

Welsh Government

**M4 Corridor around Newport**

Environmental Statement

Supplement

Volume 3: Appendix S10.6

**2016 Great Crested Newt Survey**

M4CaN-DJV-EBD-ZG\_GEN-AX-EN-0044

At Issue | September 2016

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## Summary

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- S.1** RPS has undertaken a great crested newt (*Triturus cristatus*) Presence / Absence (eDNA technique) survey for the proposed M4 Corridor around Newport (M4CaN) (referred to hereafter as ‘the Scheme’) over the period of mid-May 2016 until the end of June 2016. This survey fell within the optimum period for this type of survey. The adopted survey method was based on that described in Biggs *et al.* (2014).
- S.2** Population Size Class Estimate Surveys and Presence / Absence Surveys (traditional methods) were also undertaken from 11<sup>th</sup> April 2016 to 16<sup>th</sup> June 2016, which falls within the optimum period for this type of survey. The survey method was based on that described in the Great Crested Newt Mitigation Guidelines (English Nature, 2001).
- S.3** The 2016 surveys fell into two categories:
- land generally within 250 metres (m) of the proposed new section of motorway that was not surveyed during 2015; and
  - all watercourses within 250 m of the location of positive eDNA results identified during the 2015 eDNA sampling.
- S.4** Watercourses not covered during the 2014 Arup (March 2016 ES Appendix 10.6) or the 2015 eDNA survey (March 2016 ES Appendix 10.22) were visited during March / April 2016 to assess them in terms of accessibility (i.e. dense vegetation over watercourses) and in terms of the safety of surveyors (i.e. steep banks and / or deep water). Habitat Suitability Index (HSI) scores from the 2014 Arup Phase 1 Survey (March 2016 ES Appendix 10.2) were taken into account when assessing each watercourse. Four areas were surveyed:
- TATA Steel Land;
  - adjacent to the eastbound carriageway of the existing M4 north of Undy;
  - adjacent to the westbound carriageway of the existing M4 north of Undy; and
  - within Marshall Land on the east bank of the River Usk.
- S.5** Watercourses within 250 m of the locations of positive 2015 eDNA were identified. Three locations of positive eDNA results were identified during the 2015 eDNA sampling including:
- the Bareland Street area south of Llandeenny;
  - land to the east of Tonew Kennels; and
  - land south of Tatton Farm.
- S.6** A total of 210 watercourses were identified. Of the watercourses identified:
- a total of 105 watercourses were identified for Presence / Absence survey (using either eDNA techniques or traditional methods);
  - a total of 94 watercourses were found to not provide suitable habitat for great crested newts at the time of the survey; and

- a further eleven watercourses were not surveyed because they were inaccessible, either due to barriers preventing access or due to health and safety reasons.

**S.7** Where traditional survey methods were used, no great crested newts were found.

**S.8** The results of the 2016 surveys demonstrate that great crested newt DNA was detected at the same locations as 2015 (March 2016 ES Appendix 10.22). In addition, great crested newt eDNA was detected at one location where it was not previously detected. This was at watercourse 1,250, which is adjacent to the location where three individual great crested newts were found underneath reptile survey mats during the 2015 Reptile Survey (March 2016 ES Appendix 10.27).

**S.9** Based upon the traditional survey data and the eDNA data results, it is considered that a number of small populations are present within the 2016 survey areas, and that where they are present the great crested newts are in small, potentially isolated pockets. The presence of populations of predatory fish and the availability of habitat suitable for great crested newt (with no extensive 'fish-free' areas within which a significant and sustainable metapopulation of great crested newt could exist) is likely to be a significant constraint on the population within the vicinity of the proposed new section of motorway and more widely within the Gwent Levels.



# 1 Introduction

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- 1.1.1** RPS has undertaken a great crested newt Population Size Class Estimate Survey and a Presence / Absence Survey (using both traditional and eDNA methodologies) of land within the corridor of the proposed new section of motorway forming part of the M4 Corridor around Newport project (referred to hereafter as 'the Scheme') between Castleton and Magor. The survey areas are shown in Figure 1.
- 1.1.2** The survey included land generally within 250 m of the proposed alignment that was not surveyed during 2015 (March 2016 ES Appendix 10.22) or where there were positive results during great crested newt eDNA sampling during 2015, following the methodologies set out in the Great Crested Newt Mitigation Guidelines (English Nature, 2001) and in the Analytical and Methodological Development for Improved Surveillance of the Great Crested Newt (Biggs *et al.*, 2014).
- 1.1.3** During 2014, a Habitat Suitability Index (HSI) assessment and Great crested newt Presence / Absence survey was undertaken by Arup on behalf of Welsh Government (March 2016 ES Appendix 10.6).
- 1.1.4** A great crested newt Presence / Absence (eDNA Technique) Survey was undertaken by Thompson Ecology on behalf of RPS in June 2015 using standard Biggs *et al.* (2014) methodology. This is reported in March 2016 ES Appendix 10.22.
- 1.1.5** This report describes the methods used in the 2016 survey (Section 2) and the findings of the survey (Section 3). A discussion of the survey findings is provided in Section 4.

## 2 Survey Methods

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### 2.1 Introduction

**2.1.1** The great crested newt Population Size Class Estimate and Presence / Absence surveys were carried out between April and mid-June 2016, which falls within the optimum period for this type of survey.

**2.1.2** The eDNA sampling was carried out between mid-May and the end of June which falls within the optimal time for this type of survey.

### 2.2 Methodologies

#### Scoping Assessment

**2.2.1** The 2016 surveys fell into two categories, these were:

- areas not surveyed during the 2015 eDNA sampling; and
- all watercourses within 250 m of the location of positive eDNA results identified during the 2015 eDNA sampling (Appendix 10.22 of the March 2016 ES).

**2.2.2** The findings of the scoping survey are presented in Annex A and are shown on Figure 2.

**2.2.3** Watercourses that were not covered during the 2015 eDNA sampling were visited during March / April 2016 to ground truth in terms of accessibility (i.e. dense vegetation over watercourses) and in terms of the safety of surveyors (i.e. steep banks and / or deep water). The suitability of each watercourse was undertaken based upon the HSI scores from the 2014 Arup survey (March 2016 ES Appendix 10.2) and on the professional judgement of the experienced Great crested newt surveyor. HSI assessment was not repeated as part of the 2016 survey.

**2.2.4** Four areas were surveyed:

- TATA Steel Land;
- adjacent to the eastbound carriageway of the existing M4 north of Undy;
- adjacent to the westbound carriageway of the existing M4 north of Undy; and
- within Marshall Land on the east bank of the River Usk.

**2.2.5** Watercourses from within 250 m of the location of positive 2015 eDNA were identified. Three locations of positive eDNA results were identified during the 2015 eDNA sampling including:

- the Bareland Street area south of Llandeenny;
- land to the east of Tonew Kennels; and
- land south of Tatton Farm.

**2.2.6** A total of 210 watercourses were identified. Of the watercourses identified:

- a total of 105 watercourses were identified for Presence / Absence survey (using either eDNA techniques or traditional methods);

- a total of 94 watercourses were found to not provide suitable habitat for great crested newts at the time of the survey; and
- a further 11 watercourses were not surveyed because they were inaccessible, either due to barriers preventing access or due to health and safety reasons.

## Great Crested Newt Sampling Techniques

**2.2.7** Three survey methods were used. These included:

- eDNA sampling as described in Biggs *et al.* (2014) and Natural Resources Wales (2015);
- Presence / Absence surveys as described in the Great Crested Newt Mitigation Guidelines (English Nature, 2001); and
- Population Size Class Estimate surveys as described in the Great Crested Newt Mitigation Guidelines (English Nature, 2001).

## eDNA Sampling

**2.2.8** The surveyors were experienced in carrying out traditional great crested newt surveys and were specifically trained in the eDNA surveying technique, as described by Biggs *et al.* (2014), by either Freshwater Habitats Trust or by ADAS prior to collecting water samples.

**2.2.9** The 2016 eDNA sampling areas fell into two distinct types, these were:

- areas that were not surveyed during 2015 due to lack of access; and
- watercourses where great crested newt eDNA was detected in 2015.

**2.2.10** The location of each watercourse and waterbody subject to eDNA sampling during 2016 is shown in Figure 3.

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

**2.2.12** Surveyors collected 30 millilitre (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 constraints meant this was not possible in some locations but samples were spread out as much as practicable. Further details of the accessibility of each waterbody are provided in the full data set provided in Annex B of this report.

**2.2.13** 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.

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

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

#### **Laboratory Protocol**

- 2.2.16** Water sample analysis was conducted by SureScreen Scientifics with ISO9001 and ISO13485 accreditation working to a quality policy that adheres to the ISO17025 standard.
- 2.2.17** The water samples were analysed using the quantitative Polymerase Chain Reaction (qPCR) eDNA test, following the protocols provided by Biggs *et al.* (2014).
- 2.2.18** 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.
- 2.2.19** 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.

#### **Population Size Class Estimate & Presence / Absence Surveys**

- 2.2.20** The survey methodology was based on guidelines set out in the Great Crested Newt Mitigation Guidelines (English Nature, 2001).
- 2.2.21** For Population Size Class Estimate Surveys, a total of six surveys were undertaken between mid-March and mid-June with at least three surveys undertaken between mid-April and mid-May.
- 2.2.22** For Presence / Absence surveys, a total of four surveys were undertaken between mid-March and mid-June with at least two surveys undertaken between mid-April and mid-May. In many cases, one or two extra visits were undertaken as part of the Presence / Absence Surveys. This was done to provide further confidence in the survey results because it was not possible to undertake bottle-trapping due to the presence of water shrew (see paragraph 2.3.6 and 2.3.7).
- 2.2.23** For each survey visit, three of each of the following methodologies were used in accordance with the Great Crested Newt Mitigation Guidelines (English Nature, 2001).

#### **Torching**

- 2.2.24** Torch surveying was conducted between 30 minutes after sunset and midnight using 1,000,000 candlepower torches. During the torch survey, all accessible watercourses were systematically checked for great crested newt within the water column. Other aquatic fauna seen during the survey was also recorded, along with details of factors that could impact upon the effectiveness of the search such as water clarity; accessibility; or vegetation covering the surface of the water. This technique was not used during periods of heavy rain or wind as it is not an effective method in these conditions.
- 2.2.25** A 2 m stand-off was implemented where the bankside was steep and uneven ground prevented safe access to the water's edge.

## Egg Searching

- 2.2.26** Great crested newts typically lay eggs on pliable leaves of aquatic vegetation, although dead leaves of aquatic vegetation, leaf litter or discarded litter may be used.
- 2.2.27** To increase the likelihood of identifying the presence of breeding great crested newt, egg-laying strips were placed throughout all watercourses at intervals of 10 m along the sections of safely accessible watercourse at each of the survey locations. Where sections of watercourse were not accessible, then egg laying strips were concentrated in areas that were accessible.
- 2.2.28** The egg-laying strips comprised long strips of polyethylene attached to canes, which mimic the vegetation typically used by great crested newts. Each of the strips was inspected for the eggs of great crested newt.

## Netting

- 2.2.29** Netting was undertaken where watercourses were safely accessible. A long handled net with a fine mesh small enough to catch adult newts and larvae with minimal risk of injury was used. After each sweep the contents of the net was carefully inspected.

## Bottle Trapping

- 2.2.30** This method involves setting bottle traps (normally made from 2-litre plastic bottles) around the pond margin, and leaving the traps set overnight. A density of one trap per two metres of shoreline is recommended for general survey purposes. Bottle trapping should only be used when the night-time air temperature is >5°C. This method was carried out in accordance with guidelines on animal welfare (English Nature, 2001).
- 2.2.31** The watercourses subject to Population Size Class Estimate and Presence / Absence Surveys are shown in Figure 4.

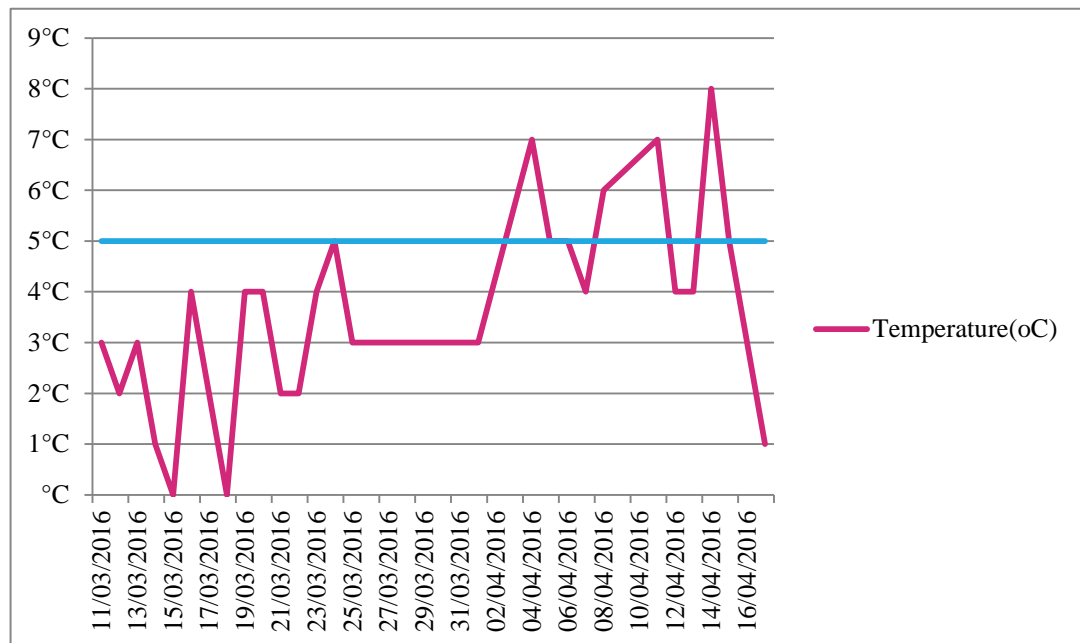
## 2.3 Limitations

- 2.3.1** A variety of constraints were encountered during the survey, these included:
- dense emergent and bank vegetation;
  - dense floating vegetation;
  - hedge preventing access;
  - high turbidity;
  - high water level;
  - steep banks; and
  - presence of water shrew.
- 2.3.2** The limitations to the survey for each watercourse (where relevant) are shown in the constraints tables in Annex B.

## Weather Conditions during Late March and Early April 2016

**2.3.3** Great crested newt surveys during late March and early April could not proceed due to a sustained cold period when night-time temperatures were consistently below 5°C. These temperatures were recorded and are shown in the graph below.

### Inset 1 March - April 2016 Temperatures



## Safe Access to Watercourse

**2.3.4** The watercourses included within the survey varied in character and ease of access. Consequently, egg searching and netting of watercourse was not always possible, and visibility during torching was reduced. Duckweed coverage and high turbidity also occasionally obscured the visibility of the watercourse during torching.

**2.3.5** A small number of watercourses within the TATA survey area were not accessible and as such their suitability to be used by great crested newts was not able to be determined. This includes watercourses 537, 1,069, 1,136 and 1,219. Watercourses to the west and east were considered unsuitable for great crested newts. To the north, watercourse 1,257 was included in the survey. However no GCN were recorded.

## Water Shrew and Bottle Trapping

**2.3.6** Water shrews were captured in bottle traps at two locations, and Bareland West (638) and Tatton South (1099) on 5<sup>th</sup> April 2016 and 20<sup>th</sup> April 2016 respectively.

**2.3.7** Bottle trapping is potentially lethal to water shrews therefore following the captures bottle traps were no longer used during survey visits. As bottle trapping is often the most effective method of survey, survey effort was increased by installing extra egg laying strips (installed every 10 m along watercourses) and undertaking longer searches with torching.

## 3 Results

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### 3.1 Introduction

**3.1.1** This section provides the findings of the great crested newt Presence / Absence (eDNA technique) survey and the Population Size Class Estimate / Presence Absence Surveys using methods cited in the Great Crested Newt Mitigation Guidelines (English Nature, 2001).

### 3.2 Results

**3.2.1** Full results are presented in Annex C and Annex D and are shown in Figure 5. Positive results in terms of identifying the presence of great crested newt or of great crested newt DNA are presented in the following section.

#### eDNA Sampling

**3.2.2** The location of each positive eDNA result is shown on Figure 5.

**3.2.3** Positive results for the presence of great crested newt DNA were identified in the following watercourses:

- 637 in Bareland Street Area south of Llandeenny;
- 827 in the land to the east of Tonew Kennels;
- 1,111 in the land south of Tatton Farm; and
- 1,250 in TATA Steel Landholding.

**3.2.4** The Bareland Street, land east of Tonew Kennels and the Tatton Farm positive results were at the same locations as previous positive eDNA results from surveys undertaken during 2015.

**3.2.5** The positive result within watercourse 1,250 was a new finding in terms of great crested newt eDNA presence. This watercourse is adjacent to the location where three individual great crested newts were found under survey mats during reptile surveys in 2015 (March 2016 ES Appendix 10.27).

#### Population Size Class Estimate & Presence / Absence Surveys

**3.2.6** The full results of the 2016 surveys are presented in Annex D.

**3.2.7** No great crested newts were identified during the Population Size Class Estimate and Presence / Absence Surveys.



## 4 Discussion

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### 4.1 Introduction

- 4.1.1** This section summarises the main findings of the surveys and refers also to the results of the previous surveys in 2014 (March 2016 ES Appendix 10.6) and 2015 (March 2016 ES Appendix 10.22). It sets out the key considerations for the proposed new section of motorway and requirements for any further surveys.

### 4.2 Survey Findings

- 4.2.1** Out of a total of 35 watercourses surveyed using traditional methods as set out in the Great Crested Newt Mitigation Guidelines (English Nature, 2001), no great crested newt were identified.
- 4.2.2** As noted within the limitations section, bottle trapping was halted following the discovery of water shrew at two locations. These were at Tatton Farm (1,099) and at Bareland Street (636). Therefore torching, netting and egg searching were the survey techniques used, where safe / possible to do so. To increase the effectiveness of survey techniques, egg laying strips were installed at intervals of every 10 m and were extensively searched during each survey where possible and safe torching effort was extended.
- 4.2.3** eDNA sampling was undertaken at 29 watercourses and ponds in locations that were not sampled during 2015 (March 2016 ES Appendix 10.22). A single watercourse (1,250) was found to contain great crested newt eDNA. This watercourse was adjacent to the locations where three individual great crested newts were identified during reptile surveys during the summer of 2015 (March 2016 ES Appendix 10.27). Sampling on all other watercourses did not detect any great crested newt eDNA.
- 4.2.4** To support the findings of the traditional surveys, great crested newt eDNA sampling was undertaken at three locations where great crested newt eDNA was found during 2015 (March 2016 ES Appendix 10.22). The samples for all of these watercourses were positive.
- 4.2.5** Fish, including extensive populations of three spined stickleback *Gasterosteus aculeatus*, were found in moderate to high abundance throughout the areas surveyed during 2016. Fish are known predators of great crested newt eggs and efts and where this species occurs, the likelihood that a breeding population will be present is significantly reduced.

### 4.3 Key Considerations

- 4.3.1** The key survey technique for the traditional surveys would, under usual circumstances, be trapping. However this was not undertaken due to the presence of water shrew within the survey area. To compensate for not being able to trap, egg laying strips were installed at a significantly higher density and given that female great crested newt will lay approximately 250 eggs per season, it is considered that there would be a high likelihood of detecting great crested newt eggs using this method with an increased number of egg laying strips installed.



- 4.3.2** Using traditional survey methods no great crested newts were found. Although it should be noted that eDNA data does not allow population size estimates but purely presence / absence of DNA. The presence of positive eDNA results at four locations within the survey area indicates that great crested newt are present in numbers that were not detected using traditional survey methods.
- 4.3.3** Great crested newt DNA was detected at the same locations as 2015 (March 2016 ES Appendix 10.22). In addition, great crested newt eDNA was detected at one location where not previously detected. This was watercourse 1,250 that is adjacent to the location where three individual great crested newts were found underneath reptile survey mats during the 2015 Reptile Survey (March 2016 ES Appendix 10.27).
- 4.3.4** Based upon the traditional survey data and the eDNA data results, it is considered that a number of small populations are present within the 2016 survey areas, and that, where they are present, the great crested newts are in small, potentially isolated pockets. The presence of populations of predatory fish and the availability of habitat suitable for great crested newt (with an absence of extensive 'fish-free' areas within which a significant and sustainable metapopulation of great crested newt could exist) is likely to be a significant constraint on the population within the vicinity of the proposed new section of motorway and more widely within the Gwent Levels.

