Sheringham Shoal and Dudgeon Offshore Wind Farm Extension Projects

Environmental Statement

Volume 3

Appendix 20.2 – Great Crested Newt Survey Report (Revision B) (Clean)

Revision B
Deadline 3
May 2023
Document Reference: 6.3.20.2
APFP Regulation: 5(2)(a)

China Resources
Better Life Together

Masdar
A MUBADALA COMPANY

equinor

Report produced by	Produced on behalf of
Written by: William Riddett BA ACIEEM Checked by: Robert Yaxley BSc CEcol CEnv MCIEEM Ptolemy McKinnon BSc MSc Authorised by: Robert Yaxley BSc CEcol CEnv MCIEEM Mary Goddard BSc MSc ACIEEM Final edit authorised by William Riddett BA ACIEEM Update produced and authorised by: William Riddett BA ACIEEM	Equinor New Energy Limited Forusbeen 50 4035 Stavanger Norway
Wild Frontier Ecology Ltd. Unit 2, Cold Blow Farm Great Snoring, Fakenham Norfolk, NR21 0HF Tel: 01328 864633 will@wildfrontier-ecology.co.uk	

Company Registered in England and Wales No 4942219.

VAT Reg No. 887 4692 54

Registered Office: Saxon House, Hellesdon Park Road, Drayton High Road, Norwich NR6 5DR

Director: Robert Yaxley BSc (Hons) CEcol CEnv MCIEEM.

The data which we have prepared and provided is accurate, and has been prepared and provided in accordance with the CIEEM's Code of Professional Conduct. We confirm that any opinions expressed are our best and professional bona fide opinions.

This report conforms to the British Standard 42020:2013 Biodiversity – Code of practice for planning and development.

CONTENTS

LIST OF ACRONYMS

DCO	Development Consent Order
DEFRA	The Department for the Environment, Food and Rural Affairs
DEP	Dudgeon Offshore Wind Farm Extension Project
DLL	District Level Licence/Licensing
DNA	Deoxyribonucleic Acid
eDNA	Environmental DNA
EIA	Environmental Impact Assessment
EP1HS	Extended Phase 1 Habitat Survey
EPS	European Protected Species
ETG	Expert Topic Group
EU	European Union
GCN	Great Crested Newt
HSI	Habitat Suitability Index
NBIS	Norfolk Biodiversity Information Service
OS	Ordnance Survey
PEIR	Preliminary Environmental Information Report
SEP	Sheringham Shoal Offshore Wind Farm Extension Project
SSL	SureScreen Scientifics Ltd.
UCL	University College London
UCLPRRG	University College London Pond Restoration Research Group
UK	United Kingdom
WFE	Wild Frontier Ecology Ltd.

GLOSSARY OF TERMS

Term	Definition
DCO boundary	The area subject to the application for development consent, including all permanent and temporary works for SEP and DEP.
Dudgeon Offshore Wind Farm Extension Project (DEP)	The Dudgeon Offshore Wind Farm Extension onshore and offshore sites including all onshore and offshore infrastructure.
DEP onshore site	The Dudgeon Offshore Wind Farm Extension onshore area consisting of the DEP onshore substation site, onshore cable corridor, construction compounds, temporary working areas and onshore landfall area.
European site	Sites designated for nature conservation under the Habitats Directive and Birds Directive. This includes candidate Special Areas of Conservation, Sites of Community Importance, Special Areas of Conservation and Special Protection Areas, and is defined in regulation 8 of the Conservation of Habitats and Species Regulations 2017.
Evidence Plan Process (EPP)	A voluntary consultation process with specialist stakeholders to agree the approach, and information to support, the EIA and HRA for certain topics.
Expert Topic Group (ETG)	A forum for targeted engagement with regulators and interested stakeholders through the EPP.
Horizontal directional drilling (HDD) zones	The areas within the onshore cable route which would house HDD entry or exit points.
Jointing bays	Underground structures constructed at regular intervals along the onshore cable route to join sections of cable and facilitate installation of the cables into the buried ducts.
Landfall	The point at the coastline at which the offshore export cables are brought onshore, connecting to the onshore cables at the transition joint bay above mean high water
Onshore cable corridor	The area between the landfall and the onshore substation sites, within which the onshore cable circuits will be installed along with other temporary works for construction.
Onshore export cables	The cables which would bring electricity from the landfall to the onshore substation. 220 – 230kV.
Onshore Substation	Compound containing electrical equipment to enable connection to the National Grid.
PEIR boundary	The area subject to survey and preliminary impact assessment to inform the PEIR.
Sheringham Shoal Offshore Wind Farm Extension Project (SEP)	The Sheringham Shoal Offshore Wind Farm Extension onshore and offshore sites including all onshore and offshore infrastructure.
SEP onshore site	The Sheringham Shoal Wind Farm Extension onshore area consisting of the SEP onshore substation site, onshore cable corridor, construction compounds, temporary working areas and onshore landfall area.
Study area	Area where potential impacts from the project could occur, as defined for each individual Environmental Impact Assessment (EIA) topic.
The Applicant	Equinor New Energy Limited

EXECUTIVE SUMMARY

Wild Frontier Ecology Ltd. was commissioned by Equinor New Energy Ltd. to undertake great crested newt *Triturus cristatus* (GCN) surveys of ponds within and up to 250 metres (m) of the onshore grid connection cable corridor associated with the proposed Sheringham Shoal Offshore Wind Farm Extension Project (SEP) and Dudgeon Offshore Wind Farm Extension Project (DEP). The GCN survey effort comprised Habitat Suitability Index (HSI) appraisals of all accessible ponds for their suitability to support GCN, and environmental DNA (eDNA) surveys of all accessible ponds to confirm the presence or likely absence of GCN. The HSI appraisals and eDNA surveys were undertaken between March and June 2020 and between April and June 2021, within the appropriate survey seasons and by GCN licensed ecologists or Accredited Agents.

The GCN surveys took place between 2020 and 2021, at the same time as the ongoing site selection process. The survey area was initially based on the Preliminary Environmental Information Report (PEIR) boundary and the surrounding 250 m buffer. The boundary was refined throughout 2020 and 2021, and by June 2021 had become the narrower preliminary Development Consent Order (DCO) boundary. This boundary was subject to further refinement, drawing on consultation responses and incoming survey data on ecological and other constraints. This report presents information on GCN surveys of ponds within and up to 250 m of the final DCO boundary.

