Numbers on paper map (832) & electronic mapper differ		Negative	
243	823	17/06/2015	2							No Sample: Ditch dry	Dry		
244	857	17/06/2015	2							No Sample: Ditch dry	Dry		
245	1391	17/06/2015	2	328160.00	183495.00	1	40	0	No	No Sample: Ditch dry	Dry		
246	1393	17/06/2015	2	328108.00	183529.00	0	2	0	No	Eight samples taken from three sites only. Very overgrown perimeter resulting in minimal access		Negative	
247	9956	17/06/2015	2	327902.00	183576.00	3	5	0	No	Samples taken from a single 5m stretch		Negative	
248	456	18/06/2015	4	335103.00	185519.00	2	40	0	No	Sample taken but dry along 60% of its length		Negative	
249	461	18/06/2015	4	334945.00	185497.00	1	20	0	No	Dense hedgerows on either side only able to get in gaps		Negative	
250	463	18/06/2015	4	334884.00	185543.00	3	60	0	No	Ditch dry in sections (20%) high shade cover		Negative	
251	465	18/06/2015	4	334958.00	185351.00	1	30	0	No	Dense vegetation on both banks. Highly shaded.		Negative	
252	466	18/06/2015	4			1	10	0	No	No sample: Dry ditch; Very limited access due to vegetation	Dry		
253	467	18/06/2015	4			1	15	0	No	No sample: Dry ditch	Dry		
254	468	18/06/2015	4	334612.00	185202.00	2	60	0	No	Pink invasive pond weed		Negative	

Index	WB	Date	Area	OS Grid X	OS Grid Y	Number of inflows	% of perimeter accessible	Number of GCN on site during sampling	Flow	Notes	Unsampled	eDNA Result	eDNA Score
										covering ditch			
255	469	18/06/2015	4			0	10	0	No	No sample: Dry ditch	Dry		
256	470	18/06/2015	4			0	0	0	No	No sample: Very small reen inaccessible due to fences & thick vegetation on both banks	Inaccessible		
257	471	18/06/2015	4			0	80	0	No	No sample: Dry ditch	Dry		
258	516	18/06/2015	4	335799.00	185922.00	2	90	0	No	N/A		Negative	
259	741	18/06/2015	4	335011.00	185536.00	3	40	O	No	N/A		Negative	
260	834	18/06/2015	4	334853.00	185580.00	2	90	0	No	838 on Mapper		Negative	
261	839	18/06/2015	4			2	1	0	No	No sample: Dry; Mapper 454, map 839. Mostly inaccessible and where was, was dry	Dry		
262	1005	18/06/2015	4							No sample: Dry	Dry		
263	1015	18/06/2015	4			1	O	O	No	No access except through Travellers Park	Inaccessible		
264	1016	18/06/2015	4			0	30	0	No	No sample: Dry ditch	Dry		
265	1017	18/06/2015	4			0				No access except through Travellers Park	Inaccessible		
266	1019	18/06/2015	4			0				No access except through Travellers Park	Inaccessible		
267	1023	18/06/2015	4			1	10	0	No	No sample: Dry ditch; Field which borders 1023, 1105 etc had >20 Meadow Brown butterflies	Dry		
268	1024	18/06/2015	4			3	10	0	No	No sample: Dry ditch	Dry		
269	1090	18/06/2015	4	335671.00	185818.00	1	80	0	No	N/A		Negative	
270	1091	18/06/2015	4	335633.00	185910.00	0			No	N/A		Negative	
271	1105	18/06/2015	4	335068.00	185756.00	2	5	0	No	N/A		Negative	
272	1122	18/06/2015	4	335126.00	185726.00	3	60	0	No	N/A		Negative	
273	1329	18/06/2015	4	335047.00	185772.00	1	100	0	No	No sample: Dry ditch	Dry		