## 5 References

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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.

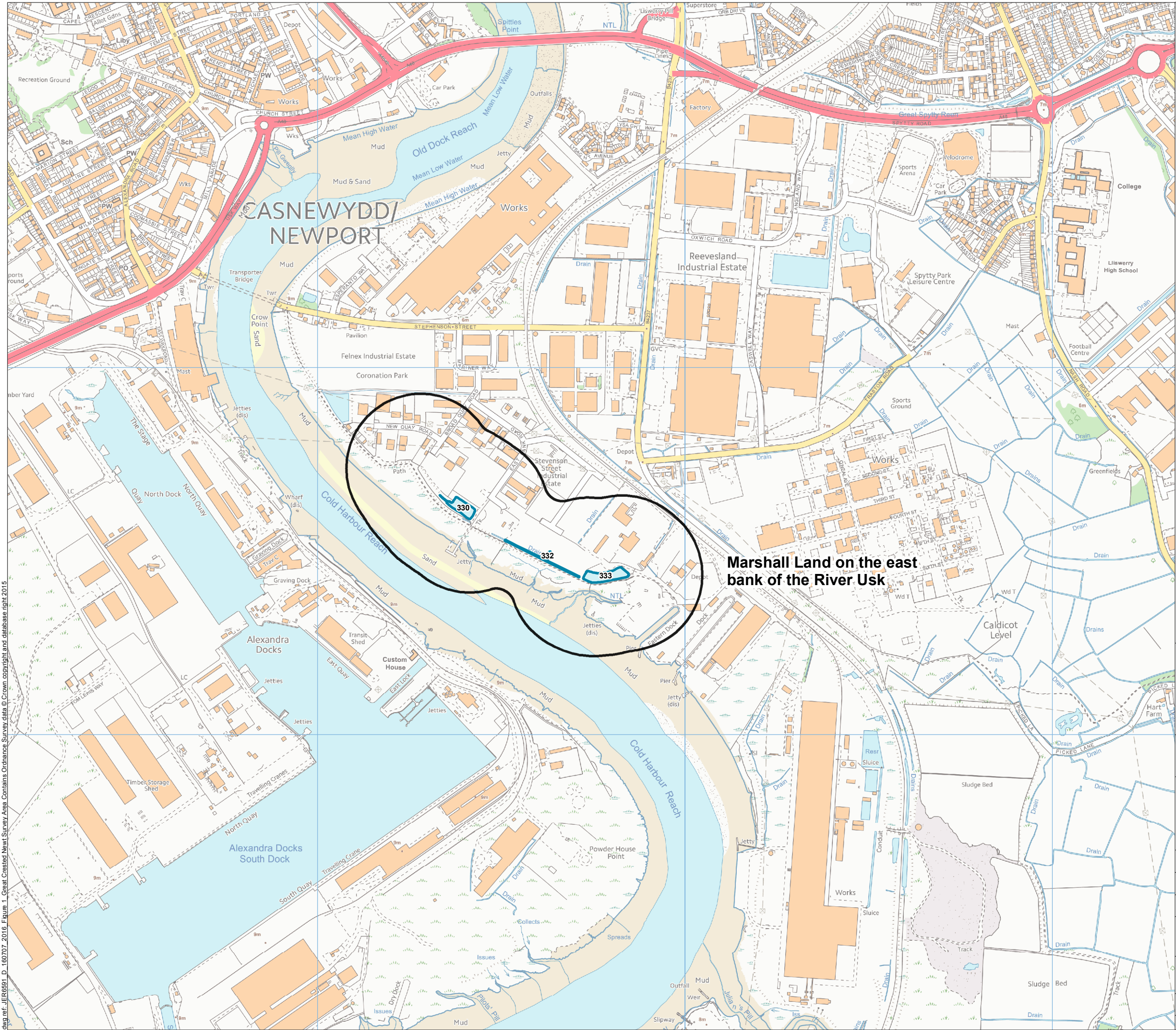
English Nature (2001) Great Crested Newt Mitigation Guidelines. English Nature: Peterborough

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

# Figures

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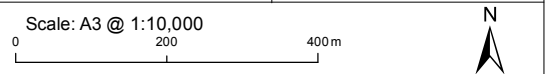
- Legend**
- Great Crested Newt Survey
  - Watercourses



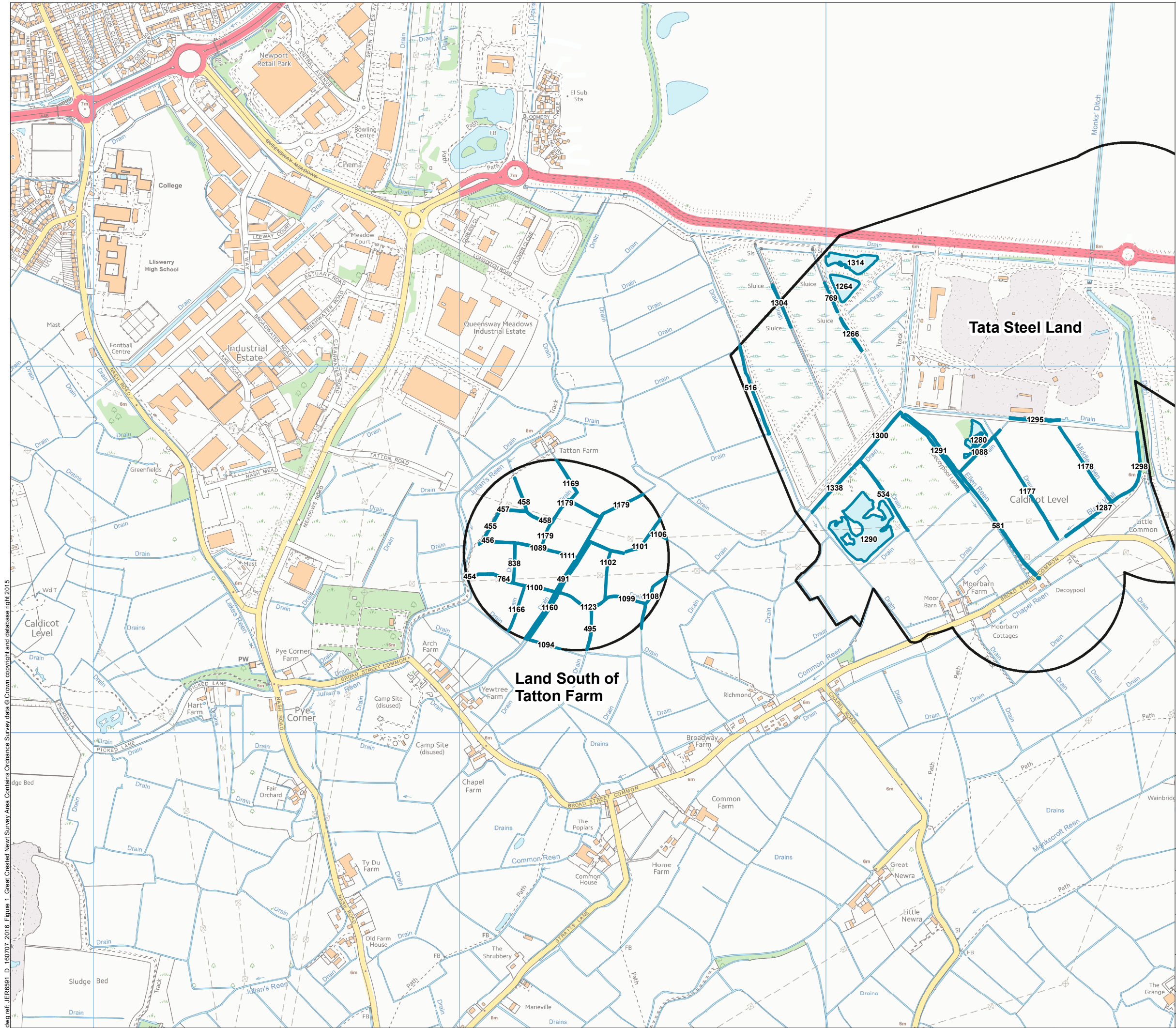
Llywodraeth Cymru  
Welsh Government

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2016 Great Crested Newt Survey  
Area


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**Legend**

 Great Crested Newt Survey

 Watercourses

  
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**2016 Great Crested Newt Survey Area**

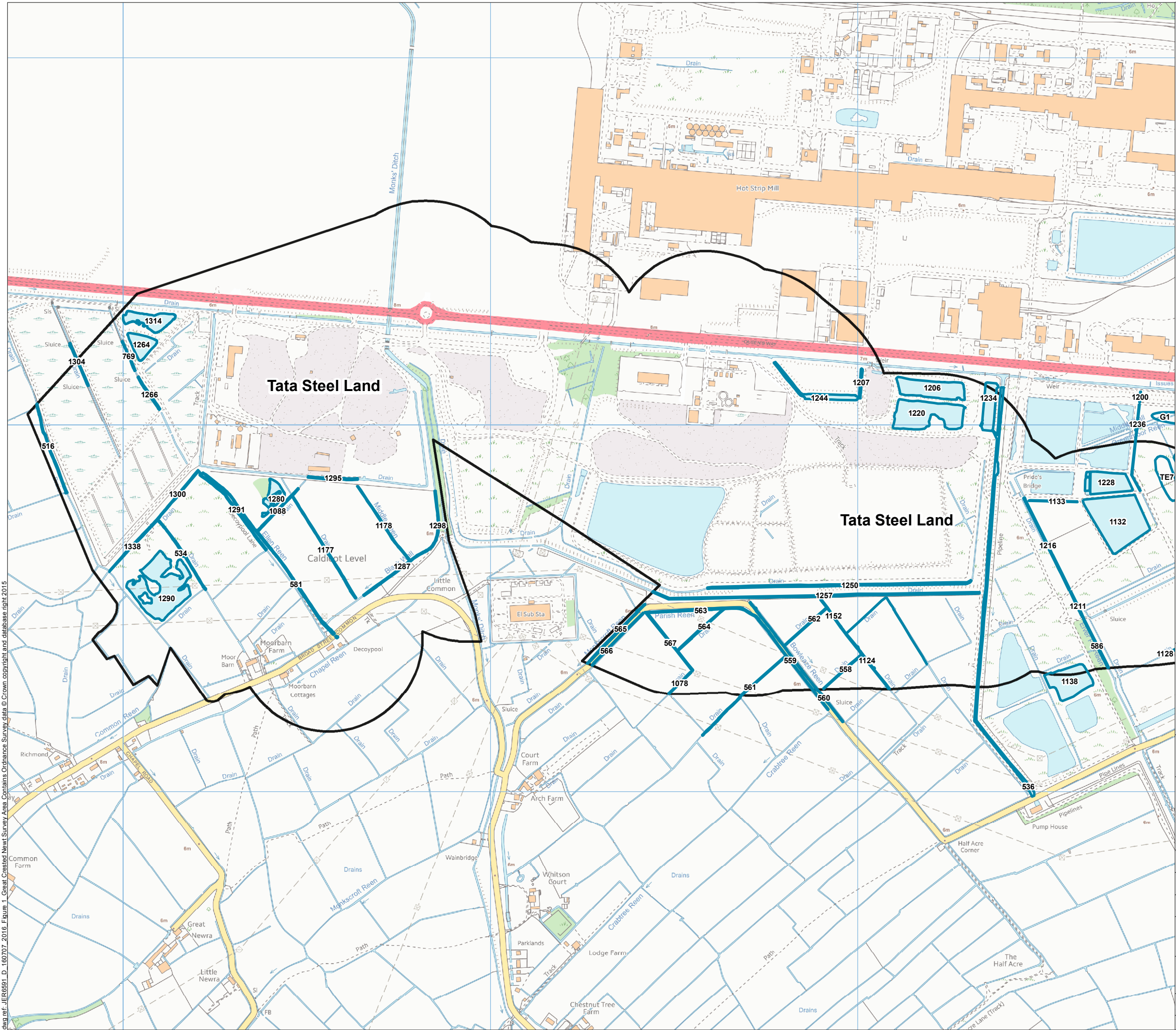
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 Great Crested Newt Survey

 Watercourses



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2016 Great Crested Newt Survey Area

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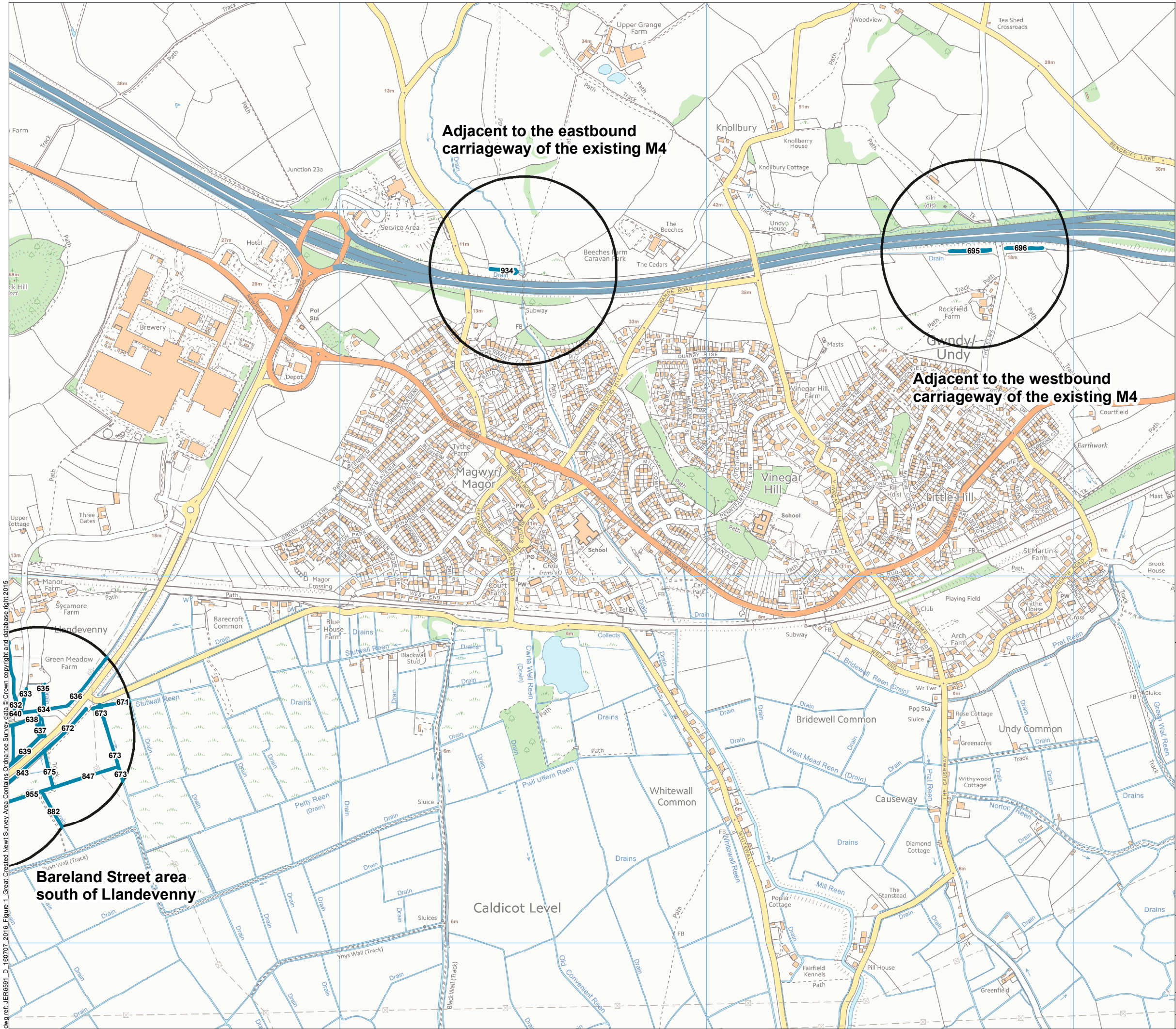
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**Legend**

- Great Crested Newt Survey
- Watercourses

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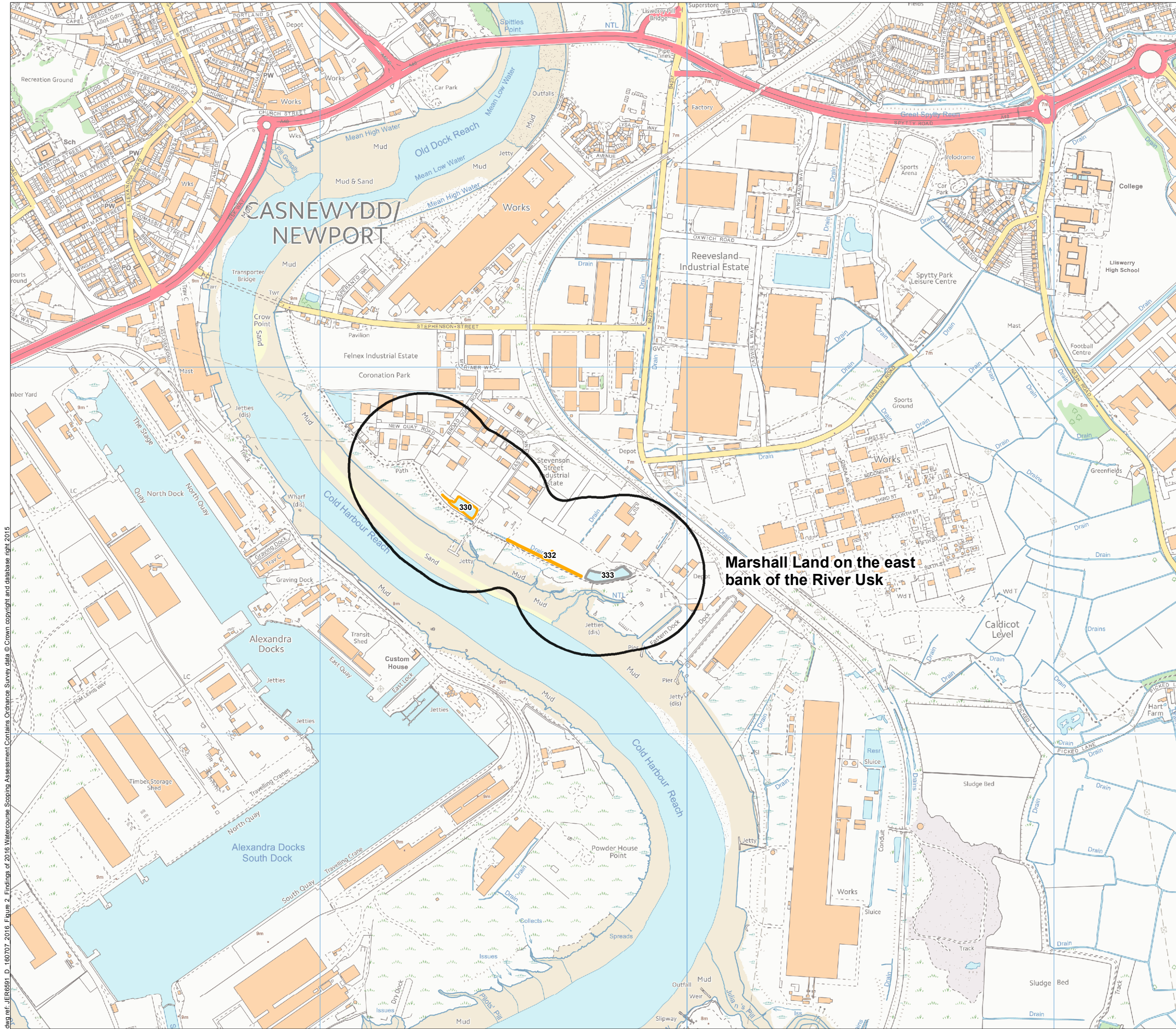
**2016 Great Crested Newt Survey Area**