Ponds within and up to 250 m from this PEIR/DCO boundary were identified using Ordnance Survey (OS) maps and other freely availably mapping programmes such as Google Earth. Any additional ponds within the survey area that were noted during other ecological field surveys were also included in the 2020 and/or 2021 GCN survey effort.

There are a total of 180 known ponds within the DCO boundary and the surrounding 250m buffer area, of which:

- 140 ponds were HSI appraised and eDNA surveyed in full;
- 14 ponds were found to be dry so were not surveyed;
- 4 ponds were physically inaccessible due to barriers such as fences or dense vegetation;
- 15 ponds were not surveyed because landowner access was not granted; and,
- 7 ponds were not surveyed because they were originally beyond 250m from the DCO boundary during the 2020 and 2021 survey seasons, but later refinements to the boundary brought these ponds inside the survey area.

The HSI appraisals of the 142 ponds which were accessible and surveyed (comprising the 140 fully accessible ponds plus two ponds which could be viewed remotely but not accessed for an eDNA survey) produced the following suitability classifications of the ponds as GCN breeding ponds:

- Excellent: 28 ponds;
- Good: 35 ponds;
- Average: 29 ponds;
- Below average: 35 ponds; and,

- Poor: 15 ponds.

Of the 140 ponds subject to an eDNA survey, 14 returned a positive result indicating GCN presence. GCN presence was visually confirmed by surveyors at one pond prior to the eDNA survey (GCN were seen in the pond so eDNA sampling and analysis was deemed unnecessary). The other 125 ponds returned negative results indicating the likely absence of GCN.

The results of the HSI and eDNA surveys of the 140 ponds within the survey area are provided in full, below, as are the results of the HSI appraisals of the additional two ponds which could not be accessed for eDNA surveys. The individual reasons (e.g. pond dry, landowner access not granted, etc.) for not fully surveying the other 38 ponds are also provided below. Maps are also provided below (see **Figure 1** to **Figure 16**) which show the location and distribution of ponds surveyed and the positive/negative eDNA survey results.

There are a number of clusters of ponds which returned positive results, suggesting the presence of GCN metapopulations in these areas, including around Bodham (see **Figure 3**), around Marlingford and Colton (see **Figure 12**) and around Hethersett, Ketteringham and Swardeston (see **Figure 13**, **Figure 14** and **Figure 15**).

A desk study comprising a data search with the Norfolk Biodiversity Information Service (NBIS) and consultation with the University College London Pond Restoration Research Group (UCLPRRG) returned records of GCN within the PEIR/DCO boundaries and further afield. These data largely corroborate the results of the 2020-21 eDNA surveys, with records of GCN distributed around the aforementioned metapopulation areas, particularly around Bodham. The one exception is around Saxthorpe and Itteringham where the 2020-21 eDNA surveys recorded one isolated positive result (pond reference PW166 – see **Figure 6**); the NBIS data search returned a GCN record from this same pond and another nearby pond (pond reference PN103 [see **Figure 6**], for which the 2020 eDNA survey received a negative result). This one positive eDNA result coupled with a nearby NBIS record may also indicate a metapopulation of GCN around this part of the DCO boundary.

SEP and DEP has been approved by Natural England to use District Level Licence (DLL) prior to construction to ensure compliance with the legal status of GCN and mitigate for potential impacts on this species. DLL involves providing a Conservation Payment to fund a net increase in habitat for GCN at a county level, rather than mitigate for impacts specifically within and around the DCO boundary. An initial Conservation Payment has been made by SEP and DEP to begin funding off-site mitigation for GCN (see Annex 3, Appendix 1 of the Planning Statement (document reference 9.1). The remaining Conservation Payment will be settled shortly before construction is due to commence. Further GCN surveys are not necessarily required to inform the remaining DLL Conservation Payment. However, updated survey data could be used (if available) to refine the DLL Conversation Payment calculation prior to construction commencing.

1. BACKGROUND

Equinor New Energy Limited (hereafter Equinor) is proposing to extend the existing operational Sheringham Shoal Offshore Wind Farm and Dudgeon Offshore Wind Farm, named the Sheringham Shoal Offshore Wind Farm Extension Project (SEP) and Dudgeon Offshore Wind Farm Extension Project (DEP). SEP and DEP will consist of a number of offshore and onshore elements including the offshore wind turbines, offshore export cables and offshore substation(s). The offshore export cables will connect to shore on the North Norfolk coast, with onshore infrastructure connecting the offshore wind farms to the National Grid, which will comprise underground cables from landfall at Weybourne to an onshore substation and National Grid connection at Norwich Main. A full description of SEP and DEP is provided within **ES Chapter 4 Project Description** (document reference 6.1.4).

WFE was commissioned by Equinor to undertake surveys to establish the presence and/or likely absence of Great Crested Newts in ponds within and up to 250m of the onshore grid connection cable corridor boundary to inform an ecological impact assessment of the proposed onshore grid connection for SEP and DEP. The onshore components comprise a c.60-kilometre (km) route with landfall location around Weybourne on the North Norfolk coast, with the onshore cable route then running southwards and eventually eastwards around the west and south sides of Norwich, where it is to connect with a proposed onshore electricity substation, feeding into the National Grid near Norwich Main Substation.

The GCN surveys ran concurrently with the ongoing SEP and DEP onshore site selection process. Initially, the onshore cable corridor was defined as the PEIR boundary, and this formed the basis of the surveys completed in 2020 and early 2021. Between the end of April and mid-June 2021, the PEIR boundary was subject to statutory consultation. Input from that consultation, together with the initial results of the ecology surveys (and other investigations) completed to date informed further refinement of the final DCO boundary. Therefore, the GCN survey area evolved throughout the 2020 and 2021 survey seasons, as it was based on the evolving onshore cable corridors at those times. For the purposes of the impact assessment and this technical appendix, it is only ponds which are within the final DCO boundary and the surrounding 250 m buffer which are included. Ponds which were surveyed because they were within 250 m of the wider PEIR boundary are not included if they are beyond 250 m of the DCO boundary.

Maps showing the survey area (i.e. the DCO boundary plus the surrounding 250m buffer) are provided in **Figure 1** to **Figure 16**, below.

This report outlines the aims, methods and results of the GCN surveys which have been completed in March to June 2020 and April to June 2021.