Index	WB	Date	Area	OS Grid X	OS Grid Y	Number of inflows	% of perimeter accessible	Number of GCN on site during sampling	Flow	Notes	Unsampled	eDNA Result	eDNA Score
274	1330	18/06/2015	4	3350458.00	185841.00	0	5	0	No	No sample: Dry ditch	Dry		
275	1334	18/06/2015	4	335602.00	185729.00	1	80	0	No	N/A		Negative	
276	1341	18/06/2015	4	335205.00	185875.00	1	80	0	No	N/A		Negative	
277	398	19/06/2015	4	333949.00	185026.00	2	25	0	No	50% not accessible due to dense vegetation. Highly shaded.		Negative	
278	399	19/06/2015	4			1	5	0	No	No sample: Dry ditch; poor access, dense vegetation	Dry		
279	400	19/06/2015	4	334043.00	184961.00	3	25	0	No	Heavily shaded		Negative	
280	401	19/06/2015	4	334071.00	184988.00	1	15	0	No	One side of reen accessible - other side dr, majority runs under the path		Negative	
281	404	19/06/2015	4			1	0	0	No	No sample: Dry ditch	Dry		
282	405	19/06/2015	4	334219.00	185049.00	3	30	0	No	50% dry. High level of shade		Negative	
283	406	19/06/2015	4			1	5	0	No	No sample: Dry ditch; Also considerable access issues due to vegetation	Dry		
284	440	19/06/2015	4	334203.00	185011.00	2	20	0	No	Access only in gaps in vegetation		Negative	
285	446	19/06/2015	4			1	NA	0	No	No sample: Dry ditch	Dry		
286	451	19/06/2015	4	334828.00	185870.00	1	80	0	No	Steep bank on road side		Negative	
287	801	19/06/2015	4			1	80	0	No	No sample: Dry ditch	Dry		
288	55	22/06/2015	2	328300.00	183553.00	1	90	0	SLIGHT FLOW	N/A		Negative	
289	59	22/06/2015	2			0	0	0	No	No sample: No access, dense vegetation (hawthorn & thistles)	Inaccessible		
290	60	22/06/2015	2	327616.00	184085.00	0	90	0	OBVIOUS	Steep banks both sides		Negative	
291	64	22/06/2015	2	328460.00	183662.00	3	100	0	No	N/A		Negative	
292	432	22/06/2015	4	334353.00	185136.00				No	N/A		Negative	

Index	WB	Date	Area	OS Grid X	OS Grid Y	Number of inflows	% of perimeter accessible	Number of GCN on site during sampling	Flow	Notes	Unsampled	eDNA Result	eDNA Score
293	447	22/06/2015	4	334715.00	185133.00	3	100	0	No	N/A		Negative	
294	473	22/06/2015	4	334439.00	185261.00	3	50	0	No	N/A		Negative	
295	474	22/06/2015	4	334358.00	185433.00	1	15	0	No	Polluted with tyre pile. Very shallow.		Negative	
296	476	22/06/2015	4	334356.00	185396.00	4	50	0	No	No access permitted from farm side.		Negative	
297	477	22/06/2015	4	334298.00	185476.00	4	60	0	No	No access permitted from farm side.		Negative	
298	479	22/06/2015	4	334272.00	185575.00	1	40	0	No	No access for far side. - opposite road side.		Negative	
299	485	22/06/2015	4	334501.00	185116.00	0	50	0	No	Overgrown		Negative	
300	486	22/06/2015	4	334490.00	185127.00	2	40	0	No	Ditch hard to reach due to vegetation.		Negative	
301	863	22/06/2015	2			1	0	0	No	No sample: Ditch completely dry & inaccessible due to o/g vegetation	Inaccessible		
302	9999	22/06/2015	2			1	1	0	No	No sample: Dry ditch	Dry		
303	62	23/06/2015	2			1	5	0	No	No sample: Dry ditch	Dry		
304	63	23/06/2015	2			0	50	0	No	No sample: Dry ditch	Dry		
305	101	23/06/2015	2	328884.00	183687.00	1	25	0	No	N/A		Negative	
306	652	23/06/2015	6	340313.00	186230.00	0	100	0	No	Vertical banks so cautious access.		Negative	
307	820	23/06/2015	2	327819.00	183739.00	1	5	0	SLIGHT FLOW	Access only at inlet		Negative	
308	861	23/06/2015	2			1	0	0	No	No sample: Inaccessible	Inaccessible		
309	862	23/06/2015	2			0	30	0	No	No sample: Dry ditch	Dry		
310	902	23/06/2015	6			0	100	0	No	No sample: Within fenced off compound in separate fenced area. From vantage point looked dry.	Inaccessible		
311	954	23/06/2015	6	340387.00	186129.00	3	35	0	No	Couldn't access industrial site bank and only certain areas along roadside bank		Negative	