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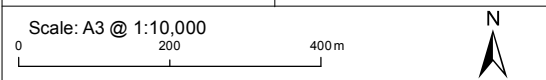
- Legend**
- Great Crested Newt Survey
  - Suitable
  - Suitable But Not Safely
  - Not Suitable
  - Access Not Possible Due to Dense Vegetation



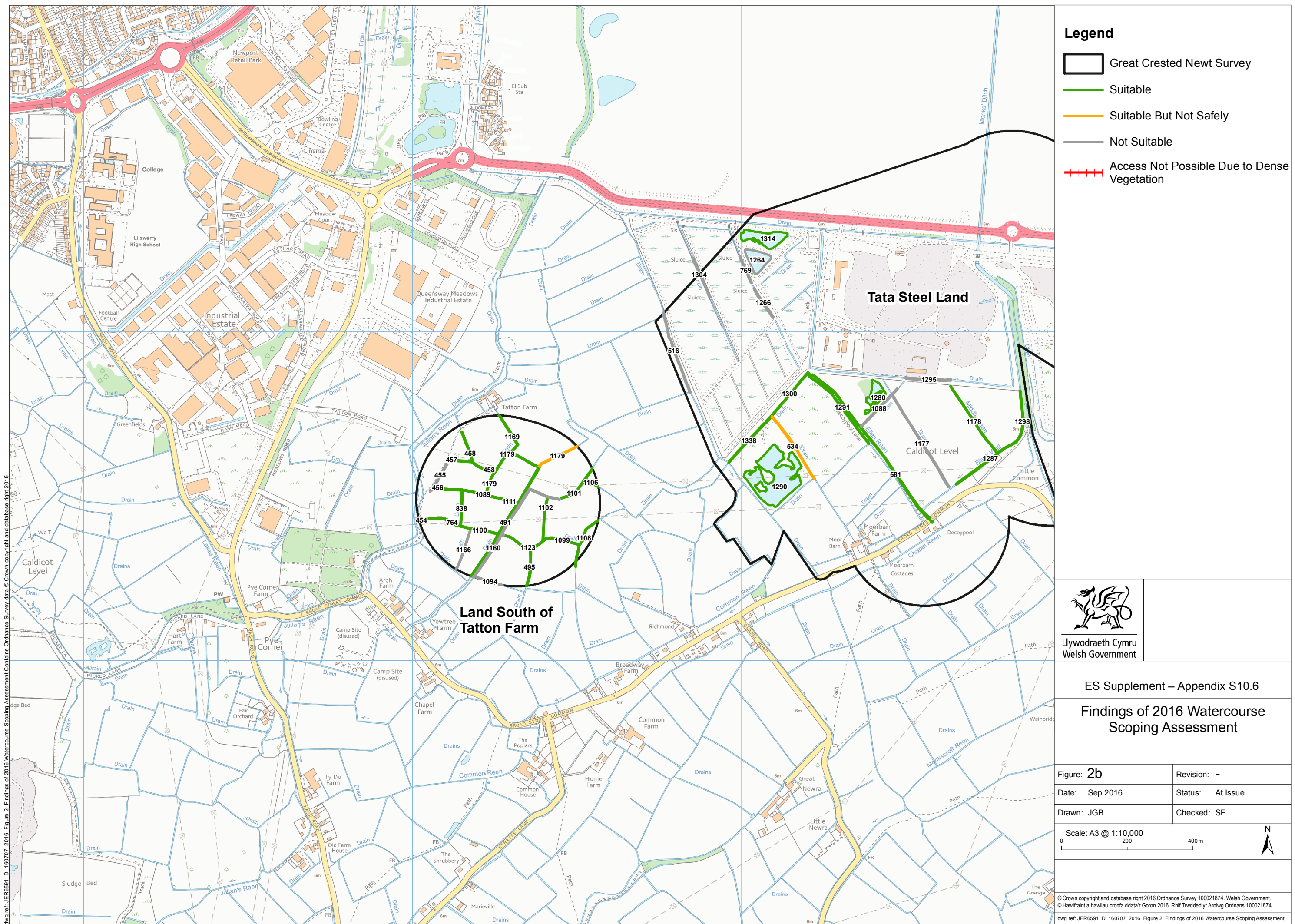
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Findings of 2016 Watercourse Scoping Assessment

Figure: 2a	Revision: -
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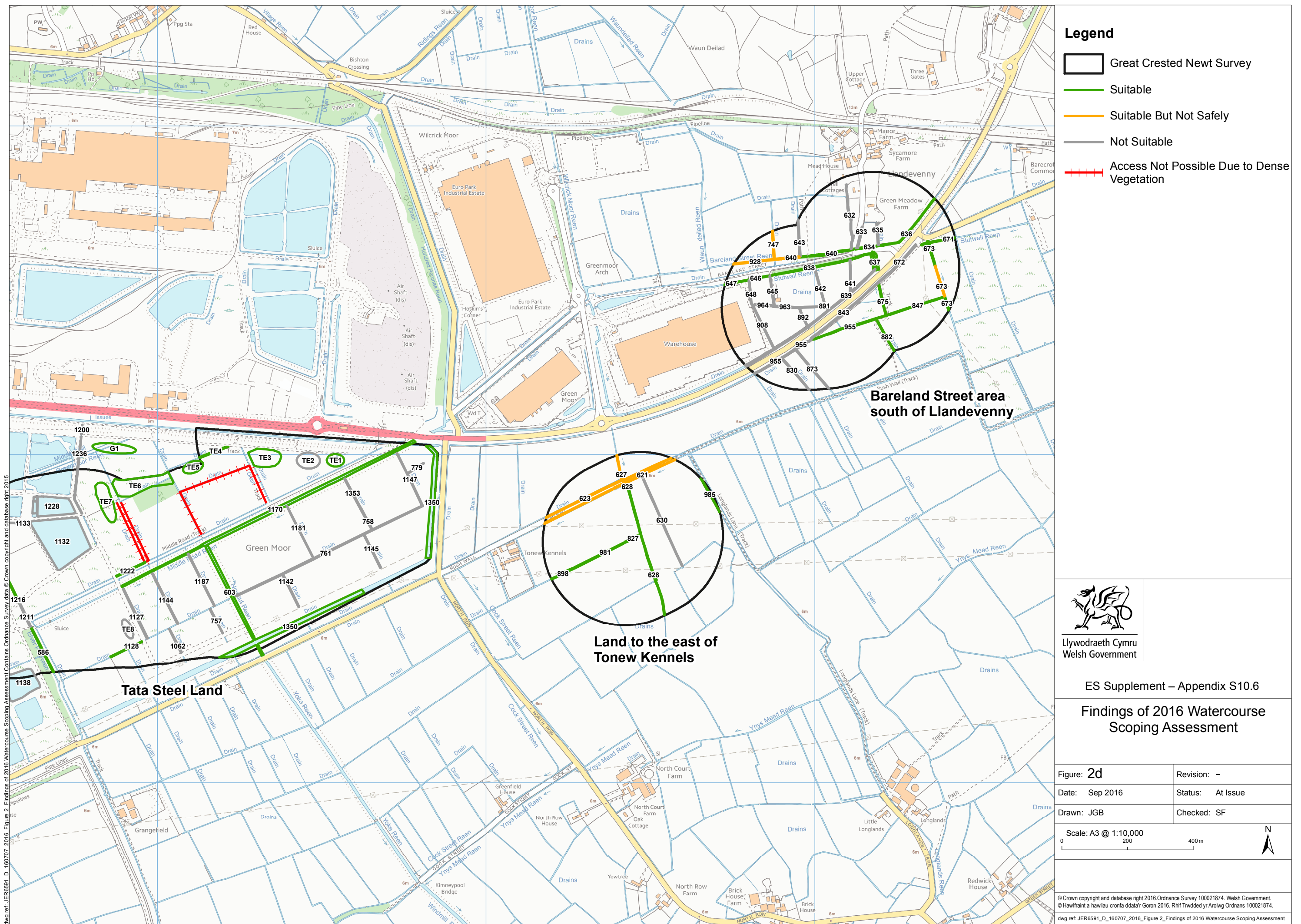




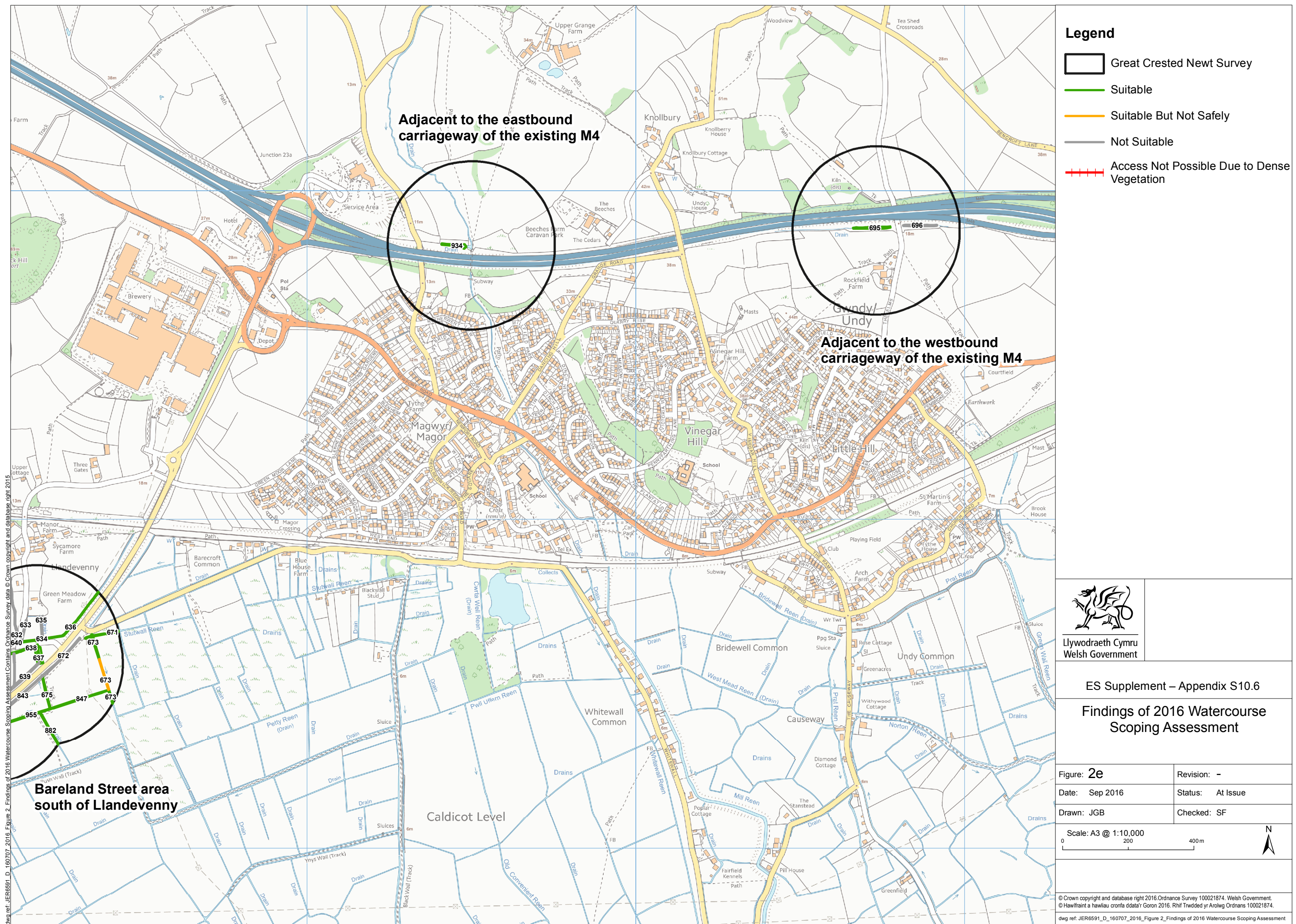




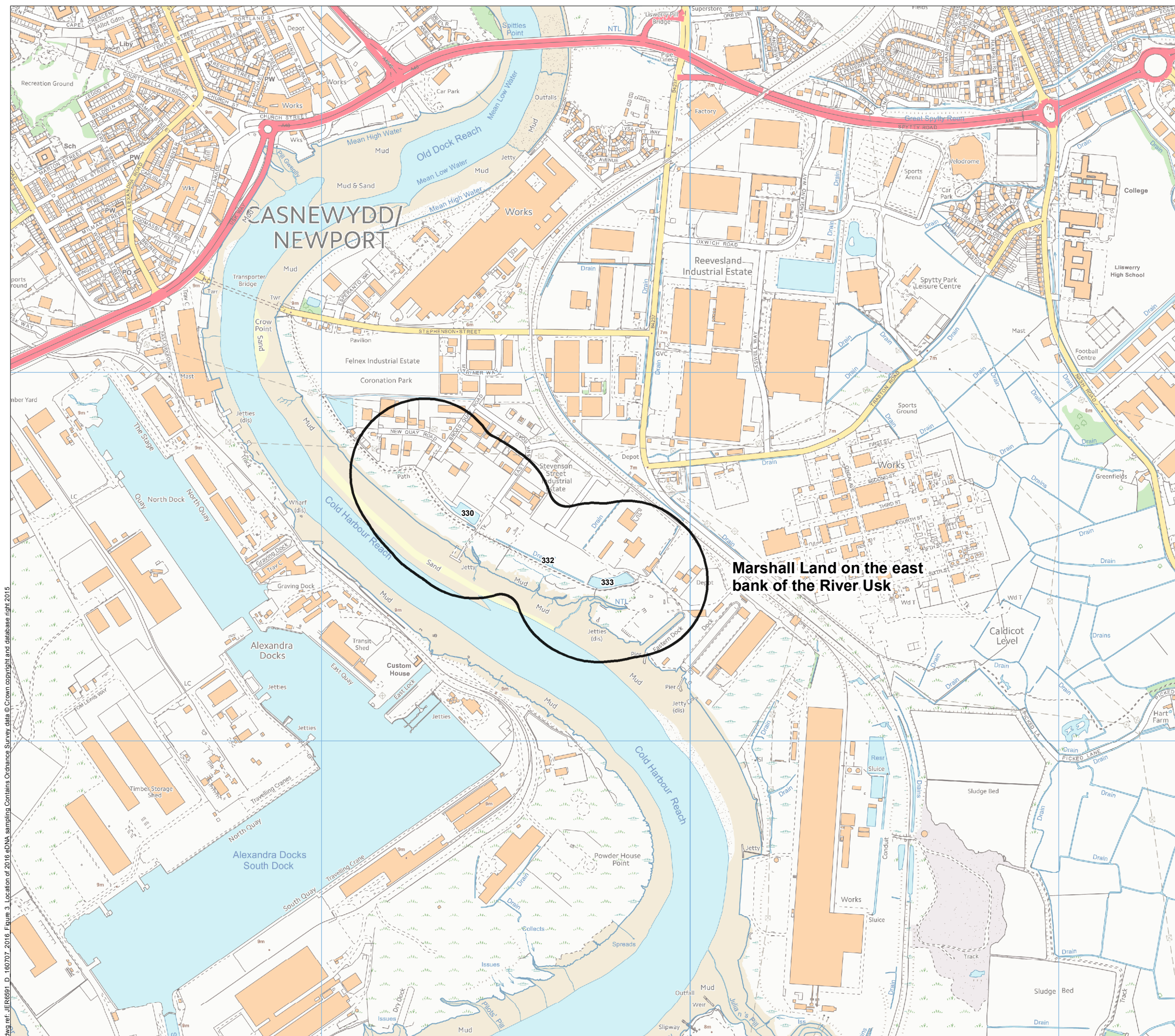














dwg ref: JER6591\_D\_160707\_2016 Figure 3 Location of 2016 eDNA sampling Contains Ordnance Survey data © Crown copyright and database right 2015

### Legend

 Great Crested Newt Survey Area  
 eDNA Sample (New Site for 2016)  
 eDNA Re-sampled (Following Positive Result During 2015)



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### Location of 2016 eDNA sampling