2. RELEVANT LEGISLATION AND POLICY

The GCN is fully protected in accordance with both national and international legislation. The species is listed under Annexes IV and II of European Directive 92/43/EEC, and Schedule 2 of The Conservation of Habitats and Species Regulations 2017. EU laws supporting species protection are, from 31st January 2020, transposed into UK law through changes made to existing legislation by the Conservation of Habitats and Species (Amendment) (EU Exit) Regulations 2019. The GCN is also protected by Sections 9(4) and 9(5) of the Wildlife and Countryside Act 1981 as amended.

It is an offence to knowingly or recklessly kill, injure, disturb, handle or sell the animal, and this protection is afforded to all life stages. It is unlawful to deliberately or recklessly damage, destroy, or obstruct the access to any structure or place used for shelter or protection; this includes both the terrestrial and aquatic components of its habitat.

3. SURVEY METHODS

3.1. Desk Study

During the Terrestrial Ecology and Ornithology Expert Topic Group (ETG) meeting on 28th January 2020, attended by Natural England, the Environment Agency, Broadland District Council, Norfolk County Council, North Norfolk District Council and South Norfolk District Council, it was agreed that ponds within and up to 250m from the PEIR boundary (which constituted the extents of the onshore grid connection cable corridor at that time) should be surveyed for GCN.

Ponds within this area (i.e. within and up to 250 m from the boundary) were identified from a desk-based review of Ordnance Survey (OS) maps and other freely available mapping software such as Google Earth. Ponds were mapped as points onto the Quantum Geographic Information System (QGIS) programme, and assigned a unique individual reference, typically a P (denoting Pond) followed by a three-digit number (e.g. P123). Other ponds were assigned a unique reference of two letters followed by a three-digit number (e.g. PA001, PN101, PS004, PW203, PX018), which related to ponds that were subsequently added to the survey effort, typically following the adjustments to the PEIR/DCO boundaries as part of the ongoing site selection process.

In general, numbering started at 001 at the southern end of the PEIR/DCO boundary and increased moving northwards, so, for example, pond P001 is at the very southern end of the survey area and pond PW204 is at the northernmost point of the survey area near the proposed landfall location near Weybourne.

At the end of the 2020 GCN survey season, 50 ponds within the survey area had not been surveyed because the ponds were either dry or landowner access had not been granted. These ponds were added to the 2021 survey scope, as it was possible that they would hold water and/or landowner access would be agreed in 2021. The 2021 surveys therefore aimed to cover three groups of ponds overall: those which had been newly added to the survey area following refinements to the PEIR boundary since the 2020 GCN survey season (a total of 34 ponds) along with any ponds newly identified during other ecological surveys (a total of six ponds); those which remained within the survey area but which had been found to be dry in 2020 (a total of 21 ponds); and those which remained within the survey area but which had not been accessible (due to withheld landowner access) in 2020 (a total of 29 ponds).

As shown in Section 4 (Results), 12 ponds (out of the 21 ponds which had been found to be dry in 2020) were found to be holding water in 2021 and could therefore be surveyed (the remaining nine ponds continued to be dry). Landowner access was permitted to survey 20 ponds (out of the 29 ponds which had previously been inaccessible), allowing them to also be surveyed in 2021, although three of these ponds were then found to be dry in 2021.

3.1.1. GCN Survey Data Provided by UCL Pond Restoration Research Group

One of the landowners of a parcel of land within the survey area has connections to the University College London (UCL) Pond Restoration Research Group (UCLPRRG) which studies ponds and engages in the restoration and conservation of ponds in various parts of Norfolk, including part within the DCO boundary. The studies include recording whether ponds support breeding GCN. The UCLPRRG provided WFE with GCN survey

data compiled between 2011 and 2020 for ponds between Baconsthorpe and Bodham[1]. A review of the data revealed seven of these ponds are within the survey area, all of which were also surveyed by WFE in 2020-21.

Precise survey methodologies used by the UCLPRRG are not outlined in the report. However, the studies on which the GCN data is based dates back to 2011 (before eDNA for GCN was known to be available), and relate to breeding GCN, which cannot be determined by eDNA surveys alone. Therefore, the UCLPRRG surveys are not expected to have used eDNA sampling; instead, more conventional pond survey techniques such as dip-netting or setting of bottle traps in the ponds overnight are thought to have been used.

3.1.2. NBIS GCN Records

A data search was undertaken with the Norfolk Biodiversity Information Service (NBIS) in January 2021, to obtain all biological records (including records of GCN) within and up to 2km from the PEIR boundary. A wider search area was used than the survey area (restricted to the PEIR/DCO boundary and the surrounding 250m) because some biological records are defined to a 1km grid square, so a wider search area is required to ensure all relevant records are obtained.

3.2. Habitat Suitability Index

All accessible ponds within the survey area were appraised for their suitability to support GCN using the HSI per Oldham (2000)[2] and the classification guide defined by the Amphibian and Reptile Groups of the United Kingdom (2010)[3]. All pond appraisals took place during March to June 2020, and April to June 2021.

The HSI is an indicative tool used to rate the suitability of ponds for GCN, based on ten characteristics and features such as size, water quality, vegetation cover and quality of surrounding terrestrial habitat. These features are assessed, classified according to prescribed criteria and assigned a numerical score. These scores allow the HSI to categorise ponds into one of five ratings which indicate their suitability for use by GCN. The five categories and the score parameters (between 1 and 0) are as follows:

- Excellent: >0.8
- Good: 0.7 – 0.79
- Average: 0.6 – 0.69
- Below average: 0.5 – 0.59
- Poor: <0.5

The HSI appraisals were completed by the following WFE staff (always working in pairs):

---

[1] Sayer C. (2020). *Threats to pond networks associated with the Equinor cable – Information provided by Carl Sayer and the Norfolk Ponds Project.* Unpublished report.

[2] Oldham R., Keeble J., Swan M. & Jeffcote, M. (2000). Evaluating the suitability of Habitat for Great Crested Newt (*Triturus cristatus*). *Herpetological Journal* **10**: 143-155.

[3] ARG UK. (2010). *ARG UK Advice Note 5, Great Crested Newt Habitat Suitability Index*. Amphibian and Reptile Groups of the United Kingdom

- Alex Lowe BSc MArborA
- Ptolemy McKinnon BSc MSc
- Justin Parry BSc
- Alice Petherick BA
- William Riddett BA ACIEEM (Natural England class licence reference 2015-19075-CLS-CLS).
- Graham Riley BSc ACIEEM (Natural England class licence reference 2019-43743-CLS-CLS)
- Katrina Salmon BSc
- Adam Stickler BSc MSc ACIEEM (Natural England class licence reference 2019-43544-CLS-CLS)
- Robert Yaxley BSc CEnv CEcol MCIEEM (Natural England class licence reference 2016-19382-CLS-CLS)

All surveys were completed by Natural England licensed surveyors or accredited agents (i.e. surveyors permitted to do the surveys under the permission of the licence holder).