Index	WB	Date	Area	OS Grid X	OS Grid Y	Number of inflows	% of perimeter accessible	Number of GCN on site during sampling	Flow	Notes	Unsampled	eDNA Result	eDNA Score
312	971	23/06/2015	6	340350.00	186245.00	1	100	0	No	Very steep banks on both sides		Negative	
313	1344	23/06/2015	6	339884.00	186270.00	2	50	0	No	N/a		Negative	
314	1378	23/06/2015	2	327820.00	183815.00	1	10	0	No	Waterbody much bigger than indicated by map. May connect to 1386.		Negative	
315	1382	23/06/2015	2	328123.00	183622.00	2	100	0	OBVIOUS	Very steep banks		Negative	
316	1384	23/06/2015	2			2	0	0	No	No sample: Dry ditch	Dry		
317	1386	23/06/2015	2	327840.00	183847.00	0	40	0	No	Lots of fish. Only one side accessible.		Negative	
318	1389	23/06/2015	2	327789.00	183777.00	3	50	0	SLIGHT FLOW	Dry sections (10%), very steep banks (50%).		Negative	
319	1390	23/06/2015	2	327987.00	183704.00	2	15	0	No	Only 25% with water and only some accessible.		Negative	
320	9960	23/06/2015	2			3	0	0	No	No sample: Inaccessible from sides by reen 43. Direction of reen is not as suggested by map. Surrounded by bramble	Inaccessible		
321	672	24/06/2015	6	341255.00	186593.00	3	20	0		80% dry		Negative	
322	698	24/06/2015	7	345448.00	187409.00	2	50	0	No	Far bank inaccessible, obstructed by hedgerow		Negative	
323	699	24/06/2015	7	345468.00	187364.00	2	50	0	No	Far bank inaccessible, obstructed by hedgerow		Negative	
324	700	24/06/2015	7	345291.00	187446.00	2	15	0	SLIGHT FLOW	85% of ditch inaccessible and possibly also dry		Negative	
325	701	24/06/2015	7	345431.00	187324.00	2	50	0	No	N/A		Negative	
326	702	24/06/2015	7							No sample: Dry	Dry		
327	703	24/06/2015	7	345527.00	187385.00	1	40	0	No	Access from one bank only. Steep banks in parts		Negative	
328	704	24/06/2015	7	335338.00	187319.00	2	50	0	No	N/A		Negative	
329	705	24/06/2015	7	345161.00	187387.00	0	50	0		N/A		Negative	
330	772	24/06/2015	7	345592.00	187408.00	1	50	0	No	Only one bank accessible. Nearside bank steep, but		Negative	