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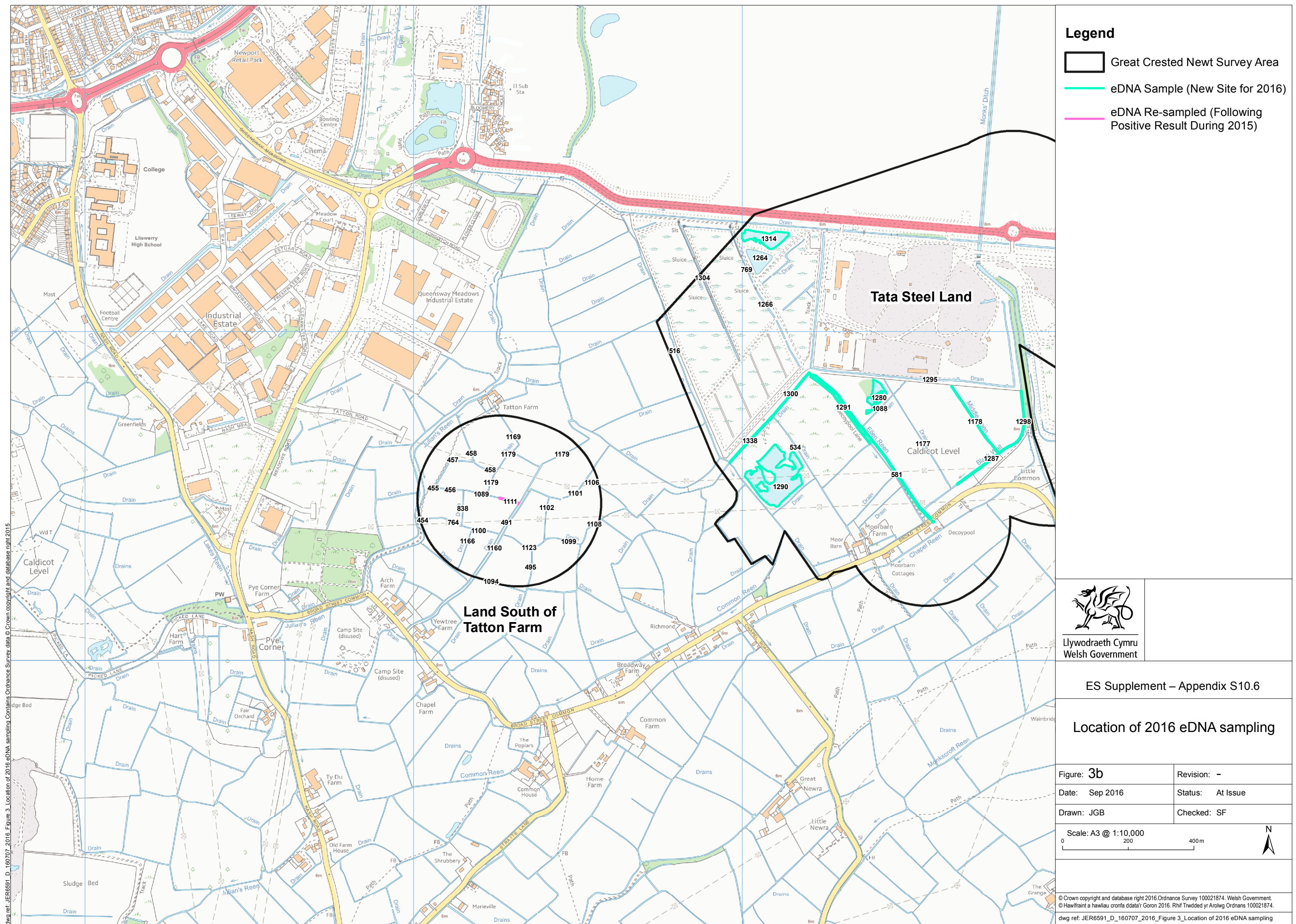
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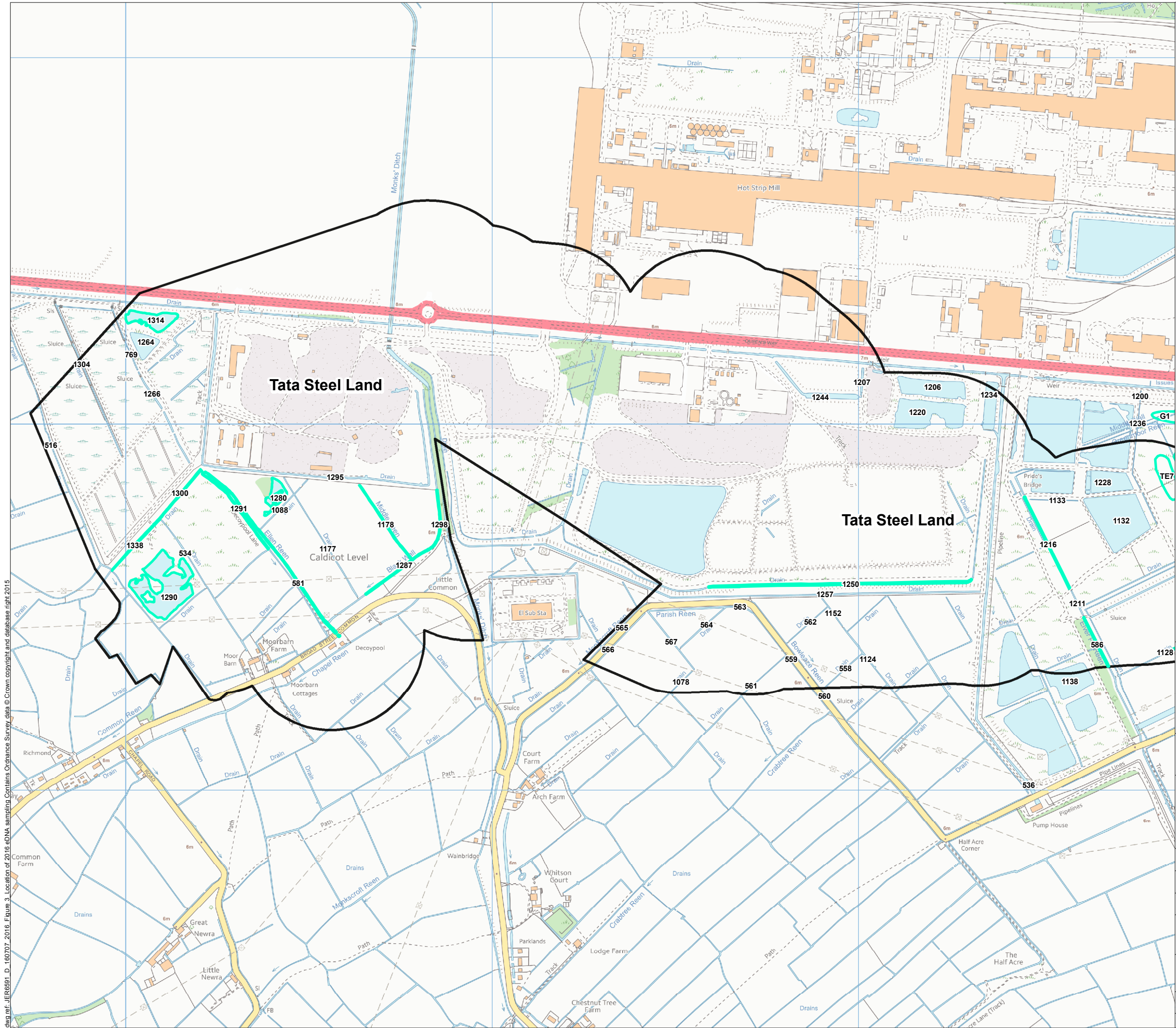
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- Legend**
- Great Crested Newt Survey Area
  - eDNA Sample (New Site for 2016)
  - eDNA Re-sampled (Following Positive Result During 2015)

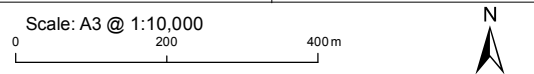


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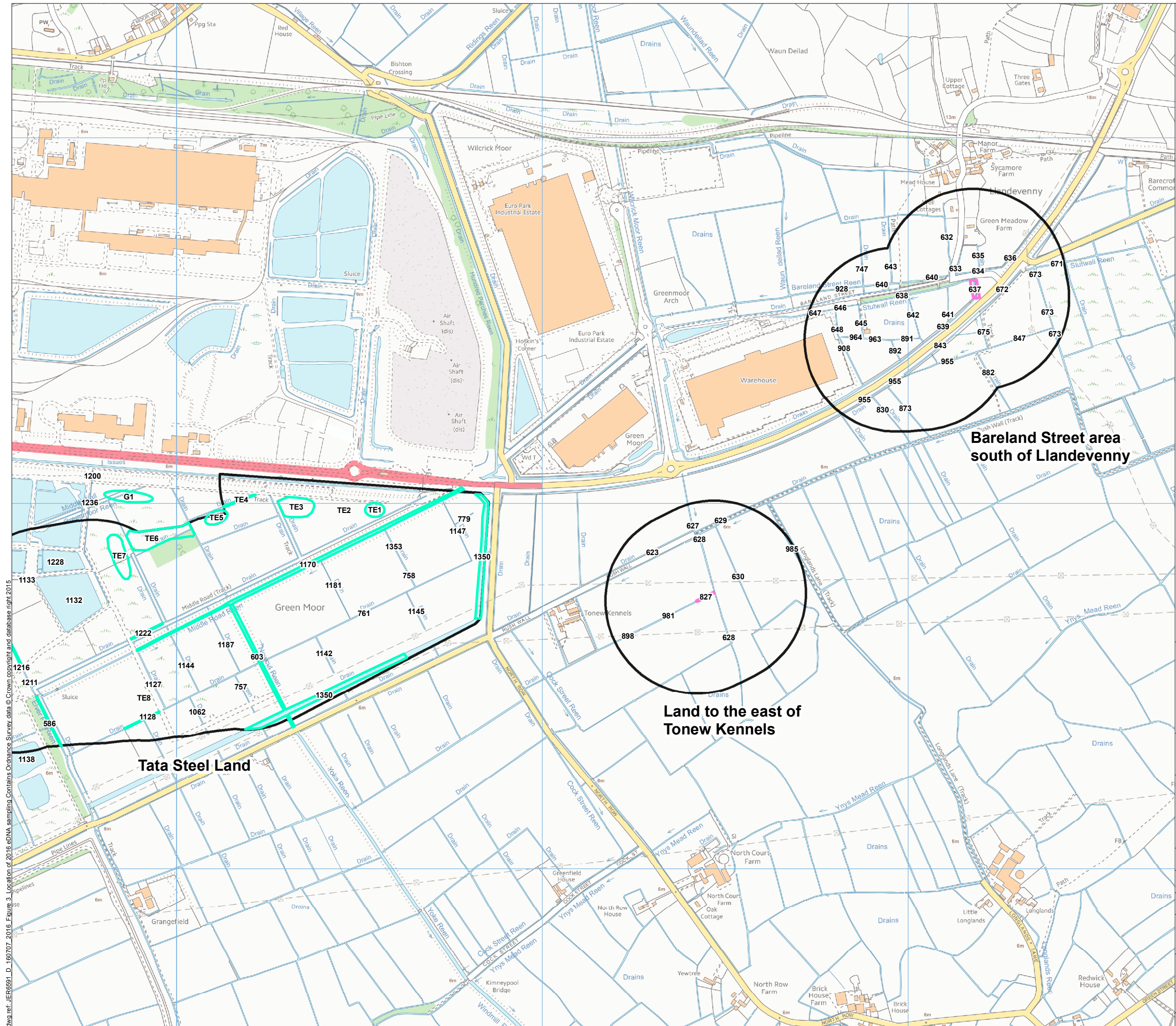
ES Supplement – Appendix S10.6

Location of 2016 eDNA sampling


Figure: <b>3C</b>	Revision: -
Date: Sep 2016	Status: At Issue
Drawn: JGB	Checked: SF







- Legend**
- Great Crested Newt Survey Area
  - eDNA Sample (New Site for 2016)
  - eDNA Re-sampled (Following Positive Result During 2015)




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Location of 2016 eDNA sampling

Figure: 3d	Revision: -
Date: Sep 2016	Status: At Issue
Drawn: JGB	Checked: SF

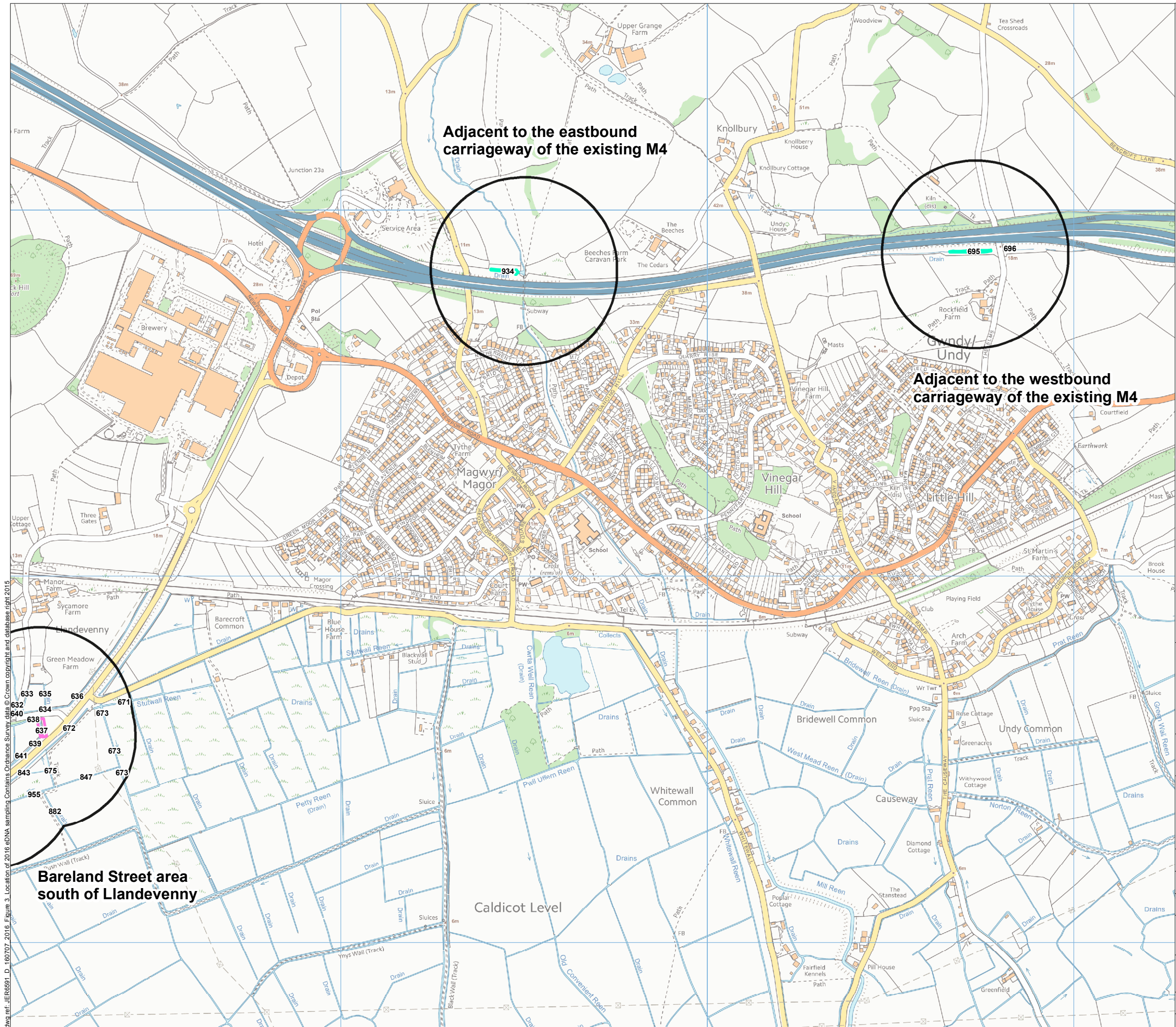
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dwg ref: JER6591\_D\_160707\_2016\_Figure 3\_Location of 2016 eDNA sampling





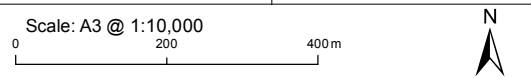
- Legend**
- Great Crested Newt Survey Area
  - eDNA Sample (New Site for 2016)
  - eDNA Re-sampled (Following Positive Result During 2015)



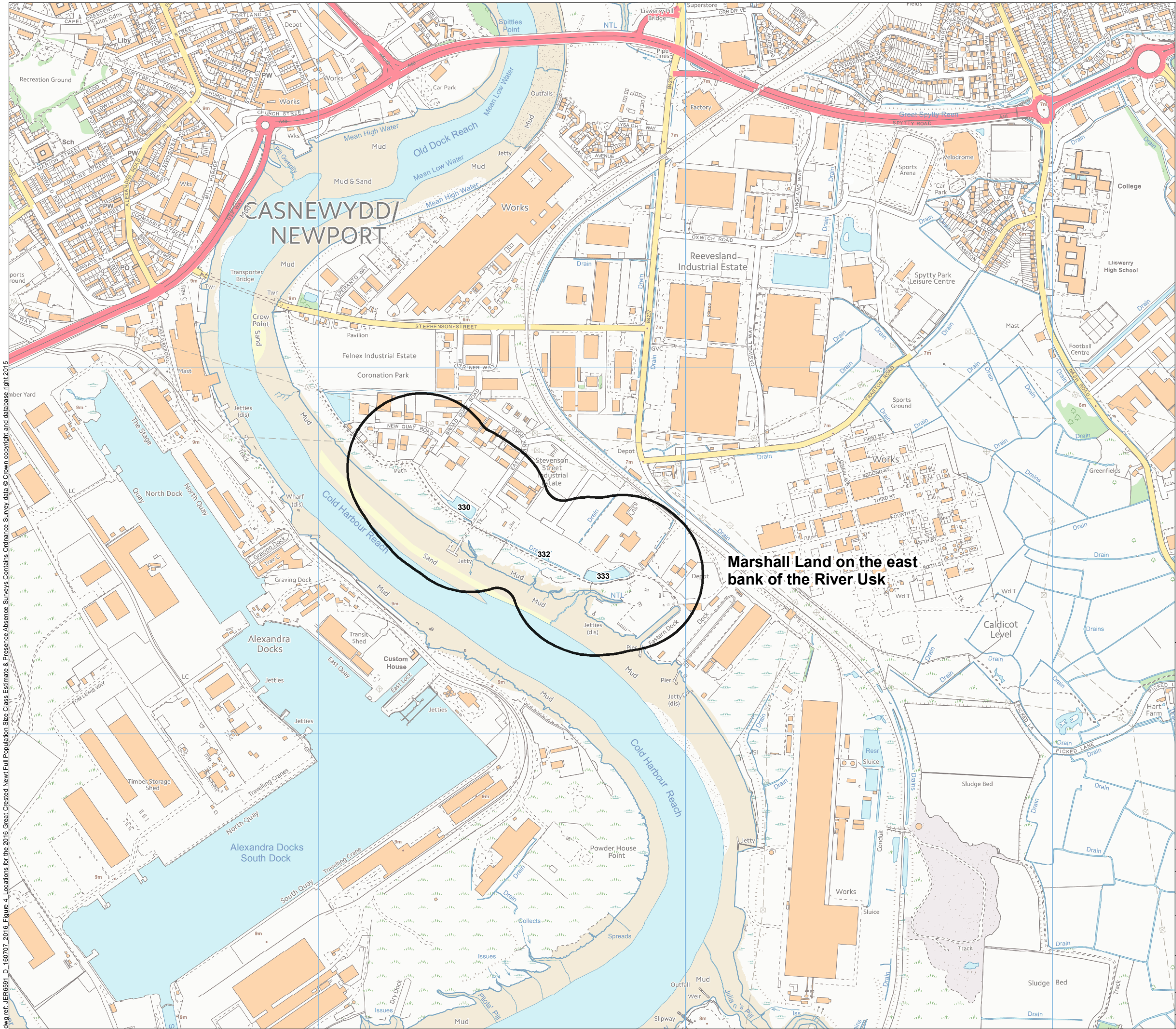
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Location of 2016 eDNA sampling

Figure: 3e	Revision: -
Date: Sep 2016	Status: At Issue
Drawn: JGB	Checked: SF








**Legend**

- Great Crested Newt Survey Area
- Full Population Size Class Estimate / Presence Absence Survey

  
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Locations for the 2016 Great Crested Newt  
Full Population Size Class Estimate &  
Presence Absence Surveys

Figure: <b>4a</b>	Revision: -
Date: Sep 2016	Status: At Issue
Drawn: JGB	Checked: SF

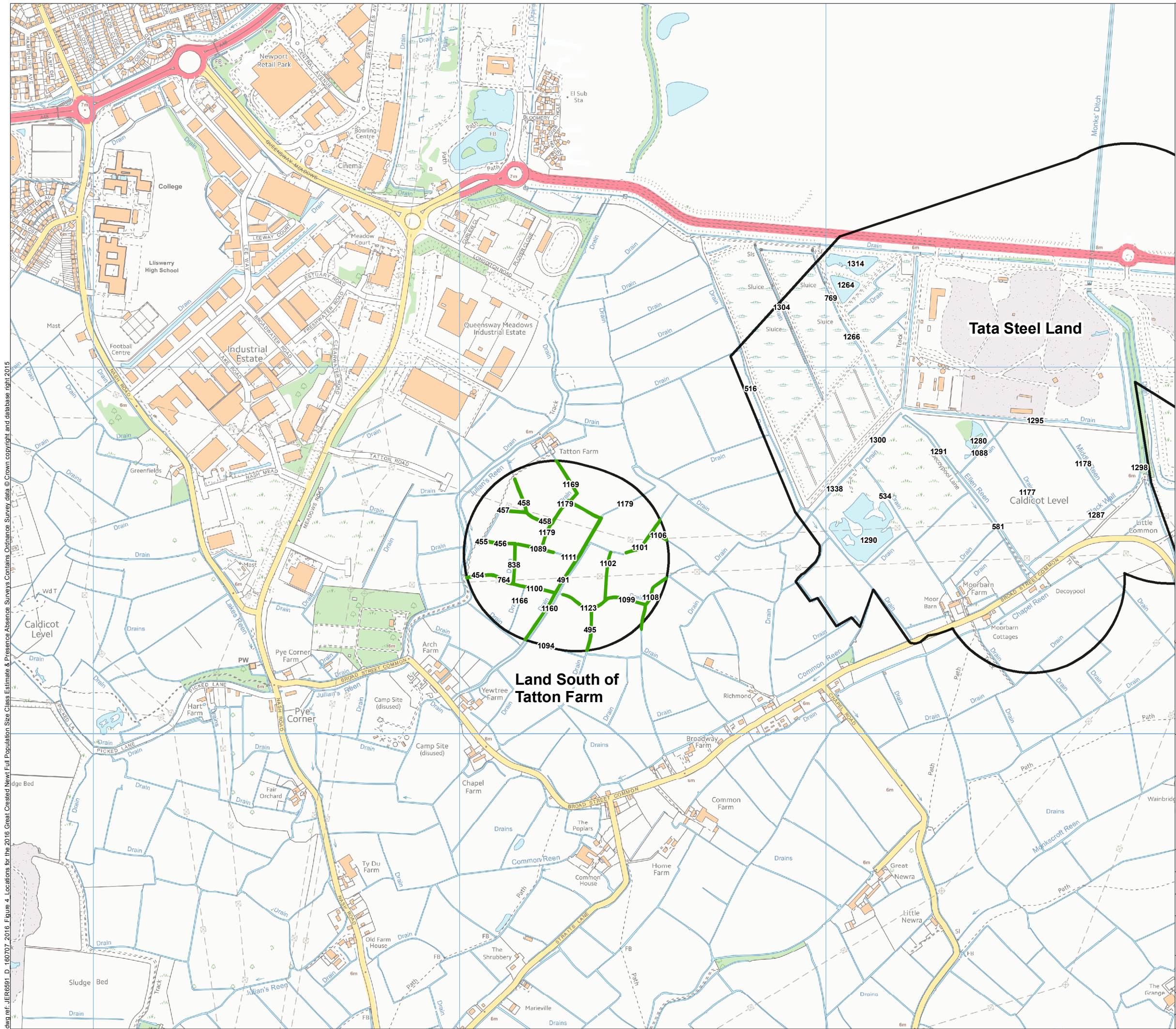
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dwg ref: JER6591\_D\_160707\_2016\_Figure 4\_Locations for the 2016 Great Crested Newt Full Population Size Class Estimate & Presence Absence Surveys Contains Ordnance Survey data © Crown copyright and database right 2015