3.3. Presence/Absence Survey using eDNA Testing

Within the survey area, each accessible pond was surveyed to collect water samples for eDNA analysis using a SureScreen Scientifics Ltd. (SSL) eDNA sampling kit. The survey employed the methodology outlined by DEFRA[4], Natural England[5] and the Freshwater Habitats Trust[6].

Twenty water samples were taken from each pond using sterile equipment: samples were taken using gloves and a ladle from across all accessible parts of each pond, concentrating on areas which the surveyor considered had greatest potential to be used by GCN.

The surveyors did not enter the water in order to ensure there was no accidental contamination (e.g. from footwear), so all samples were collected by reaching into the pond from the shoreline. For each pond, the water samples were all poured into a mixing bag and combined. Water samples were then transferred with a pipette from the mixing bag into six sealed test tubes partly pre-filled with preservative. These tubes were resealed and then posted to SSL for laboratory analysis. This process was completed for each surveyed pond. All eDNA surveys were completed from 28th April to 30th June 2020, and from 21st April to 30th June 2021.

[4] 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

[5] https://www.gov.uk/guidance/great-crested-newts-surveys-and-mitigation-for-development-projects

[6] Freshwater Habitats Trust (2015) *Pondnet: How to collect an eDNA sample*. Available online at [redacted]

4. RESULTS

4.1. Desk Study

A total of 180 ponds have been identified and mapped within the survey area (the DCO boundary and the surrounding 250 m buffer) and therefore assigned individual references. This number has fluctuated since surveys began in March/April 2020 due to ongoing refinements made to the PEIR/DCO boundaries as part of the site selection process.

4.1.1. GCN Survey Data Provided by UCL Pond Restoration Research Group

The data obtained from UCLPRRG is provided in Annex 2 and shows that these studies have found breeding GCN within six of the seven ponds within the survey area (specifically within ponds PW179, PW180, PW181, PW182, PW183 and PW186 – see **Figure 3**). The only pond in which the UCLPRRG has not confirmed breeding GCN presence is pond PW175 (see **Figure 3**), for which their report lists GCN status as 'unknown'. Where appropriate, the results from the UCLPRRG surveys are included in the Notes column of **Table 1**, below.

4.1.2. GCN Data Provided by Norfolk Biodiversity Information Service

The NBIS data search returned 18 records of GCN within and up to 250m from the DCO boundary. These records were provided by NBIS with locations defined to a grid reference. These have been mapped and overlaid with the pond location maps to attempt to assign each NBIS GCN record to a known pond (none of the records are of terrestrial GCN, so are all assignable to a waterbody). Some grid references are given to a low resolution, so it is not certain which pond these records relate to. In such cases the description of the record has been used to inform which pond the record is assigned to. Where there is a residual level of uncertainty as to which pond a record definitely relates to, this is listed in **Table 1**, below.

The records are clustered around Bodham, with 12 of the 18 NBIS GCN records attributed to five ponds (PW175 [one record], PW180 [seven records], PW181 [one record], PW182 [two records] and PW183 [one record]) in and around Pond Farm south of Bodham. All these ponds were surveyed by WFE between 2020 and 2021, with a positive result returned for pond PW180 but negative results returned for the other four ponds.

Four of the records are clustered around Ketteringham and Swardeston. Three records likely relate to ponds P024 and/or P025 near Ketteringham (see **Figure 14**), but the grid references of these records are not of sufficient accuracy to confidently assign the records to a specific pond. Another record is within the survey area but may relate to a pond which is outside the survey area (formerly referenced as pond P016 when this pond was within the survey area in 2020), near The Old Rectory in Swardeston. Whichever ponds these records relate to, they demonstrate the presence of a metapopulation in this general area. WFE surveys of ponds P016, P024 and P025 completed in 2020 confirmed GCN presence in pond P024 only.

Two of the NBIS GCN records have been assigned to ponds PW166 and PN103 near Saxthorpe and Itteringham (see **Figure 6**). Both these ponds were surveyed by WFE in 2020, with pond PW166 returning a positive eDNA result but pond PN103 returning a negative result.

Further records of GCN were provided by NBIS but mapping has revealed these are outside the survey area, so they are not included in this report.

4.2. Habitat Suitability Index and eDNA Results

The results from the HSI appraisals are presented in **Table 1**, below, along with the eDNA results. In Table 1, ponds which returned a Positive eDNA result are highlighted in green and ponds which returned a Negative eDNA result are highlighted in blue. Ponds which were found to be dry are highlighted in pink, and ponds which were not accessible are highlighted in grey.

Full details of the HSI appraisals and pond photographs are provided in a separate Annex.

The SSL eDNA analysis reports are provided within Annex 1 of this report. The SSL reports include results for many of the ponds which were surveyed but which are now outside of the survey area (due to refinements made to the DCO boundary since the 2020/2021 GCN survey effort), because those ponds would have been surveyed on the same days as ponds which remain in the survey area; SSL typically issued single reports outlining results on the daily batches of pond samples sent to them.

Maps showing the locations of the ponds subject to the HSI and eDNA survey effort are provided in **Figure 1** to **Figure 16**.

Table 1: HSI and eDNA Results (to be read in conjunction with Figures 1-16)

Pond Reference	HSI Score	HSI Classification	eDNA Result	SureScreen Sample Kit Reference	Notes
P001	0.56	Below average	Negative	5324	-
P002	0.60	Average	Negative	5322	-
P003	0.66	Average	Negative	2888	Pond dry in 2020 but holding water and therefore surveyed in 2021
P004	0.82	Excellent	Negative	2895	Pond dry in 2020 but holding water and therefore surveyed in 2021
P005	0.56	Below average	Negative	5319	-
P006	0.58	Below average	Negative	5326	-
P007	0.65	Average	Negative	5323	-
P008	0.82	Excellent	Negative	2978	Pond dry in 2020 but holding water and therefore surveyed in 2021
P009	0.75	Good	Negative	2894	Pond dry in 2020 but holding water and therefore surveyed in 2021
P010	0.58	Below average	Negative	2864	-
P012	0.68	Average	Negative	5313	-
P014	0.76	Good	POSITIVE	683	-
P015	0.54	Below average	Negative	1301	-
P018	0.73	Good	POSITIVE	1335	-
P020	0.49	Poor	Negative	2919	Landowner access not granted in 2020 but access subsequently granted and therefore pond surveyed in 2021
P022	0.80	Good	Negative	3548	-
P023	0.82	Excellent	Negative	3589	-
P024	0.81	Excellent	POSITIVE	3587	NBIS record of GCN presence from 2014. There is another NBIS record of GCN presence from 2006 inside the same 1km grid square as this pond. This may relate to P024, to P025 or to another pond within the 1km grid square but outside the survey zone.