Index	WB	Date	Area	OS Grid X	OS Grid Y	Number of inflows	% of perimeter accessible	Number of GCN on site during sampling	Flow	Notes	Unsampled	eDNA Result	eDNA Score
										accessible			
331	773	24/06/2015	7							No sample: Inaccessible	Inaccessible		
332	844	24/06/2015	6	340269.00	186108.00	1	50	0	No	N/A		Negative	
333	988	24/06/2015	7							No sample: Dry ditch	Dry		
334	1342	24/06/2015	6	340032.00	186071.00	2	50	0	OBVIOUS	N/A		Negative	
335	1350	24/06/2015	6	339825.00	185744.00	2	50	0	No	Only access via main road. Waterbody around 1.5km long		Negative	
336	378	25/06/2015	4							No sample: Dry ditch	Dry		
337	379	25/06/2015	4	333864.00	185150.00	0	60	0	No	N/A		Negative	
338	380	25/06/2015	4							No sample: Inaccessible	Inaccessible		
339	381	25/06/2015	4							No sample: Inaccessible	Inaccessible		
340	382	25/06/2015	4							No sample: Water too shallow	Too shallow		
341	383	25/06/2015	4							No sample: Dry ditch	Dry		
342	384	25/06/2015	4							No sample: Dry ditch	Dry		
343	385	25/06/2015	4							No sample: Inaccessible	Inaccessible		
344	386	25/06/2015	4							No sample: Inaccessible	Inaccessible		
345	387	25/06/2015	4	333953.00	185401.00	2	50	0	No	N/A		Negative	
346	388	25/06/2015	4							No sample: Dry ditch	Dry		
347	389	25/06/2015	4	334026.00	185385.00	3	45	0	No	N/A		Negative	
348	390	25/06/2015	4							No sample: Inaccessible	Inaccessible		
349	391	25/06/2015	4	334188.00	185398.00			0	No	N/A		Negative	
350	393	25/06/2015	4							No sample: Inaccessible	Inaccessible		
351	394	25/06/2015	4	333955.00	185176.00	1	5	0	No	Poor access due to dense vegetation of hawthorn and brambles. Only small pool sampled		Negative	
353	395	25/06/2015	4	334059.00	185115.00	3	2	0	No	Very inaccessible. All		Negative	

Index	WB	Date	Area	OS Grid X	OS Grid Y	Number of inflows	% of perimeter accessible	Number of GCN on site during sampling	Flow	Notes	Unsampled	eDNA Result	eDNA Score
										samples taken from one 5m wide point			
355	396	25/06/2015	4							No sample: Dry ditch	Dry		
356	397	25/06/2015	4							No sample: Inaccessible	Inaccessible		
357	402	25/06/2015	4							No sample: Inaccessible & dry	Dry		
358	403	25/06/2015	4							No sample: Dry ditch	Dry		
359	472	25/06/2015	4							No sample: Dry ditch	Dry		
360	478	25/06/2015	4							No sample: Dry ditch	Dry		
361	487	25/06/2015	4							No sample: Dry ditch	Dry		
362	833	25/06/2015	4							No sample: Dry ditch	Dry		
363	1007	25/06/2015	4							No sample: Inaccessible	Inaccessible		
364	1009	25/06/2015	4							No sample: Dry ditch	Dry		
365	1010	25/06/2015	4							No sample: Inaccessible	Inaccessible		
366	1020	25/06/2015	4							No sample: Dry ditch	Dry		
367	179	26/06/2015	3	330481.00	184451.00	3	50	0	No	N/A		Negative	
368	180	26/06/2015	3	330320.00	184518.00	2	60	0	No	N/A		Negative	
369	369	26/06/2015	4							No sample: Ditch dry and inaccessible	Dry		
370	374	26/06/2015	4	333519.00	185142.00	1	75	0	No	N/A		Negative	
371	375	26/06/2015	4							No sample: Ditch dry	Dry		
372	376	26/06/2015	4							No sample: Ditch dry and inaccessible	Dry		
373	377	26/06/2015	4							No sample: Ditch dry	Dry		
374	407	26/06/2015	4							No sample: Inaccessible	Inaccessible		
375	408	26/06/2015	4							No sample: Ditch dry	Dry		
376	409	26/06/2015	4	334372.00	184958.00	1	10	0	No	N/A		Negative	
377	410	26/06/2015	4	334416.00	184985.00							Negative	
378	412	26/06/2015	4							No sample: No access	Inaccessible		