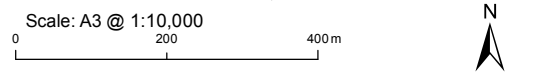
- Legend**
- Great Crested Newt Survey Area
  - Full Population Size Class Estimate / Presence Absence Survey



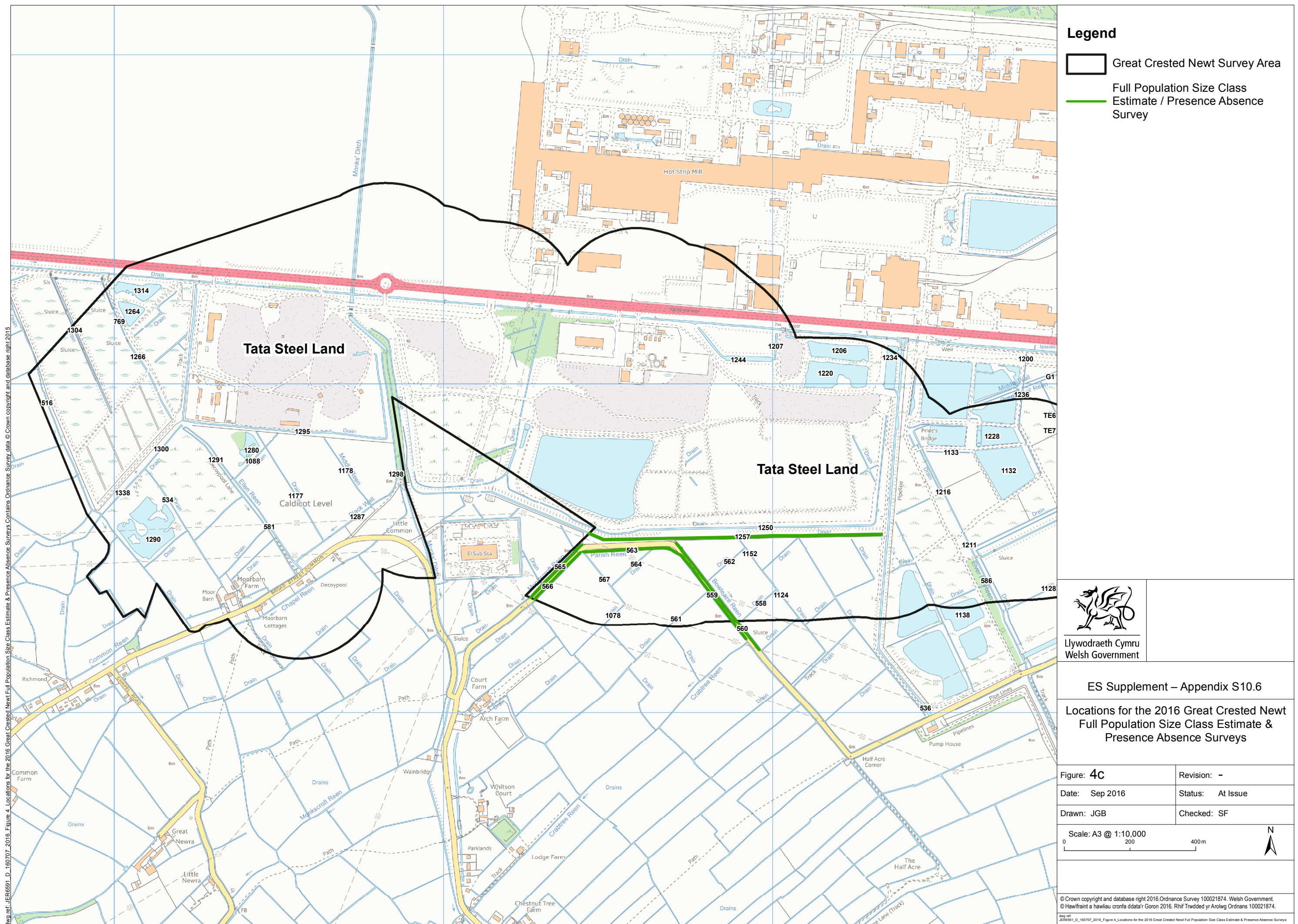
ES Supplement – Appendix S10.6

Locations for the 2016 Great Crested Newt Full Population Size Class Estimate & Presence Absence Surveys

Figure: <b>4b</b>	Revision: -
Date: Sep 2016	Status: At Issue
Drawn: JGB	Checked: SF

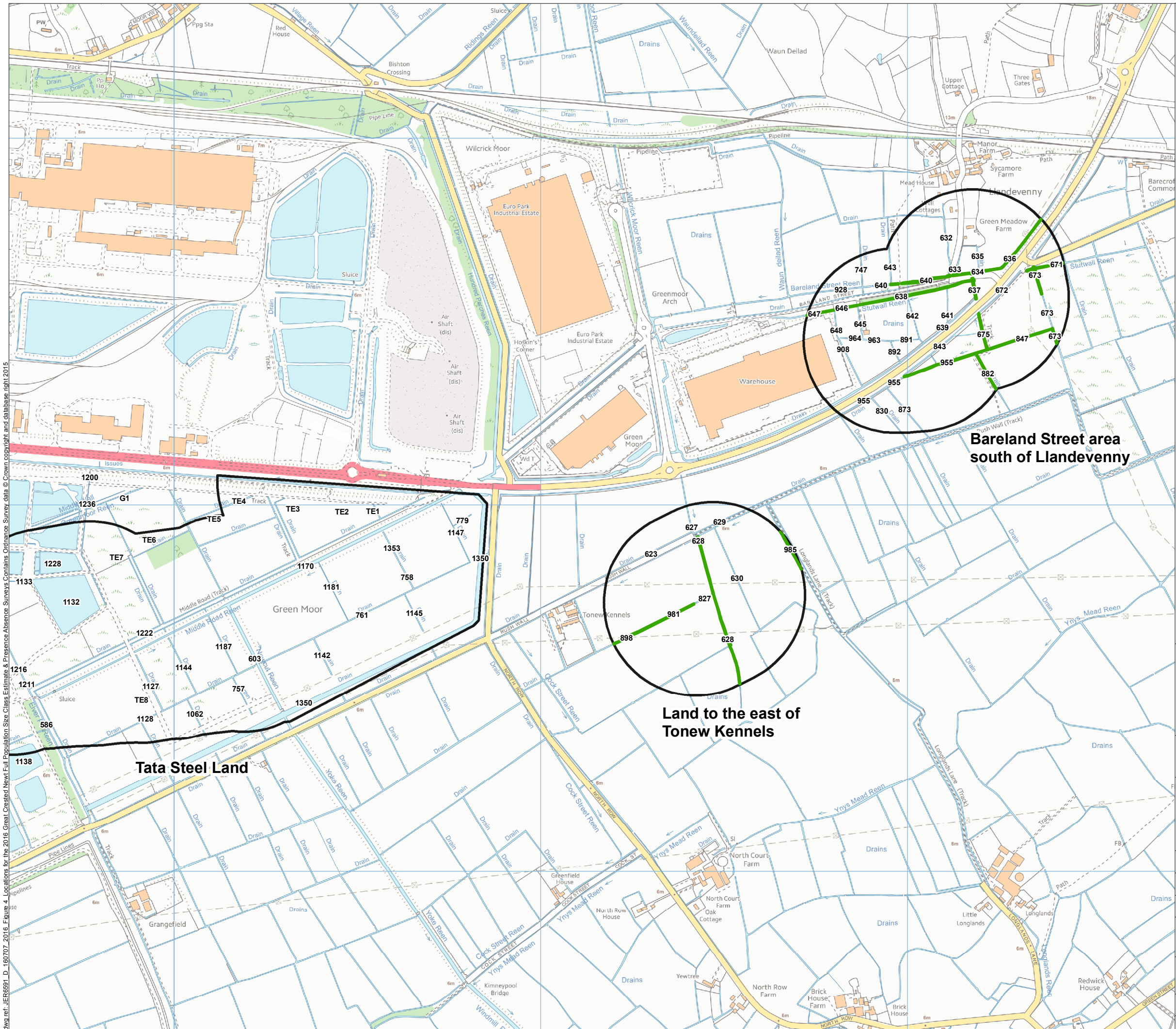









dwg ref: JER591\_D\_160707\_2016\_Figure 4\_Locations for the 2016 Great Crested Newt Full Population Size Class Estimate & Presence Absence Surveys Contains Ordnance Survey data © Crown copyright and database right 2015



**Legend**

- Great Crested Newt Survey Area
- Full Population Size Class Estimate / Presence Absence Survey



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Locations for the 2016 Great Crested Newt  
Full Population Size Class Estimate &  
Presence Absence Surveys

Figure: <b>4d</b>	Revision: -
Date: Sep 2016	Status: At Issue
Drawn: JGB	Checked: SF

Scale: A3 @ 1:10,000

0 200 400 m



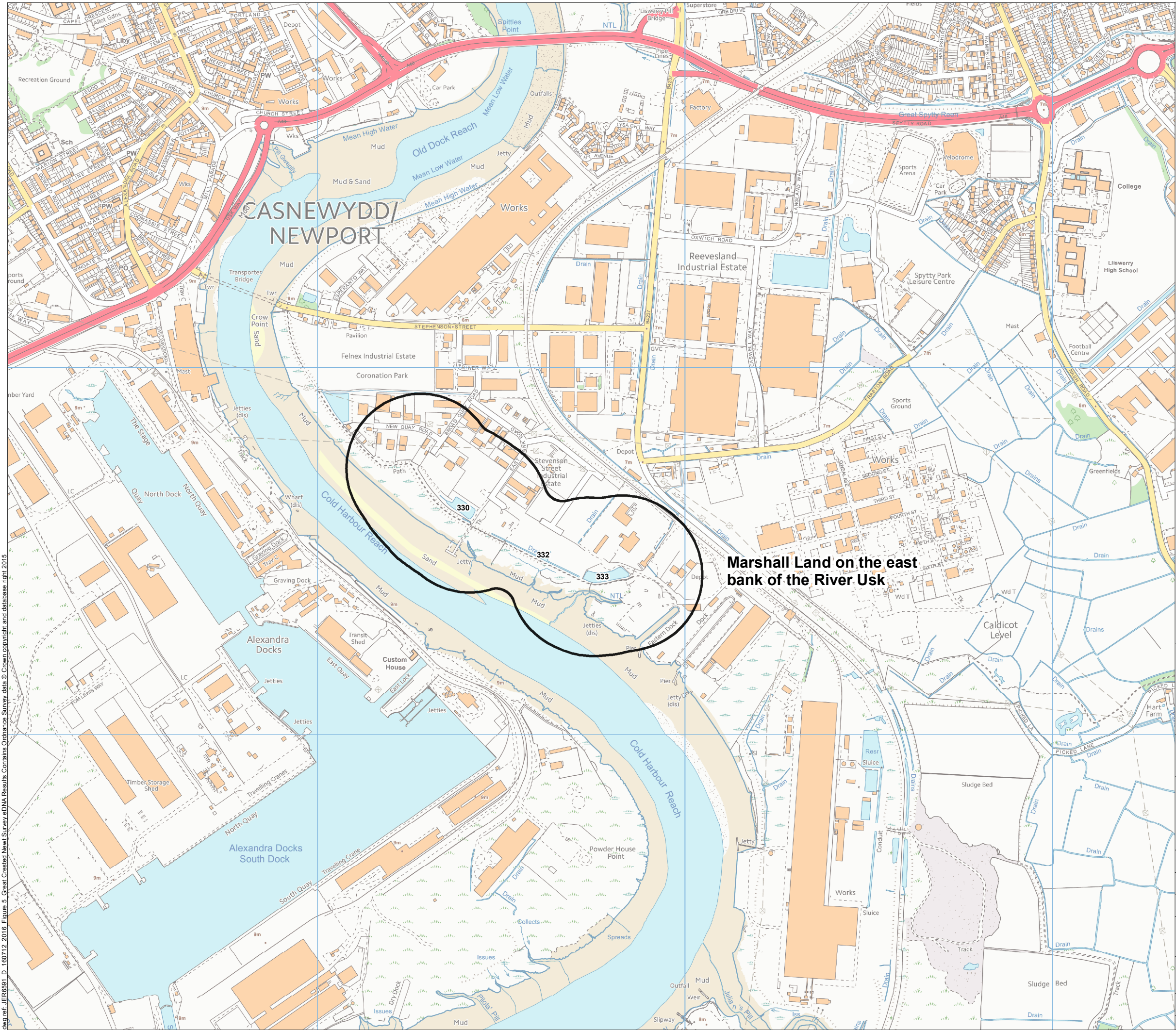
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dwg ref: JER591\_D\_160707\_2016\_Figure 4\_Locations for the 2016 Great Crested Newt Full Population Size Class Estimate & Presence Absence Surveys









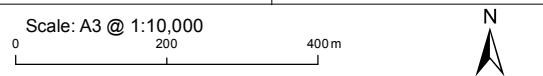
- Legend**
- Great Crested Newt Survey
  - eDNA 2016 Sampling Positive Result
  - eDNA 2016 Sampling Negative Result



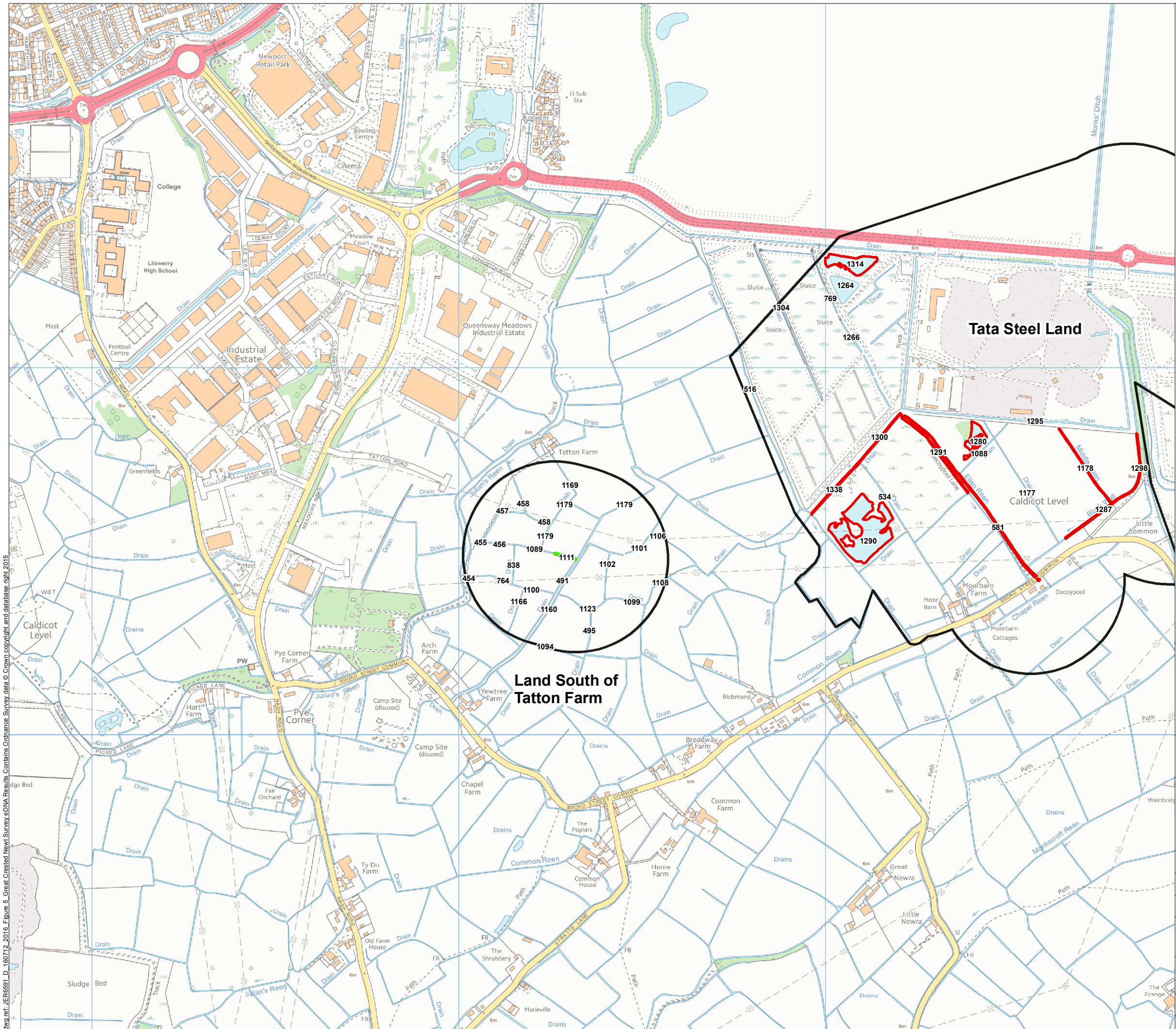
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2016 Great Crested Newt Survey  
eDNA Results

Figure: 5a	Revision: -
Date: Sep 2016	Status: At Issue
Drawn: JGB	Checked: SF








**Legend**

- Great Crested Newt Survey
- eDNA 2016 Sampling Positive Result
- eDNA 2016 Sampling Negative Result

  
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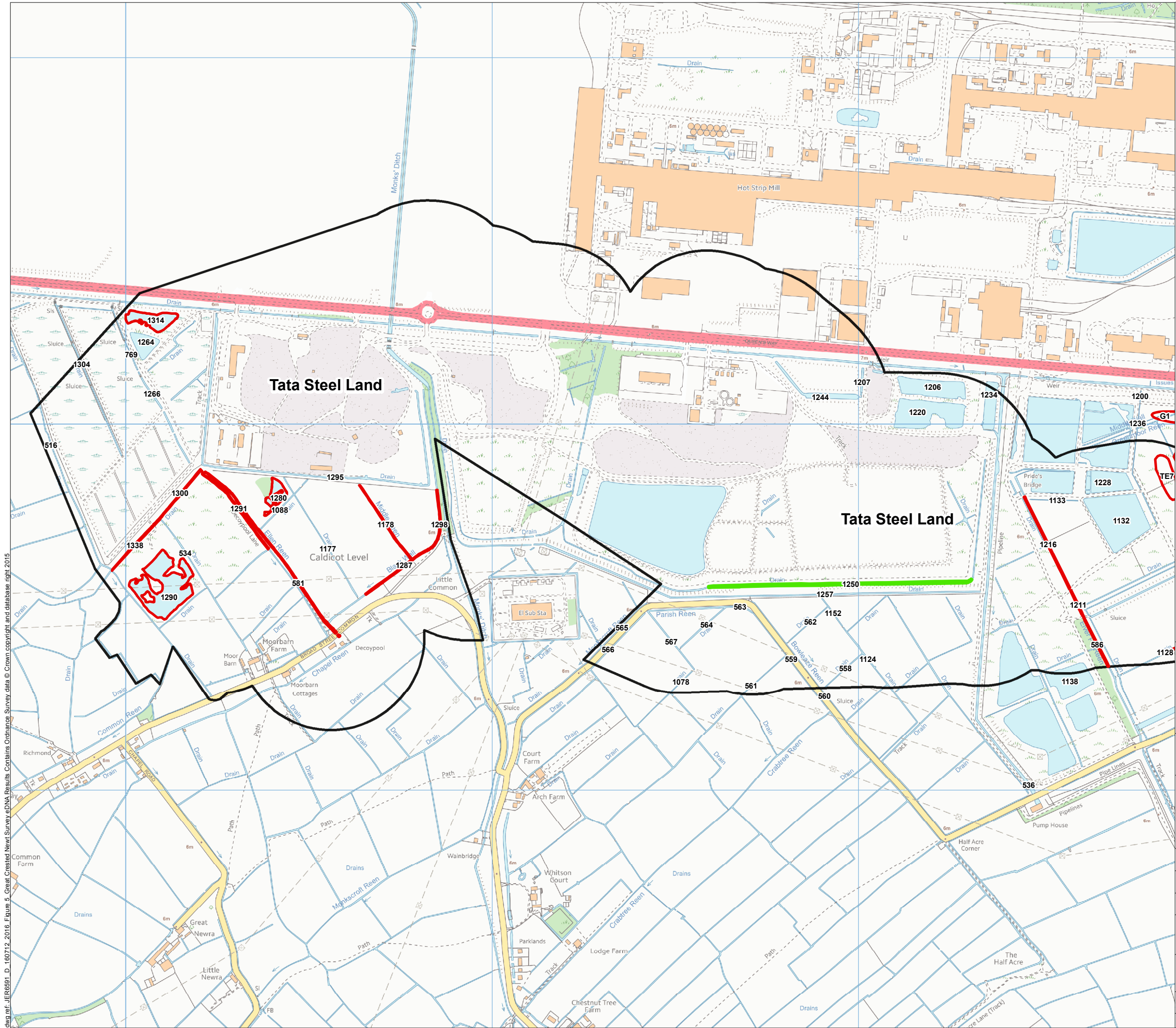
2016 Great Crested Newt Survey  
eDNA Results