Pond Reference	HSI Score	HSI Classification	eDNA Result	SureScreen Sample Kit Reference	Notes
P025	0.93	Excellent	Negative	3549	NBIS record of GCN presence from 2014. There is another NBIS record of GCN presence from 2006 inside the same 1km grid square as this pond. This may relate to this pond, to P024 or to another pond within the 1km grid square but outside the survey zone.
P039	0.70	Average	Negative	3588	-
P040	0.79	Good	Negative	3546	-
P041	0.61	Average	Negative	2873	-
P043	0.70	Average	Negative	2874	-
P051	0.77	Good	Negative	2918	Landowner access not granted in 2020 but subsequently access granted and therefore pond surveyed in 2021
P053	-	-	-	-	Access not granted
P055	-	-	-	-	Access not granted
P057	0.54	Below average	Negative	2917	Landowner access not granted in 2020 but subsequently access granted and therefore pond surveyed in 2021
P058	0.48	Poor	Negative	1303	-
P059					Landowner access not granted in 2020 or 2021
P120	0.60	Below Average	POSITIVE	1316	Pond erroneously listed as PN120 in SSL report
P121	0.63	Average	Negative	1313	Pond erroneously listed as PN121 in SSL report
P122	0.60	Below Average	Negative	1305	-
P123	-	-	-	-	Pond dry in 2020 and 2021
P130	0.53	Below Average	Negative	1329	-
P131	0.60	Average	Negative	2906	Pond dry in 2020 but holding water and therefore surveyed in 2021
P132	0.71	Good	Negative	1331	-
P133	0.59	Below Average	Negative	1371	-
P134	0.71	Good	Negative	1336	-
P135	0.56	Below Average	Negative	1367	-
P138	0.51	Below Average	Negative	1351	-
P139	-	-	-	-	Pond dry in 2020 and 2021
P140	-	-	-	-	Pond dry in 2020 and 2021
P143	0.75	Good	Negative	1341	-

Pond Reference	HSI Score	HSI Classification	eDNA Result	SureScreen Sample Kit Reference	Notes
P153	0.56	Below Average	Negative	3580	-
PA002	-	-	-	-	Landowner access not granted in 2020 or 2021
PA003	0.42	Poor	Negative	1722	-
PA004	0.52	Below average	Negative	1720	-
PA005	0.70	Average	Negative	1730	-
PA006	0.80	Excellent	POSITIVE	2923	-
PA007	0.55	Below average	Negative	2958	-
PA008	0.72	Good	Negative	2963	-
PA009	0.58	Below average	Negative	2961	-
PA010	0.58	Below average	Negative	2962	-
PA013	0.58	Below average	Negative	2959	-
PA014	0.52	Below average	Negative	2983	-
PA015	0.77	Good	Negative	2955	-
PA016	0.54	Below average	Negative	2951	-
PA019	-	-	-	-	Landowner access not granted in 2020 or 2021
PA020	0.72	Good	Negative	1705	-
PA021	0.83	Excellent	POSITIVE	1697	-
PA026	0.72	Good	Negative	1717	-
PA027	0.75	Good	Negative	2981	-
PA028	-	-	-	-	Landowner access not granted in 2020 or 2021
PA029	0.79	Good	Negative	1709	-
PA031	0.52	Below average	Negative	2980	-
PA032	0.58	Below average	Negative	2885	-
PA033	-	-	-	-	Pond dry
PA037	0.85	Excellent	Negative	2982	-
PA038	-	-	-	-	Pond dry
PA039	-	-	-	-	Pond dry
PA040	0.67	Average	Negative	1688	-
PA041	-	-	-	-	Pond dry
PA043	0.88	Excellent	Negative	1721	-
PA045	0.82	Excellent	Negative	1726	-
PA046	-	-	-	-	Landowner access not granted in 2020 or 2021
PA047	-	-	-	-	Pond dry
PA048	-	-	-	-	Pond not accessible because Schedule 1 nesting birds using the pond

Pond Reference	HSI Score	HSI Classification	eDNA Result	SureScreen Sample Kit Reference	Notes
PA049	0.66	Average	Negative	1728	-
PA050	0.89	Excellent	Negative	1706	-
PN001	0.40	Poor	Negative	1365	-
PN002	0.61	Average	Negative	2931	Pond dry in 2020 but holding water and therefore surveyed in 2021
PN003	0.71	Good	Negative	3585	-
PN004	0.44	Poor	Negative	2925	Pond dry in 2020 but holding water and therefore surveyed in 2021
PN005	-	-	-	-	Landowner access not granted in 2020 or 2021
PN006	0.43	Poor	Negative	3542	-
PN012	0.66	Average	Negative	2850	-
PN013	0.54	Below Average	Negative	3540	-
PN016	0.83	Excellent	Negative	3544	-
PN017	0.82	Excellent	-	-	Pond not accessible for eDNA, but visible for HSI appraisal
PN018	0.59	Below Average	-	-	Pond not accessible for eDNA, but visible for HSI appraisal
PN019	0.44	Poor	Negative	3534	-
PN025	0.74	Good	Negative	2838	-
PN026	0.25	Poor	Negative	1306	-
PN030	0.75	Good	Negative	1733	Pond dry in 2020 but holding water and therefore surveyed in 2021
PN031	-	-	-	-	Pond dry in 2020 and 2021
PN032	0.45	Poor	Negative	1729	Pond dry in 2020 but holding water and therefore surveyed in 2021
PN034	0.84	Excellent	Negative	1311	-
PN035	-	-	-	-	Landowner access not granted in 2020 or 2021
PN036	0.62	Average	Negative	1723	Landowner access not granted in 2020 but access subsequently granted and therefore pond surveyed in 2021
PN037	0.72	Good	Negative	1718	Landowner access not granted in 2020 but access subsequently granted and therefore pond surveyed in 2021
PN038	-	-	-	-	Pond dry in 2020 and 2021
PN039		-	-	-	Landowner access not granted in 2020 or 2021