Index	WB	Date	Area	OS Grid X	OS Grid Y	Number of inflows	% of perimeter accessible	Number of GCN on site during sampling	Flow	Notes	Unsampled	eDNA Result	eDNA Score
379	413	26/06/2015	4	334386.00	185061.00	2	30	0	No	Far side inaccessible. Vegetation obstructing much of near side		Negative	
380	414	26/06/2015	4							Waterbody does not exist	Mapping error		
381	431	26/06/2015	4							No sample: Ditch dry	Dry		
382	442	26/06/2015	4	333973.00	184923.00	0	1	50	No	N/A		Negative	
383	462	26/06/2015	4	335009.00	185397.00	1	30	0	No	N/A		Negative	
384	646	26/06/2015	6	340832.00	186543.00	2	25	0	No	N/A		Negative	
385	648	26/06/2015	6	340807.00	186493.00	2	5	0	No	Access restricted by vegetation. Field itself also very difficult to access, all thistles & nettles to ~6ft		Negative	
386	832	26/06/2015	4							No sample: Ditch dry	Dry		
387	908	26/06/2015	6							No sample: Ditch dry	Dry		
388	964	26/06/2015	6							No sample: Ditch dry	Dry		
389	673	30/06/2015	6	341381.00	186526.00	3	40	0	No	N/A		Negative	
390	847	30/06/2015	6	341315.00	186455.00	3	100	0		N/A		Negative	
391	850	30/06/2015	6	341434.00	186352.00	3	50	0	No	N/A		Negative	
392	882	30/06/2015	6	341235.00	186328.00	3	50	0		N/A		Negative	
393	1006	30/06/2015	4	334957.00	185229.00	2	40	0	No	N/A		Negative	
394	1112	30/06/2015	4							No sample: Ditch dry	Dry		
395	1166	30/06/2015	4							No sample: Ditch dry	Dry		
396	1333	30/06/2015	4	335092.00	185301.00	3	100	0	No	Whorl grass (Catabrosa aquatica)		Negative	

Annex C: Laboratory Standards

Laboratory Standards

Laboratory A: Laboratory Standards in place for great crested newt eDNA work

Environmental DNA work can involve low quantities of DNA, and therefore steps need to be taken in order to reduce the risk of false positive and negative results. We have implemented the following steps and quality controls in order to minimise the risk and assure that false results will be avoided.

- Field sampling kits are prepared in a separate room where no samples are stored and no DNA extraction or PCR amplification takes place. Personnel wear dedicated laboratory coats for this area, and bleaching of surfaces takes place daily.
- We operate a separate clean room for preparing environmental DNA samples that is performing DNA extraction and setting up PCR reactions. This room is subject to positive pressure to prevent entry of DNA from external areas. Personnel wear dedicated laboratory coats for this area, and bleaching of surfaces and UV treatment of equipment (pipettes, plastics) takes place daily.
- Positive control DNA (from GCN tissue) is prepared in a DNA extraction area separate from the clean room described above. The diluted positive control DNA standards are the final item to be added when setting up qPCR reaction plates to minimise the risk of cross contamination.
- qPCRs are performed in a dedicated area for DNA amplification, separate from those described above.
- Water samples from a pond known to contain great crested newts and from one known to be free from them were extracted and qPCR performed, giving results as expected.

Laboratory B: Procedures to prevent and detect possible contamination

All tests were carried following standard procedures to prevent and detect contamination:

- To prevent contamination, pre and post-PCR areas are in different rooms, and the working flux is always unidirectional.
- To detect contamination, 2 kinds of negative control are carried out at the laboratory:
- To detect DNA contamination during extraction, an extraction blank is added to every qPCR run. Result: 12 out of 12 technical replicates were negative.
- To detect DNA contamination during the preparation of the qPCR plate, a non-template control is added in every run. Result: 4 out of 4 technical replicates were negative.
- Besides, a DNA sample known to be positive (from a pond were it is known the presence of newts) is added to the analysis as a positive control. Result: 10 out of 12 technical replicates were positive.