Figure: 5b	Revision: -
Date: Sep 2016	Status: At Issue
Drawn: JGB	Checked: SF

Scale: A3 @ 1:10,000  
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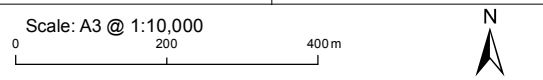
- Legend**
- Great Crested Newt Survey
  - eDNA 2016 Sampling Positive Result
  - eDNA 2016 Sampling Negative Result



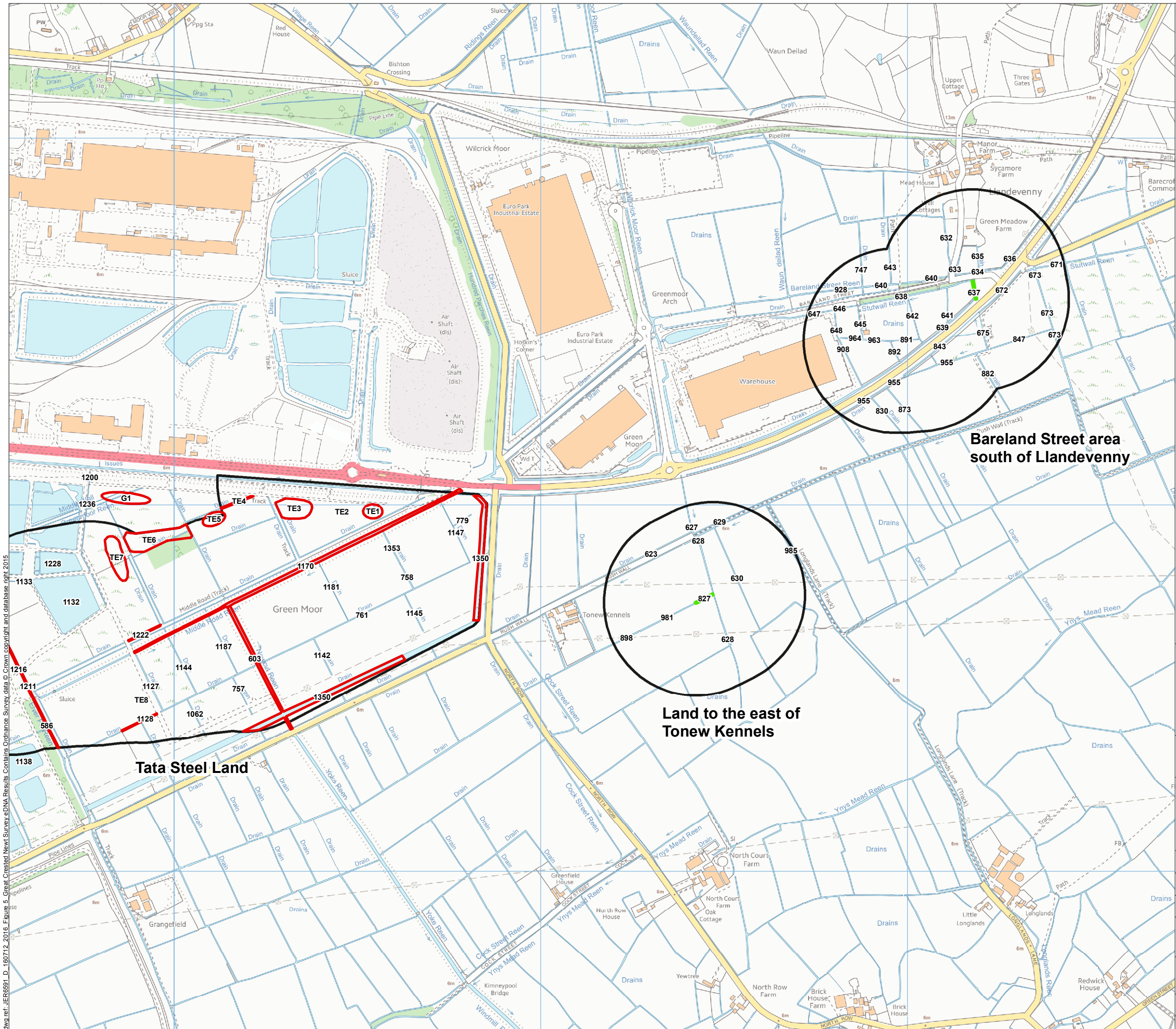
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2016 Great Crested Newt Survey  
eDNA Results

Figure: 5C	Revision: -
Date: Sep 2016	Status: At Issue
Drawn: JGB	Checked: SF








**Legend**

- Great Crested Newt Survey
- eDNA 2016 Sampling Positive Result
- eDNA 2016 Sampling Negative Result

  
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2016 Great Crested Newt Survey  
eDNA Results

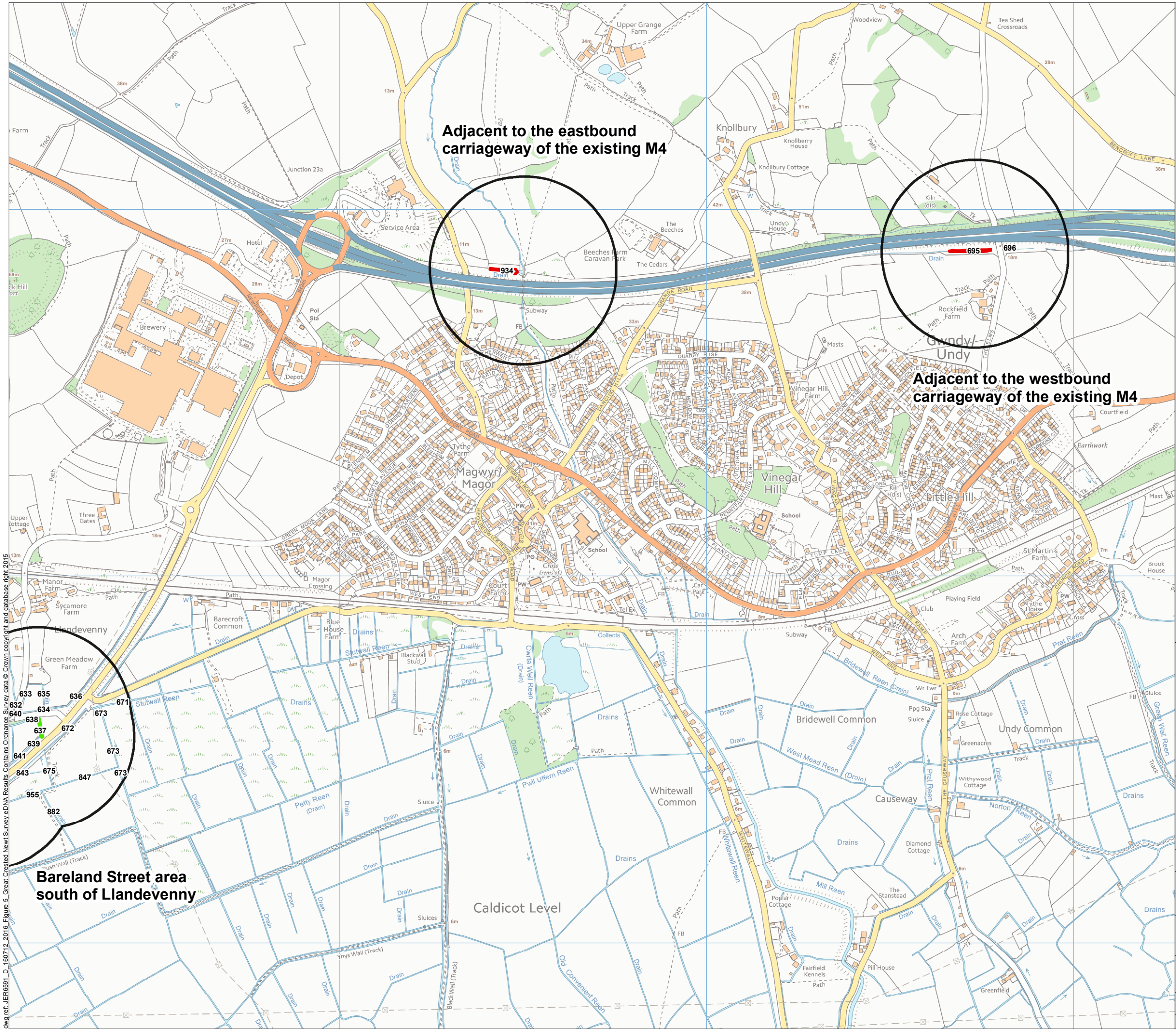
Figure: 5d	Revision: -
Date: Sep 2016	Status: At Issue
Drawn: JGB	Checked: SF

Scale: A3 @ 1:10,000

0 200 400 m

N





- Legend**
- Great Crested Newt Survey
  - eDNA 2016 Sampling Positive Result
  - eDNA 2016 Sampling Negative Result

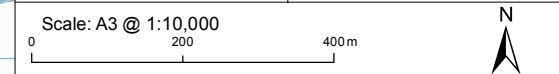


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## 2016 Great Crested Newt Survey eDNA Results

Figure: 5e	Revision: -
Date: Sep 2016	Status: At Issue
Drawn: JGB	Checked: SF



# Annexes

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## **Annex A: 2016 Watercourse Scoping Tables**

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Watercourse reference number	Location	Approximate size / length of water course / pond	Suitable for GCN (adapted HSI Score)	Suitable and safe / possible to survey	Suitable methods for survey	General notes / considerations
				Suitable and Safe		
				Suitable, not Safe		
				Not Suitable		
Magor & Undy						
695	Magor & Undy	>0.5m	Yes		Torching, bottle traps, netting	eDNA sampling undertaken
696	Magor & Undy	<0.25m	No		-	Dry at time of survey
934	Magor & Undy	>0.25m	Yes		Torching, bottle traps, netting, egg search	eDNA sampling undertaken
Bareland Street - West						
632	Bareland Street	>1m	Yes		-	Steep banks, dense scrub in sections, abundant duckweed. Can only trap if accessed from field to west.
636	Bareland Street	>1m	Yes		Torching, bottle Torching, egg searching and limited netting	Steep banks, deep watercourse. Access difficult further east (along A4810)
641	Bareland Street	>1m	Yes		-	Dense scrub prevented access
642	Bareland Street	>1m	Yes		-	Dense bramble scrub prevented access
643	Bareland Street	>0.5m	Yes		-	Steep banks, dense scrub in sections, abundant duckweed.
645						
646						
647						
648	Bareland Street	-	No		-	Steep banks, dense scrub, dry and dominated by bulrush
747	Bareland Street	>1m	Yes		Torching, bottle traps	need floating bottle traps, steep banks and patches of dense scrub
830						
873						
891						
892						
908						
928	Bareland Street		Yes			Steep banks and dense scrub prevented safe access
963						
964						
Bareland Street - East						
633						
634						
635						
637						
638						
639						
640						
671						
672						
673		>0.5m	Yes		Torching, bottle traps	50% of ditch surveyable the other 50% is inaccessible due to dense bramble either side, barbed wire fence close to steep side of water course
675		<0.15m	No		Torch, egg search, trap, net	Accessible only from east side
843						
847						
867		>0.5m	Yes		Torching, bottle traps, netting, egg search	Dense reeds to south, deep drop from sides, recently dredged and cut banks
882		<0.15m	No		Trap, egg search, net, possible torch	Small amounts of open water, though turbid and covered by duckweed.
955						
673 a		>0.5m	Yes		Torching, bottle traps, egg search	Recent dredging and cutting, patches of scrub remain
675 a		>0.25m	Yes		Torching, bottle traps, netting, egg search, terrestrial search	Bramble to west, some duckweed
Tonew Kennels						
628	Tonew Kennels	>0.25m	Yes		Torching, egg search	Steep banks, dense hedgerow did not afford access for trapping or netting
621	Tonew Kennels					
623	Tonew Kennels					
627	Tonew Kennels	0.5m	Yes		Torching, bottle traps	need floating bottle traps, few traps as dense scrub both sides, moderately steep banks, direr to north, access better from west
630	Tonew Kennels	Near dry	Yes		-	Very dense hedge over watercourse
827	Tonew Kennels					
898	Tonew Kennels					
981	Tonew Kennels					
985	Tonew Kennels					
Tatton Farm						
330	East of river Usk	1 m depth	Yes/possible		-	Steep banks, dense scrub, water looks slightly polluted, not accessible – tall, dense bramble on steep banks leading into reedbed with water of unknown depth, good terrestrial habitat quality
332	East of river Usk	0.5 m depth	Yes/possible		-	Patches of dense scrub, polluted in areas, good terrestrial habitat quality. Some areas dry and not suitable for trapping.
333	East of river Usk	<0.5 m depth	Yes		-	Steep banks, dense scrub (75%), inside fenced area, poor terrestrial habitat quality
454	Tatton Farm	1 m width, 0.15 m depth	No		Torching, netting, egg search	Dense scrub obscuring 80% of the waterbody, average terrestrial habitat quality
455	Tatton Farm	1 m width, 0.15 m depth	No		-	Very dense scrub, average terrestrial habitat quality
456	Tatton Farm	2 m width, 0.1 m depth	No		Torching	Dense scrub, average terrestrial habitat quality
457	Tatton Farm	1 m width, 0.15m depth	No		18 Torching, netting, egg search	Dense scrub, average terrestrial habitat quality
458	Tatton Farm	1 m width, 0.1 m depth	No		Torching, netting, egg search	Dense macrophyte cover obscuring 85% of the waterbody, average terrestrial habitat quality
459	Tatton Farm	2 m width, 0.15 m depth	No		-	Patches of dense scrub, average terrestrial habitat quality, lots of glyceria
491	Tatton Farm	1.5 m width, <0.5 m depth	Yes		Torching, bottle traps, netting	Shallow eastside banks, dense scrub (west), macrophytes obscuring surface, fence to west, access from east
491	Tatton Farm	3 m width, 0.25 m depth	Yes		Torching, egg search	Steep banks, dense scrub, double barbed wire fence, average terrestrial habitat quality
495	Tatton Farm	1.5m width, 0.25m depth	No		Torching, egg search and netting	Dense scrub covers 80% of the waterbody, average terrestrial habitat quality
764	Tatton Farm	1.5 m width, 0.15 m depth	No		Torching, netting, egg search	Dense scrub obscuring 80% of the waterbody, average terrestrial habitat quality
838	Tatton Farm	2 m width, 0.15 m depth	No		-	Barbwire fence on both sides with dense bramble, shallow, average terrestrial habitat quality
1089	Tatton Farm	2 m width, 0.25 m depth	No		-	Dense scrub (some open sections), probably too shallow, average terrestrial habitat quality
1089	Tatton Farm	2 m width, 0.25 m depth	Yes		Torching, netting, egg search	Dense scrub obscuring 70% of the waterbody, average terrestrial habitat quality
1094	Tatton Farm	1 m width, 0.25m m depth	No		-	
1099	Tatton Farm	3 m width, <0.5 m depth	Yes		Torching, bottle traps, netting, egg search	70% of the waterbody inaccessible due to vegetation cover, average terrestrial quality
1100	Tatton Farm	1 m width, 0.25 m depth	Yes		Torching, netting, egg search	75% of the waterbody inaccessible due to vegetation cover
1101	Tatton Farm	2 m width, <0.5 m depth	Yes		Torching (if cleared), netting, egg search	Dense scrub (some openings), macrophytes obscuring surface, average terrestrial habitat quality, few access points
1102	Tatton Farm	1 m width, 0.25 m depth	Yes		Torching (if cleared), netting, egg search	Dense scrub (some opening s), macrophytes obscuring surface, average terrestrial habitat quality
1106	Tatton Farm	2 m width, <0.5 m depth	Yes		Torching (if cleared), netting, egg search	Macrophytes obscuring surface, average terrestrial habitat quality
1108	Tatton Farm	2 m width, <0.5 m depth	Yes		Torching, bottle traps netting, egg search	Dense scrub obscuring 70% of the waterbody, average terrestrial habitat quality
1111	Tatton Farm	2 m width, 0.25 m depth	Yes		Torching, egg search, netting	Dense scrub obscuring 50% of the waterbody, average terrestrial habitat quality
1123	Tatton Farm	2 m width, <0.25 m depth	Yes		Torching, bottle traps, egg search	80% of the waterbody inaccessible due vegetation cover, average terrestrial habitat cover
1160	Tatton Farm	1 m width, 0.1 m depth			-	Dry throughout with small pockets of very shallow water
1166	Tatton Farm	1 m width, 0.1 m depth	No		-	Dense scrub obscuring 100% of the waterbody, average terrestrial habitat quality
1169	Tatton Farm	2 m width, 0.25 m depth	Yes		Torching, egg search, netting	Dense scrub obscuring 70 of the waterbody, average terrestrial habitat quality
1179	Tatton Farm	2 m width, 0.25 m depth	Yes		Torching, egg searching, netting	Dense scrub obscuring 75% of the waterbody, average habitat quality
2006	Tatton Farm	2 m width, 0.25 m depth	Yes/possible		Torching, bottle traps, netting	Dense scrub, average terrestrial habitat quality
2007	Tatton Farm	2 m width, 0.25 m depth	No		-	Steep banks to west, fence, average terrestrial habitat quality
2056	Tatton Farm	2 m width, <0.5 m depth	Yes, though dominated by duckweed		Torching (if duckweed cleared), bottle traps, netting, egg search	Marcophytes obscuring surface, average terrestrial habitat quality
2082	Tatton Farm	3 m width, <0.5 m depth	Yes		Torching, bottle traps, netting, egg search	Average terrestrial habitat quality
2083	Tatton Farm	2 m width, 0.25 m depth	Yes		Torching, bottle traps (to south, north is shallower), egg search	Dense scrub (accessible in parts), lots of glyceria and rushes
2085	Tatton Farm	1 m width, 0.1 m depth	No		-	Some scrub to west, average terrestrial habitat quality
2086	Tatton Farm	2 m width, <0.5 m depth	Yes – although dominated by duckweed		Torching (if cleared of duckweed), bottle traps, netting	Wire fence with steep drop after fence, dense scrub, macrophytes obscuring surface, average terrestrial habitat quality