Pond Reference	HSI Score	HSI Classification	eDNA Result	SureScreen Sample Kit Reference	Notes
PN040	0.80	Excellent	POSITIVE	1338	-
PN041	0.61	Average	POSITIVE	1349	-
PN088	0.89	Excellent	Negative	3557	-
PN089	0.66	Average	Negative	1345	-
PN090	0.73	Good	Negative	2907	Landowner access not granted in 2020 but access subsequently granted and therefore pond surveyed in 2021
PN091	0.89	Excellent	Negative	2886	Landowner access not granted in 2020 but access subsequently granted and therefore pond surveyed in 2021
PN092	0.93	Excellent	Negative	3532	-
PN094	0.77	Good	Negative	2846	-
PN095	-	-	-	-	Landowner access not granted in 2020 or 2021
PN098	0.43	Poor	Negative	1327	-
PN099	-	-	-	-	Pond dry in 2020 and 2021
PN100	0.73	Good	Negative	1719	Pond dry in 2020 but holding water and therefore surveyed in 2021
PN101	0.78	Good	Negative	1322	-
PN102	-	-	-	-	Landowner access not granted in 2020 or 2021
PN103	0.67	Average	Negative	1339	NBIS record of GCN presence from 2009.
PN104	0.77	Good	Negative	3571	-
PN111	-	-	-	-	Landowner access not granted in 2020 or 2021
PN112	-	-	-	-	Landowner access not granted in 2020 or 2021
PN113	0.58	Below Average	POSITIVE	1375	-
PN129	-	-	-	-	Landowner access not granted in 2020 and pond dry in 2021
PN130	0.54	Below average	Negative	6127	Landowner access not granted in 2020 but access subsequently granted and therefore pond surveyed in 2021
PN131	0.77	Good	Negative	1299	-
PS003	0.73	Good	Negative	2871	-
PS004	0.61	Average	Negative	2849	-
PS010	0.51	Below Average	Negative	1304	-
PW155	0.80	Excellent	Negative	3545	-

Pond Reference	HSI Score	HSI Classification	eDNA Result	SureScreen Sample Kit Reference	Notes
PW156	0.52	Below Average	Negative	1317	-
PW157	0.62	Average	Negative	2914	Pond dry in 2020 but holding water and therefore surveyed in 2021
PW158	0.62	Average	Negative	1318	-
PW159	0.71	Good	Negative	2889	Landowner access not granted in 2020 but access subsequently granted and therefore pond surveyed in 2021
PW166	0.59	Below Average	POSITIVE	1298	NBIS record of GCN presence from 2009
PW167	0.85	Excellent	Negative	1302	-
PW168	0.75	Good	Negative	1283	-
PW169	0.71	Good	Negative	1282	-
PW170	0.76	Good	Negative	2882	-
PW171	-	-	-	-	Landowner access not granted in 2020 and pond dry in 2021
PW172	-	-	-	-	Landowner access not granted in 2020 and pond dry in 2021
PW173	0.43	Poor	Negative	6114	Landowner access not granted in 2020 but access subsequently granted and therefore pond surveyed in 2021
PW174	0.62	Average	Negative	6121	Landowner access not granted in 2020 but access subsequently granted and therefore pond surveyed in 2021
PW175	0.86	Excellent	Negative	693	UCLPRRG has studied this pond (their ref: POFA4) but states the breeding GCN status as 'unknown'. NBIS record of GCN presence from 2007
PW176	0.62	Average	Negative	6118	Landowner access not granted in 2020 but access subsequently granted and therefore pond surveyed in 2021
PW177	0.51	Below average	Negative	6120	Landowner access not granted in 2020 but access subsequently granted and therefore pond surveyed in 2021
PW178	0.59	Below average	Negative	6110	Landowner access not granted in 2020 but access subsequently granted and therefore pond surveyed in 2021

Pond Reference	HSI Score	HSI Classification	eDNA Result	SureScreen Sample Kit Reference	Notes
PW179	0.84	Excellent	Negative	6123	Landowner access not granted in 2020 but access subsequently granted and therefore pond surveyed in 2021. UPLPRRG confirmed GCN breeding in this pond (their ref: BAW02).
PW180	0.79	Good	POSITIVE	699	UCLPRRG confirmed GCN breeding in this pond (their ref: POFA2). 7x NBIS records of GCN presence from 2007
PW181	0.77	Good	Negative	676	UCLPRRG confirmed GCN breeding in this pond (their ref: POFA1). NBIS record of GCN presence from 2013 (record describes "hundreds of eggs" found during survey).
PW182	0.61	Average	Negative	6124	Access not granted in 2020 but access granted and pond surveyed in 2021. UCLPRRG confirmed GCN breeding in this pond (their ref: BAW01). 2x NBIS records of GCN presence from 2007
PW183	0.82	Excellent	Negative	679	UCLPRRG confirmed GCN breeding in this pond (their ref POFA3). NBIS record of GCN presence from 2007
PW184	-	-	-	-	Landowner access not granted in 2020 or 2021
PW185	0.51	Below Average	POSITIVE	1370	-
PW186	0.69	Average	POSITIVE	3570	UCLPRRG confirmed GCN breeding in this pond (their ref: HART).
PW193	-	-	-	-	Landowner access not granted in 2020 or 2021
PW195	0.61	Average	POSITIVE	6108	Landowner access not granted in 2020 but access subsequently granted and therefore pond surveyed in 2021
PW197	0.86	Excellent	Negative	6109	Landowner access not granted in 2020 but access subsequently granted and therefore pond surveyed in 2021

Pond Reference	HSI Score	HSI Classification	eDNA Result	SureScreen Sample Kit Reference	Notes
PW198	0.76	Good	Negative	1281	-
PW199	0.76	Good	Negative	1286	-
PW200	0.51	Below Average	Negative	1291	-
PW201	0.82	Excellent	Negative	1280	-
PW202	0.98	Excellent	Negative	2902	Pond dry in 2020 but holding water and therefore surveyed in 2021
PW203	0.89	Excellent	Negative	1284	-
PW204	-	-	-	-	Pond not accessible because Schedule 1 nesting birds using the pond
PX001	0.42	Poor	Negative	694	-
PX003	0.64	Average	Negative	1287	-
PX004	0.59	Below Average	Negative	1285	-
PX007	0.36	Poor	Negative	1348	Pond erroneously listed as P138a in SSL report
PX012	0.72	Good	Negative	5317	-
PX015	0.56	Below average	Negative	2930	Pond erroneously listed as 'River Tud' in SSL report
PX016	0.49	Poor	Negative	1724	-
PX017	0.49	Poor	Negative	1725	-
PX018	0.82	Excellent	Negative	2911	-
PX019	0.66	Average	POSITIVE*	N/A*	*GCN seen in pond so eDNA sampling aborted
PX021	0.58	Below average	Negative	6133	-
PX100	-	-	-	-	Ponds were outside the survey area (beyond 250m from the DCO boundary) in 2020 and 2021 but changes to the DCO boundary in 2022 have brought these ponds to within 250m of it. These ponds have therefore not been surveyed.
PX101	-	-	-	-	
PX102	-	-	-	-	
PX103	-	-	-	-	
PX104	-	-	-	-	
PX105	-	-	-	-	

Figure 1: Great Crested Newt Survey Results Map 1 (Landfall to Weybourne)

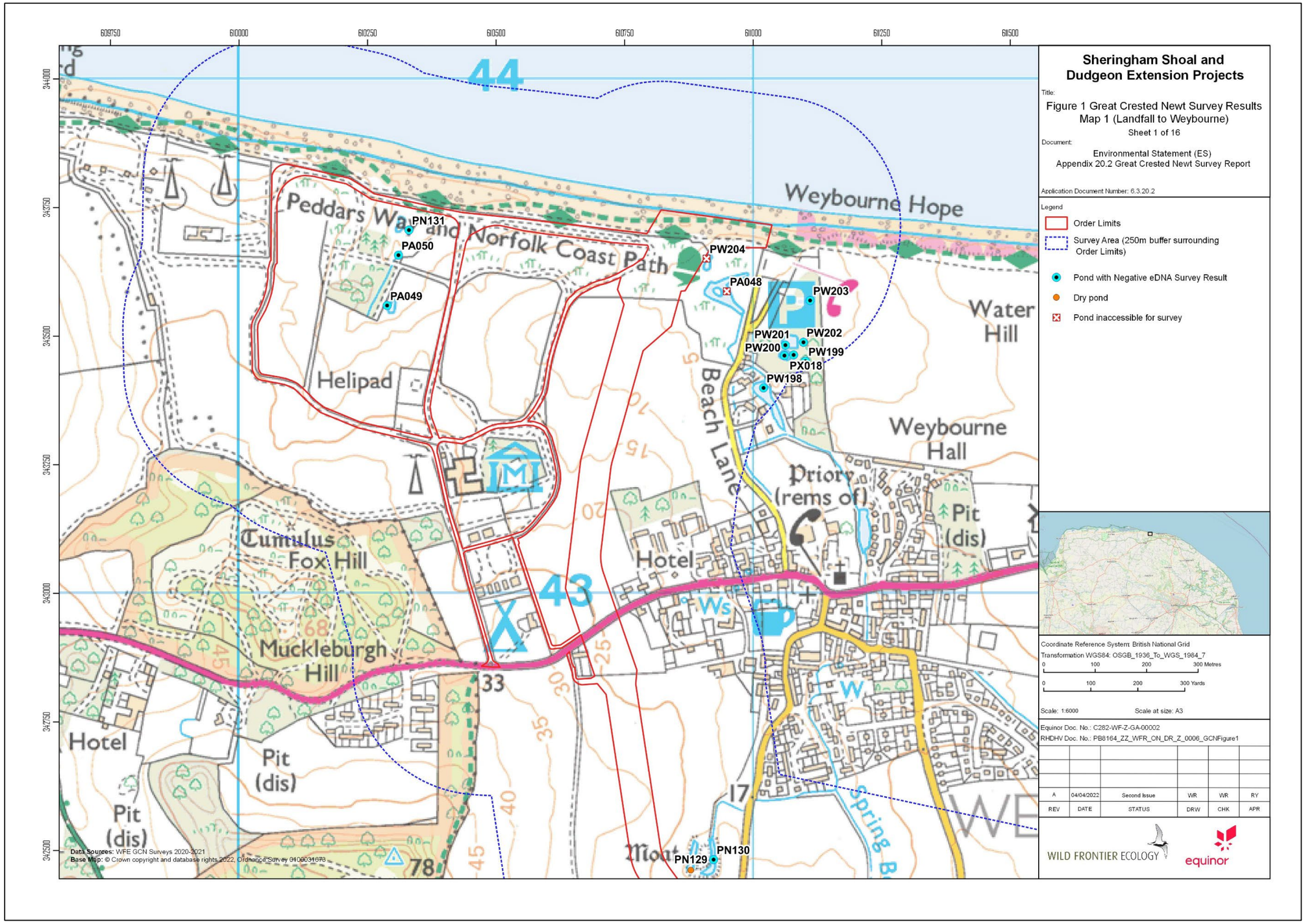


Figure 2: Great Crested Newt Survey Results Map 2 (Weybourne to Bodham)