2088	Tatton Farm	2 m width, 0.15 m depth	No		-	Dense scrub (in sections), average terrestrial habitat quality, shallow
1101a	Tatton Farm	2 m width, <0.25 m depth	Yes		Torching (if cleared), bottle traps, egg search	Dense scrub in parts, macrophytes obscuring surface
1163a	Tatton Farm	3 m width, <0.5 m depth	Yes		Torching, bottle traps (canes), netting, egg search	Banks – moderately steep, accessible with caution, macrophytes partially obscuring surface, average terrestrial habitat quality
2007b	Tatton Farm	3 m width, <0.5 m depth	Yes		Torching (if removed duckweed), bottle traps (---), egg search	Patches of dense scrub, macrophytes obscuring surface, lots of emergent vegetation, average terrestrial habitat quality
2083a	Tatton Farm	3 m width, <0.5 m depth	Yes		Torching (if cleared), bottle traps, netting, egg search	Macrophytes obscuring surface, average terrestrial habitat quality
2086a	Tatton Farm	2 m width, <0.25 m depth	Yes (possibly)		Torching	Dense scrub, average terrestrial habitat quality, deeper in corners
491a	Tatton Farm	2 m width, <0.5 m depth	Yes		Torching (if cleared of duckweed), bottle traps (canes), netting, egg search	Moderate steep banks, dense scrub (partial), macrophytes obscuring surface, average terrestrial habitat quality
TATA - West						
516	TATA	2 m width, 0.15 m depth	No			Very shallow
534	TATA	3 m width, 1 m depth (est)	Yes		-	Dense bramble, no access, good terrestrial habitat quality
581	TATA	3 m width, 0.5 m depth	Yes		Torching, bottle traps, netting	Access to the eastern side only due to dense scrub to the west, steep banks
769		60m length, 1 m width, 0.1 m depth	No		-	
1088	TATA	40 m width, 1 m depth	Yes		Torching, bottle traps, netting, egg search	Dense reeds on route, gentle banks at the edge, merges with 1280, good terrestrial habitat quality
1088	TATA	2 m width, 0.25 m depth	No		-	Very shallow, bramble, inaccessible in most parts, good terrestrial habitat quality
1177	TATA	2 m width, 0.75-0.5 m depth	Yes			Depth variable, dense scrub obscures 95 of the waterbody, good terrestrial habitat quality
1178	TATA	1 m width, 0.25m depth	Yes		Torching, bottle traps, netting, egg search	Moderately steep banks, dense scrub on the western side of the waterbody, average terrestrial habitat quality
1264	TATA	-	No		-	Completely dry
1266	TATA	2 m width, <1 m depth	Yes			Tall reeds obscuring the bankside throughout the watercourse, good terrestrial habitat quality
1280	TATA					
1287	TATA	2 – 3 m width, 0.5 m depth	Yes		Torching, bottle traps, netting, egg search	Moderately steep banks, dense scrub on the southern side of the waterbody, good terrestrial habitat quality
1290	TATA	<1 m depth at margin	Yes		Torching, bottle traps, netting, egg search	Reeds present, swan present, gentle slope at edge, good terrestrial habitat quality. *If access gained to entire perimeter
1291	TATA	2 m width, <0.5 m depth	Yes		-	Very dense bramble, average terrestrial habitat quality
1295	TATA	4 m width, 1 m depth	No		-	Steep banks to east, dense scrub, very polluted, good terrestrial habitat quality
1298	TATA	3 m width	Yes		Torching, bottle traps, netting, egg search	Steep banks on either side of the waterbody, good terrestrial habitat quality
1300	TATA	3 m width, 1 m depth	Yes		Torching, bottle traps (floating), netting	Moderately steep banks, dense scrub towards southwest, macrophytes obscuring surface in sections, average – good terrestrial habitat quality, silty/muddy base – unsure of depth
1304	TATA	1 m width, 0.15 m depth	No		-	Very shallow
1314	TATA	80 m width, <1 m depth	Yes		Torching	Steep unstable banks and dense scrub prevents access to 75 of the pond
1338	TATA	3 m width, 1 m depth	Yes		Torching, bottle traps (floating), netting	Moderately steep banks, dense scrub towards southwest, macrophytes obscuring surface in sections, average – good terrestrial habitat quality, silty/muddy base – unsure of depth
2069	TATA		-		-	Very shallow
2069A	TATA		-		-	Very shallow
2069B	TATA		Yes		Torching, bottle traps, terrestrial search	Very tall, dense reeds along most of watercourse and deep. Can trap along whole length.
2069C	TATA	5 m width, 0.5 m depth	No		-	Very shallow, very polluted towards northwest
Pond 4	TATA	<0.5 m depth	Yes		Torching, bottle traps (canes), netting, egg search	Reeds present, good terrestrial habitat quality, mallards present
TATA – Central						
536						
558	South of TATA	<0.15m	No			Dense scrub, double hedgerow, average terrestrial habitat quality
559	TATA	2 m width, 0.5 m depth	Yes		Torching, netting, egg search	Steep banks, dense vegetation obscuring the water edges, average terrestrial habitat quality
560	South of TATA	>0.5m	Yes		Torching, bottle traps, netting	Steep drop from banks, slightly polluted, areas of fly tipping, average terrestrial habitat quality
561	South of TATA	<0.15m	No		-	Shallow, scrub to south, difficult to access
562	TATA	1 m width, 0.25 depth	No			Dense scrub preventing access to the waterbody, average terrestrial habitat quality.
563	South of TATA	3 m width, >0.5m depth	Yes		Torching, bottle traps, netting, terrestrial search	Steep banks in section, 20% macrophytes cover, average terrestrial habitat cover
564	South of TATA	<0.15m	No		-	Dense scrub, channel filled with reeds
565	South of TATA	3 m width, 1 m depth	Yes		Torching, bottle traps, netting	Steep drop from banks, fly tipping to north, small patches of duckweed
566	South of TATA	2 m width, <0.75 m depth	Yes		Torching	Steep banks preventing safe access, average terrestrial habitat quality
567	South of TATA	1 m width, 0.1 m depth	No		-	Shallow water, channel dense with Common reed, average terrestrial habitat quality
568	TATA	2 m width, 0.15 m depth	No		-	Shallow, double hedgerow
569	TATA	2 m width, 0.5 m depth	Yes		Torching, bottle traps (canes or floating), netting	
578	TATA		No		-	Dry
586						
1078	South of TATA	1 m width, 0.1 m depth	No			Dense scrub, channel filled with reeds
1082	South of TATA	<0.15m	No			Dense scrub
1124	South of TATA	1 m width, 0.25 m depth	No			Shallow, dense hedgerow, average terrestrial habitat quality
1128						
1132						
1133						
1138						
1152	TATA	1 m width, 0.25 m depth	No		-	Shallow, dense hedgerow, average terrestrial habitat quality
1200						
1206						
1207						
1211						
1216						
1220						
1228						
1234						
1236						
1244						
1250	TATA	3 m width, <1 m depth	Yes			Steep banks, dense reeds obscuring the water edge
1257	TATA	3 m width, <1 m depth	Yes		Torching	Steep banks, dense scrub to the south, average terrestrial habitat quality, waterfowl present.
2092	South of TATA	<0.15m	No			Dense scrub
1263 & 2002	TATA	2 m width, 0.5 m depth	Yes		Torching, bottle traps (floating), netting	Moderately steep banks, slightly polluted, 2002: higher turbidity and access restricted by reen
2092a	South of TATA	<0.15m	No			Dense scrub
TATA - East						
586	TATA	2 m width, 0.5 m depth	Yes		Torching, bottle traps, netting	Moderately steep banks, average terrestrial habitat quality quality
586	TATA					
603						
757						
758						
761						
778	TATA	2 m width, <0.25 m depth	No		-	Too shallow, average terrestrial habitat quality
779	TATA					
1062						
1127	TATA - East	<0.15m	No		-	Dense scrub, too shallow
1128						
1132	TATA	>1m depth	Yes		Torching, bottle traps, netting	Reeds present, waterfowl presence, average terrestrial habitat quality
1133	TATA	2 m width, 0.5 m depth	No		Torching, bottle traps (if deep enough)	Mid steep banks, reeds in channel, water level may fall below suitable levels, average terrestrial habitat quality
1138	TATA					Unable to find
1142	TATA - East	0.15m	No		-	Too fast flowing and shallow
1144	TATA					
1145	TATA					
1145						
1147						
1170						
1181						
1187	TATA - East	<0.15m	No		-	Moderately steep banks, dense scrub
1200	TATA	3 m width, >0.5 m depth	No			Mid steep banks, polluted
1200						



1206	TATA		No			Dry
1207	TATA	3 m width, 0.5 m depth	No			Steep banks, very polluted, average terrestrial habitat quality
1211	TATA	2 m width, 0.5 m depth	Yes		Torching, bottle traps, netting	Moderately steep banks, average terrestrial habitat quality
1211						
1216	TATA	2 m width, 0.5 m depth	Yes		Torching, bottle traps, netting	Moderately steep banks, average terrestrial habitat quality
1216						
1220	TATA		No			Dry
1222	TATA	3 m width, >0.5 m	Yes		Torching, bottle traps (floating)	Steep banks, looks muddy / soft at base, swan present, average terrestrial habitat quality
1222						
1228	TATA	<1 m depth	Yes		Torching, bottle traps, netting	Reeds present, average terrestrial habitat quality
1228						
1234	TATA	10 m width, 1 m depth	No			Polluted, sharp banks, average terrestrial habitat quality
1236	TATA	3 m width, >0.5 m depth	No			Mid steep banks, polluted
1236						
1244	TATA	5 m width, 0.5 m depth	No		-	Steep banks, very polluted, average terrestrial habitat quality
1350						
1353						
2090	TATA	3 m width, 0.75 m depth	Yes		Torching, bottle traps, netting	Moderately steep banks, dense scrub but reeds accessible in parts, base likely muddy soft, average terrestrial habitat quality
1359 A	TATA	3 m width, >0.5 m depth	Yes		Torching, bottle traps, netting	Dense scrub in places but can still access, good terrestrial habitat quality
778 A	TATA	2 m width, <0.25 m depth	No		-	Too shallow, steep banks, dense scrub, slightly polluted, average terrestrial habitat quality
G1	TATA	0.5 m depth	Yes		Torching, bottle traps, netting, egg search	Reeds and some scrub present, average terrestrial habitat quality
TE1	TATA - East	<0.5m	Yes		Torching, bottle traps, netting	Moderately steep banks, reeds and bramble make access difficult
TE2	TATA - East	0.5m	No		Torching, bottle traps, egg search	Rushes, expansive submerged rush pasture, rocky substrate need floating traps
TE3	TATA - East	>0.5m	Yes		Torching, bottle traps, netting, egg search	Rushes, expansive submerged rush pasture, rocky substrate need floating traps
TE4	TATA - East	<0.25m, 3m wide	Yes		Torching, netting, egg search	Dense scrub, macrophytes obscuring surface, likely dries out
TE5	TATA - East	>0.5m	Yes		Torching, bottle traps, netting, egg search	Reeds and trees within waterbody, still accessible, rocky substrate need floating traps
TE6	TATA - East	>0.5m	Yes		Torching, bottle traps, netting, egg search	Dense scrub in patches (still accessible). May dry out, appears to be ditch which has flooded outwards into surroundings
TE7	TATA - East	0.5m	Yes		Torching, bottle traps, netting, egg search	Expansive submerged rush pasture, good terrestrial habitat quality
TE8	TATA - East	0.25-0.50m	Yes		Torching, bottle traps, egg search	Rushes and bulrush
Lagoon 14	TATA	-	-		-	Very steep banks and very polluted
Pond 6 +7	TATA	-	-		-	Dry
Pond 7	TATA - East	<0.25m	No		-	Dense scrub, inaccessible, 100% duckweed cover
Total Count	S + S	105	S not S	11	NS	94

## **Annex B: Limitations of 2016 Population Size Class Estimate & Presence/Absence Surveys**

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Tata

Constraints	Watercourse Reference No.					
	559	560	563	565	566	1257
Dense emergent and bank vegetation			✓	✓		
Dense floating vegetation						
Hedge preventing access						
High turbidity					✓	
High water level				✓		
Recently cut bank vegetation damaging/removing the artificial egg strips	✓				✓	
Steep banks						✓
Water shrew	Water shrews were found in two different locations and are considered likely to be present throughout the area.					



## Tatton Farm

Constraints to survey	Watercourse Reference No.																		
	454	456	457	458	491	495	634	636	764	1089	1099	1101	1102	1106	1108	1111	1123	1169	1179
Dense emergent and bank vegetation					✓	✓								✓					
Dense floating vegetation					✓													✓	✓
Hedge preventing access	✓	✓	✓	✓?		✓			✓			✓	✓	✓	✓	✓	✓		✓
High turbidity																			
High water level																			
Steep banks							✓				✓		✓						
Water shrew	Water shrew was found and are considered likely to be present throughout the area.																		

Bareland Street

Constraints	Watercourse Reference No.										
	637	638	639	640	646	647	671	673	675	847	882
Dense emergent and bank vegetation					✓						✓
Dense floating vegetation	✓	✓	✓		✓	✓					✓
Hedge preventing access											✓
High turbidity		✓			✓	✓					
High water level											
Steep banks		✓				✓					
Water shrew	Water shrew was found in watercourse and are considered likely to be present throughout the area.										



# Kennels

Constraints	Watercourse Reference No.				
	628	827	898	981	985
Dense emergent and bank vegetation					
Dense floating vegetation					
Hedge preventing access	✓				✓
High turbidity					
High water level					
Steep banks					
Water shrew	Water shrew was found in watercourse and are considered likely to be present throughout the area.				

## Annex C: Full eDNA Survey Reports

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Technical Report  
Confidential

Folio No           D1651  
Report No:        1  
Client:            RPS Group  
Order No:  
Attn:             Sean Flynn  
Date:             11<sup>th</sup> May 2016

## **TECHNICAL REPORT**

# **EXAMINATION OF ENVIRONMENTAL DNA** **IN POND WATER FOR THE DETECTION OF** **GREAT CRESTED NEWTS**

**J.Campbell**



Forensic Scientists and Consultant Engineers  
SureScreen Scientifics Division Ltd, Morley Retreat, Church Lane, Morley, Derbyshire, DE7 6DE, UK  
Tel: +44 (0)1332 292003 Email: [scientifics@suresscreen.com](mailto:scientifics@suresscreen.com)  
Company Registration No. 2733607

## **Methodology**

When Great Crested Newts (GCN) inhabit a pond, they deposit traces of their DNA in the water as evidence of their presence. By sampling the water we can analyse these small environmental traces to detect GCN inhabitation.

The laboratory testing is conducted in two phases. The sample first goes through an extraction process where all 6 tubes are pooled together to acquire as much eDNA as possible. The pooled sample is then tested via real time PCR (or q-PCR). This process amplifies select part of DNA allowing it to be detected and measured.

qPCR combines PCR amplification and detection into a single step. This eliminates the need to detect products using gel electrophoresis. With qPCR, fluorescent dyes specific to the target sequence are used to label PCR products during thermal cycling. The accumulation of fluorescent signal during the exponential phase of the reaction is measured for fast and objective data analysis.

The primers used in this process are specific to a part of mitochondrial DNA only found in GCN ensuring no other DNA is amplified.

Samples are tested in a clean room and the different phases of testing are kept separate to reduce any risk of cross contamination.

Each pooled sample is replicated 12 times to ensure results are accurate. If one of the twelve replicates tests positive the sample is declared positive. The sample is only declared negative if no replicates show amplification.

Inhibition and degradation checks are also carried out on each sample using a known DNA marker. Results of these quality control tests are recorded with each sample.





## Results

Lab Ref	Sample	Co-Ordinates	Inhibition Check	Sample integrity	Result
21981	1298/1287 TATA Newport	1298	Acceptable	Acceptable	<b>Negative</b>
21982	TATA Newport, South Wales	1350	Acceptable	Acceptable	<b>Negative</b>
21983	TATA Newport, South Wales	G1	Acceptable	Acceptable	<b>Negative</b>
21984	TATA Newport, South Wales	586	Acceptable	Acceptable	<b>Negative</b>
21985	TATA Newport, South Wales	TE6 / TE7	Acceptable	Acceptable	<b>Negative</b>
21986	TATA Newport, South Wales	603	Acceptable	Acceptable	<b>Negative</b>
21987	TATA Newport, South Wales	1216	Acceptable	Acceptable	<b>Negative</b>
21988	TATA Newport, South Wales	603	Acceptable	Acceptable	<b>Negative</b>
21989	TATA Newport, South Wales	TE1 29/4/16 TE2 / TE3	Acceptable	Acceptable	<b>Negative</b>
21990	TATA Newport, South Wales	1170	Acceptable	Acceptable	<b>Negative</b>
21991	TATA Newport, South Wales	1222	Acceptable	Acceptable	<b>Negative</b>
21992	TATA Newport, South Wales	1128	Acceptable	Acceptable	<b>Negative</b>



21993	TATA Newport, South Wales	1290	Acceptable	Acceptable	<b>Negative</b>
21994	TATA Newport, South Wales	1314	Acceptable	Acceptable	<b>Negative</b>
21995	TATA Newport, South Wales	1178/1287	Acceptable	Acceptable	<b>Negative</b>
21996	581 Elle (6) TATA, Newport	1291	Acceptable	Acceptable	<b>Negative</b>
21997	TATA Newport, South Wales	1280 / 1088	Acceptable	Acceptable	<b>Negative</b>
21998	TATA Newport, South Wales	T4 / T5	Acceptable	Acceptable	<b>Negative</b>
21999	TATA Newport, South Wales	1300/1338	Acceptable	Acceptable	<b>Negative</b>
22000	TATA Newport, South Wales	695 25/4/16	Acceptable	Acceptable	<b>Negative</b>
22001	934, St Brides Road, Motorway Drainage Pond	934 St Brides Rd	Acceptable	Acceptable	<b>Negative</b>
22002	TATA Newport, South Wales	1291 / 591	Acceptable	Acceptable	<b>Negative</b>
22003	1178, Middle Reen, TATA, Newport	1178 Middle Reen	Acceptable	Acceptable	<b>Negative</b>





## **Advice**


Negative results may not indicate the absence of GCN just the presence of eDNA below the detection limits of the method. However this method is extremely sensitive. It is still advised to survey a pond using traditional methods within 2km of a positive result or a known habitat for GCN.

Positive results may be true positives but also may be due to contamination of samples from another pond or improper sampling technique. Please ensure traditional surveys are performed on positive ponds and care is taken to avoid spreading GCN DNA.


Samples undergo integrity scores to check for degradation post sampling. Samples which are not acceptable should be re-sampled. Sample integrity scores are based on the amount of degradation of an artificial DNA marker placed in the kits and analysed by qPCR.

PCR inhibitors can cause false results. Every effort is made to clean the sample pre-analysis however some inhibitors cannot be extracted. An unacceptable inhibition check will cause an indeterminate sample and must be sampled again.

Analysed and reported By: **J. Campbell BSc CEng.**



Checked and approved: **Troy Whyte BSc**



Technical Report  
Confidential

Folio No: D1787  
Report No: 1  
Client: RPS Group  
Order No:  
Attn: Sean Flynn  
  
Date: 01/06/16

## **TECHNICAL REPORT**

# **EXAMINATION OF ENVIRONMENTAL DNA** **IN POND WATER FOR THE DETECTION OF** **GREAT CRESTED NEWTS**

**A.Green**



Forensic Scientists and Consultant Engineers  
SureScreen Scientifics Division Ltd, Morley Retreat, Church Lane, Morley, Derbyshire, DE7 6DE, UK  
Tel: +44 (0)1332 292003 Email: [scientifics@suresscreen.com](mailto:scientifics@suresscreen.com)  
Company Registration No. 2733607



## **Methodology**

When Great Crested Newts (GCN) inhabit a pond, they deposit traces of their DNA in the water as evidence of their presence. By sampling the water we can analyse these small environmental traces to detect GCN inhabitation.

The laboratory testing is conducted in two phases. The sample first goes through an extraction process where all 6 tubes are pooled together to acquire as much eDNA as possible. The pooled sample is then tested via real time PCR (or q-PCR). This process amplifies select part of DNA allowing it to be detected and measured.

qPCR combines PCR amplification and detection into a single step. This eliminates the need to detect products using gel electrophoresis. With qPCR, fluorescent dyes specific to the target sequence are used to label PCR products during thermal cycling. The accumulation of fluorescent signal during the exponential phase of the reaction is measured for fast and objective data analysis.

The primers used in this process are specific to a part of mitochondrial DNA only found in GCN ensuring no other DNA is amplified.

Samples are tested in a clean room and the different phases of testing are kept separate to reduce any risk of cross contamination.

Each pooled sample is replicated 12 times to ensure results are accurate. If one of the twelve replicates tests positive the sample is declared positive. The sample is only declared negative if no replicates show amplification.

Inhibition and degradation checks are also carried out on each sample using a known DNA marker. Results of these quality control tests are recorded with each sample.

## Results

Laboratory Reference	Sample	Co-Ordinates	Inhibition Check	Sample integrity	Result
eDNA22399	Tatton North	1111	Acceptable	Acceptable	Positive
eDNA22400	Bareland Street, West	637	Acceptable	Acceptable	Positive
eDNA22401	Elver Pill Reen	1216	Acceptable	Acceptable	Negative
eDNA22402	Plot 422	827	Acceptable	Acceptable	Positive
eDNA22403	1250 West Tata	1250	Acceptable	Acceptable	Negative
eDNA22404	1250 East Tata	1250	Acceptable	Acceptable	Positive

## Advice

Negative results may not indicate the absence of GCN just the presence of eDNA below the detection limits of the method. However this method is extremely sensitive. It is still advised to survey a pond using traditional methods within 2km of a positive result or a known habitat for GCN.

Positive results may be true positives but also may be due to contamination of samples from another pond or improper sampling technique. Please ensure traditional surveys are performed on positive ponds and care is taken to avoid spreading GCN DNA.

Samples undergo integrity scores to check for degradation post sampling. Samples which are not acceptable should be re-sampled. Sample integrity scores are based on the amount of degradation of an artificial DNA marker placed in the kits and analysed by qPCR.

PCR inhibitors can cause false results. Every effort is made to clean the sample pre-analysis however some inhibitors cannot be extracted. An unacceptable inhibition check will cause an indeterminate sample and must be sampled again.

Reported By: **Arthur Green**



Verified By: **Andrew Penny**





Technical Report  
Confidential

A. P. Perry

Analysed By: **Thomas Wood**

Wood



## **Annex D: 2016 Population Size Class Estimate & Presence/Absence Survey Results**

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Area		Waterbody Ref	Date of survey	Number of survey	Techniques used				Results				Other species	Notes
					Bottle	Torch	Egg	Net	Bottle	Torch	Egg	Net		
Bareland street		634	160411	1		✓	✓			0	0		Stickleback	Unable to survey within 2m of
			160412	2		✓				0			Stickleback	
			160503	3		✓	✓			0	0		Stickleback	
			160504	4		✓	✓			0	0		Stickleback	
			160505	5		✓	✓			0	0		Stickleback	
			160512	6		✓	✓	✓		0	0	0	Stickleback	
		636												20% duckweed cover, steep banks leading to torching 2m away from the water, uneven ground surrounding the waterbody
			160503	1		✓	✓			0	0			
			160504	2		✓	✓			0	0			
			160505	3		✓	✓			0	0			
			160512	4		✓	✓	✓		0	0	0		
			160601	5										
		637	160405	1	✓	✓	✓		0	0	0			100% duckweed cover
														Unable to survey within 2m of the water, making egg searches and netting impossible
			160411	2		✓				0				
														Unable to survey within 2m of the water, making egg searches and netting impossible
			160412	3		✓				0				
			160503	4		✓	✓	✓		0	0	0		
			160504	5		✓	✓	✓		0	0	0		
			160505	6			✓	✓						100% duckweed cover
		647, 646, 638											Water shrew (638), sticklebacks, great diving beetle	Thick duckweed covered the water surface making it unsuitable for torching. EW fall in the reed before the other survey methods could begin
			160405	1	✓		✓		0					
			160411	2		✓	✓			0	0		Sticklebacks	Banks too steep to net
														Not allowed within 2m of the water making it impossible to net or egg search
			160412	3		✓				0			Sticklebacks	
			160503	4		✓	✓	✓		0	0	0	Sticklebacks	
			160504	5		✓	✓	✓		0	0	0	Sticklebacks	
			160505	6		✓	✓	✓		0	0	0	Sticklebacks	
			160512	7		✓	✓	✓		0	0	0	Sticklebacks	
														Very turbid, with willow catkins present on watersurface reducing visibility
			160525	8			✓	✓			0	0		
														Very turbid, with willow catkins present on watersurface reducing visibility
			160526	9			✓	✓			0	0		
		640												Standing 2m away meant we were unable to net
			160411	1		✓	✓			0	0			
			160412	2		✓				0				Standing 2m away meant we were unable to net
			160512	3		✓	✓	✓		0	0	0		
		671	160405	1	✓	✓	✓		2SM	0	0			
			160512	2		✓	✓	✓		0	0	0		
		675	160405	1	✓	✓	✓		1SM	0	0			
														1m away from the deep water, meant netting was too dangerous
			160503	2		✓	✓			0	0			
													Stickleback	1m away from the deep water, meant netting was too dangerous
			160504	3		✓	✓			0	0			
			160505	4		✓	✓			0	0			
			160512	5		✓	✓			0				
		847 - 955											Stickleback, water vole. common frog (x4)	Extensive water vole burrows
			160405	1	✓	✓	✓		2SM	0	0			Egg laying strips removed by unknown third party. Access watercourse for extensive egg searching and netting not possible due to abundance of water voles burrows
			160503	2		✓	✓			0		0	Stickleback, water vole.	

[illegible]



Area	Waterbody Ref	Date of survey	Number of survey	Techniques used				Results				Other species	Notes / constraints
				Bottle	Torch	Egg	Net	Bottle	Torch	Egg	Net		
TATA	559	160503	1		✓	✓	✓		0	0	0	Stickleback	Clear water
		160510	2		✓	✓	✓		0	0	0	Stickleback	Clear water
		160512	3		✓	✓	✓		0	0	0	Stickleback	Fairly turbid (2/5), torching was possible
		160523	4		✓	✓	✓		0	0	0	Stickleback	Fairly turbid (2/5), torching was possible
		160607	5		✓	✓	✓		0	0	0	Stickleback	Bank vegetation was recently cut removing/damaging all the egg strips in the area
		160614	6		✓	✓	✓		0	0	0	Stickleback	
	560	160503	1		✓	✓	✓		0	0	0	Stickleback	Fairly turbid (2/5), torchable to 1ft, water level higher
		160510	2		✓	✓	✓		0	0	0	Stickleback	Fairly turbid (2/5), torchable to 1ft, water level higher
		160512	3		✓	✓	✓		0	0	0	Stickleback	Fairly turbid (3/5), torchable to 1ft
		160523	4		✓	✓	✓		0	0	0	Stickleback	Fairly turbid (3/5), torchable to 1ft
		160607	5		✓	✓	✓		0	0	0	Stickleback, common frog	Fairly turbid (3/5), torchable to 1ft
		160614										Stickleback	
	563	160503	1		✓	✓	✓		0	0	0	Stickleback	Slightly turbid (2/5), torching still possible
		160510	2		✓	✓	✓		0	0	0	Stickleback	Slightly turbid (2/5), torching still possible
		160512	3		✓	✓	✓		0	0	0	Stickleback	Turbid (4/5), torching to 1ft
		160523	4		✓	✓	✓		0	0	0	Sticklebacks	Turbid (4/5), torching to 1ft
		160607	5		✓				0			Stickleback	Bank vegetation has grown making it hard to assess the waters edge, making it too dangerous to egg search or net
		160614										Stickleback	
	566	160503	1		✓	✓	✓		0	0	0	Stickleback	Quite turbid (4/5), only visible to 1ft
		160510	2		✓	✓	✓		0	0	0	Stickleback	Very turbid (5/5), difficult to see within the watercourse
		160512	3		✓		✓		0		0	Stickleback	Very turbid (5/5), difficult to see within the watercourse, water level too high to egg search safely
		160523	4		✓	✓	✓		0	0	0	Stickleback	Very turbid (5/5), difficult to see within the watercourse
		160607	5		✓	✓			0	0		Stickleback	Bank vegetation was recently cut removing/damaging all the egg strips in the area
		160614										Stickleback	
	565	160503	1		✓		✓		0		1SM	Stickleback	unable to egg search due to the southern side being steep. Access from the north will allow egg searching (need TATA land access)
		160510	2		✓		✓		1SM		0	Stickleback	Water level up too high to egg seach
		160512	3		✓	✓	✓		2SM	0	0	Stickleback	Turbid (3/5), torching was ok
		160523	4		✓	✓	✓		2SM	0	0	Stickleback	Turbid (3/5), torching was ok
		160607	5		✓				0			Stickleback	Bank vegetation has grown making it hard to assess the waters edge, making it too dangerous to egg search or net
		160614										Stickleback	
	1257	160510	1		✓				0			Stickleback	Banks too steep to safely egg seach and net
		160512	2		✓				1SM			Stickleback	Banks too steep to safely egg seach and net
		160523	3		✓				2SM			Stickleback	Banks too steep to safely egg seach and net
	Water surface has become increasing turbid throughout the survey period making torching less effective. This factor alongside the steep banks make torching												

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Area	Waterbod y Ref	Date of survey	Number of survey	Techniques used				Results				Other species	Notes	
				Bottle	Torch	Egg	Net	Bottle	Torch	Egg	Net			
Tatton Farm	454-764	160504	1		✓					0			surveyed through small gaps in the hedge, unable to egg search and net	
		160505	2		✓					0			surveyed through small gaps in the hedge, unable to egg search and net	
		160525	3		✓					0			surveyed through small gaps in the hedge, unable to egg search and net	
		160526	4		✓					0			surveyed through small gaps in the hedge, unable to egg search and net	
		160601	5		✓					0			surveyed through small gaps in the hedge, unable to egg search and net	
		160607			✓					0				
		456	160504	1		✓	✓				0	0		surveyed through small gaps in the hedge, limited access to egg search and unsuitable to net
	160505		2		✓	✓				0	0		surveyed through small gaps in the hedge, limited access to egg search and unsuitable to net	
	160509		3		✓	✓	✓			0	0	0	70% of the waterbody inaccessible due to vegetation cover	
	160510		4		✓	✓	✓			0	0	0	70% of the waterbody inaccessible due to vegetation cover	
	160511		5		✓	✓	✓			0	0	0	70% of the waterbody inaccessible due to vegetation cover	
	160525		6			✓	✓				0	0	90% of the waterbody inaccessible due to duckweed cover	
	160526		7			✓	✓				0	0	90% of the waterbody inaccessible due to duckweed cover	
	457	160509	1		✓	✓	✓			0	0	0	85% of the waterbody inaccessible	
		160510	2		✓	✓	✓			0	0	0	85% of the waterbody inaccessible	
		160511	3		✓	✓	✓			0	0	0	85% of the waterbody inaccessible	
		160525	4		✓	✓	✓			0	0	0	85% of the waterbody inaccessible	
		160526	5		✓	✓	✓			0	0	0	85% of the waterbody inaccessible	
		160601	6											
	458	160509	1		✓	✓	✓		1SM		0	0	85% of the waterbody inaccessible	
		160510	2		✓	✓	✓			0	0	0	85% of the waterbody inaccessible	
		160511	3		✓	✓	✓			0	0	0	85% of the waterbody inaccessible	
		160525	4		✓	✓	✓			0	0	0		
		160526	5		✓	✓	✓			0	0	0		
		160601	6											
	491	160525	1	✓		✓		3SM			0		Torching and netting not possible as the waterbody was covered in duckweed and emergent veg	
	495	160420	1		✓	✓				0	0		Goldfish	RAMS not yet signed off for bottle trapping. 80% of the waterbody innaccessible for torching and netting due to vegetation cover
		160421	2		✓	✓		b	1PN, 2SN		0	0	Goldfish	RAMS not yet signed off for bottle trapping. 80% of the waterbody innaccessible for torching and netting due to vegetation cover
		160425	3	✓	✓		✓	0	0	0	0	0	Goldfish	80% of the waterbody innaccessible for torching due to vegetation cover
		160509	4		✓	✓	✓			0	0	0	Goldfish	80% of the waterbody innaccessible for torching due to vegetation cover
		160510	5		✓	✓	✓			0	0	0	Goldfish	80% of the waterbody innaccessible for torching due to vegetation cover
		160511	6		✓	✓	✓			0	0	0	Goldfish	80% of the waterbody innaccessible for torching due to vegetation cover
		1089	160509	1		✓	✓	✓			0	0	0	
	160510		2		✓	✓	✓			0	0	0		
	160511		3		✓	✓	✓			0	0	0		
	160525		4		✓	✓	✓			0	0	0		50% of the waterbody inaccessible
	160526		5		✓	✓	✓			0	0	0		
	160601		6											
	1099	160420	1		✓	✓				0	0		Stickleback	RAMS not yet signed off for bottle trapping
		160421	2		✓	✓				0	0		Stickleback	RAMS not yet signed off for bottle trapping
		160425	3	✓	✓	✓		0	0	0	0		Water shrew found in a bottle trap.	
		160509	4		✓	✓				0	0	0	Stickleback	Banks too steep to safely access for netting
		160510	5		✓	✓				0	0	0	Stickleback	Banks too steep to safely access fpr netting
		160511	6		✓	✓				0	0	0	Stickleback	Banks too steep to safely access fpr netting
		1101	160420	1		✓		✓			0	0		Stickleback
	160421		2		✓		✓			0	0		Stickleback	RAMS not yet signed off for bottle trapping. 90% of the waterbody inaccessible to egg seach due to vegetation cover, Turbidity 3, lots of stickleback
	160425		3		✓		✓			0	0		Stickleback	90% of the waterbody inaccessible to egg seach due to vegetation cover, Turbidity 3, lots of stickleback



	160509	4		✓	✓	✓		0	0	0		90% of the waterbody inaccessible to egg seach due to vegetation cover, Turbidity 3, lots of stickleback		
	160510	5		✓	✓	✓		0	0	0		90% of the waterbody inaccessible to egg seach due to vegetation cover, Turbidity 3, lots of stickleback		
	160511	6		✓	✓	✓		0	0	0		90% of the waterbody inaccessible to egg seach due to vegetation cover, Turbidity 3, lots of stickleback		
1102	160420	1		✓	✓			0	0		Stickleback	95% of the waterbodt innaccessible due to vegeation cover. Banks too steep to safely access for netting		
	160421	2		✓	✓			0	0		Stickleback	95% of the waterbodt innaccessible due to vegeation cover. Banks too steep to safely access for netting		
	160425	3	✓	✓	✓			0	0		Stickleback	95% of the waterbodt innaccessible due to vegeation cover. Banks too steep to safely access for netting		
	160601	4										95% of the waterbodt innaccessible due to vegeation cover. Banks too steep to safely access for netting		
	160607	5												
1106	160411	1										Waterbody inaccessible due to the vegetation cover		
	160509	2		✓	✓	✓		0	0	0				
	160510	3		✓	✓	✓		0	0	0				
	160511	4		✓	✓	✓		0	0	0				
	160601	5												
1108	160420	1		✓	✓	✓		1P		0		Stickleback	70% of the waterbody innaccessible due to vegetation cover	
	160421	2		✓	✓	✓		0	0		Stickleback	70% of the waterbody innaccessible due to vegetation cover		
	160425	3		✓	✓	✓		0	0		Stickleback	70% of the waterbody innaccessible due to vegetation cover		
	160509	4		✓	✓	✓		0	0	0	Stickleback			
	160510	5		✓	✓	✓		0	0	0	Stickleback			
	160511	6		✓	✓	✓		0	0	0	Stickleback			
1111	160504	1		✓	✓	✓		3sn		0	0			
	160505	2		✓	✓	✓		0	0	0				
	160509	3		✓	✓	✓		0	0	0	0			
	160510	4		✓	✓	✓		0	0	0				
	160511	5		✓	✓	✓		0	0	0				
	160525	6		✓	✓	✓		0	0	0		80% of the waterbody inaccesable		
	160526	7		✓	✓	✓		0	0	0				
1123	160420	1		✓	✓				0	0			80% of the waterbody innaccessible due to vegeation cover, netting not possible, turbidity 3,	
	160421	2		✓	✓				0	0			80% of the waterbody innaccessible due to vegeation cover, netting not possible, turbidity 3,	
	160425	3	✓	✓	✓		4SM, 1P		0	0			80% of the waterbody innaccessible due to vegeation cover, netting not possible, turbidity 3,	
	160509	4		✓	✓	✓		0	0	0				
	160510	5		✓	✓	✓		0	0	0				
	160511	6		✓	✓	✓		0	0	0		Waterlevel 10cm higher		
1169	160504	1		✓	✓	✓		0		0	Stickleback	90% duckweed cover		
	160505	2		✓	✓	✓		0			Stickleback	100% duckweed cover		
	160509	3			✓	✓				0	0	Stickleback	Waterbody 100% covered in duckweed making torching not possible	
	160510	4			✓	✓				0	0	Stickleback	Waterbody 100% covered in duckweed making torching not possible	
	160511	5			✓	✓				0	0	Stickleback	Waterbody 100% covered in duckweed making torching not possible	
	160525	6			✓	✓			1SM		0	Stickleback	Waterbody 100% covered in duckweed making torching not possible	
1179	160504	1		✓	✓			5SM			0	Stickleback	Survyed from the south side only, due to vegetation cover. Too shallow to net	
	160505	2		✓	✓			2SM			0	Stickleback	Survyed from the south side only, due to vegetation cover. Too shallow to net	
	160509	3										Stickleback	Not accessable for any survey	
	160525	4			✓	✓				0	0	Stickleback	Duckweed cover and hedgerow prevented torching	
	160526	5			✓	✓				0	0	Stickleback	Duckweed cover and hedgerow prevented torching	
	160601	6										Stickleback		

Area	Ref.	Date of survey	Number of survey	Techniques used				Results				Other species	Notes
				Bottle	Torch	Egg	Net	Bottle	Torch	Egg	Net		
Tonew Kennels	628	160503	1		✓	✓			0	0			Some gaps in the hedge suitable for torching, unable to net due to the steep banks and dense vegetation
		160504	2		✓	✓			0	0			Some gaps in the hedge suitable for torching, unable to net due to the steep banks and dense vegetation
		160505	3		✓	✓			0	0			Some gaps in the hedge suitable for torching, unable to net due to the steep banks and dense vegetation
		160525	4		✓	✓	✓		0	0	0		Some gaps in the hedge suitable for torching, unable to net due to the steep banks and dense vegetation
		160526	5		✓	✓	✓		0	0	0		Some gaps in the hedge suitable for torching, unable to net due to the steep banks and dense vegetation
	827, 981, 898	160503	1		✓	✓			0	0			Some gaps in the hedge suitable for torching, unable to net due to the steep banks and dense vegetation
		160504	2		✓	✓			0	0			Some gaps in the hedge suitable for torching, unable to net due to the steep banks and dense vegetation
		160505	3		✓	✓			0	0			Some gaps in the hedge suitable for torching, unable to net due to the steep banks and dense vegetation
		160525	4			✓	✓			0	0		Some gaps in the hedge suitable for torching, unable to net due to the steep banks and dense vegetation
		160526	5			✓	✓			0	0		Some gaps in the hedge suitable for torching, unable to net due to the steep banks and dense vegetation
	985	160405	1	✓				0					Personnel fell into reen before the other survey methods could start
		160503	2		✓	✓			0	0			Dense hedgerow prevent access for netting
		160504	3		✓	✓			0	0			Dense hedgerow prevent access for netting
		160505	4		✓	✓			0	0			Dense hedgerow prevent access for netting
		160525	5		✓	✓	✓		0	0	0		Dense hedgerow prevent access for netting
		160526	6		✓	✓	✓		0	0	0		Dense hedgerow prevent access for netting