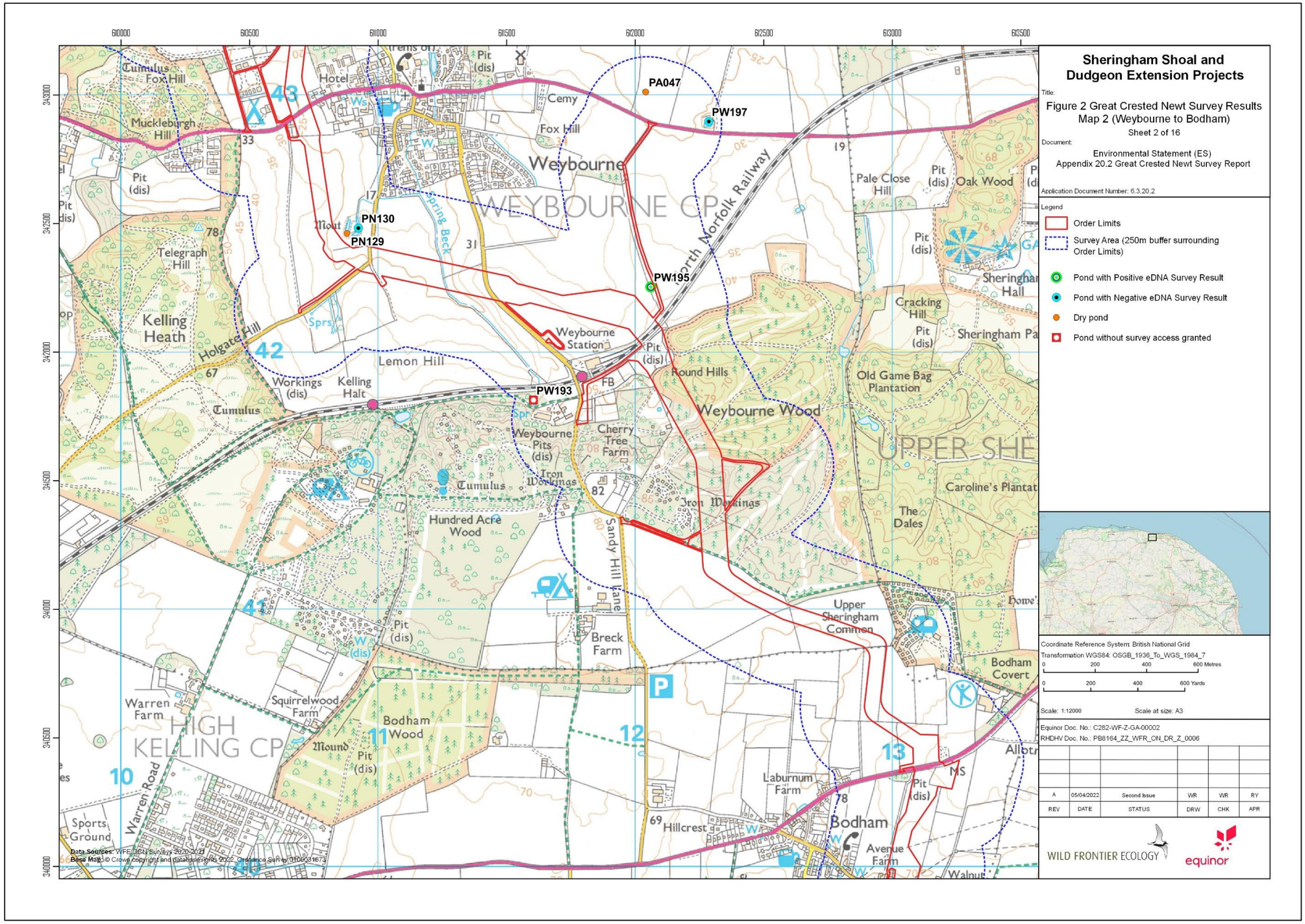


Figure 3: Great Crested Newt Survey Results Map 3 (Bodham to Baconsthorpe)

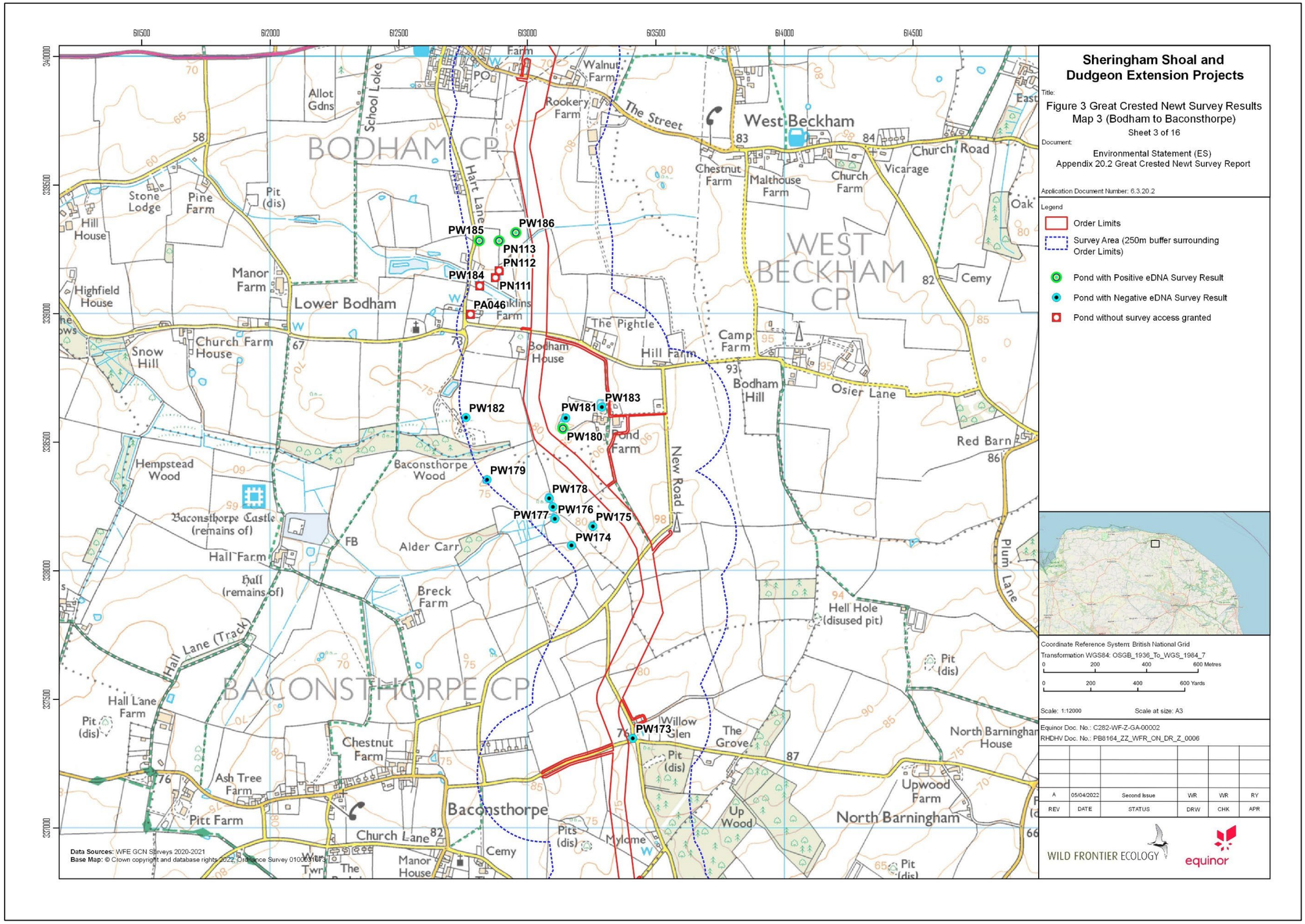


Figure 4: Great Crested Newt Survey Results Map 4 (Baconsthorpe to Little Barningham)

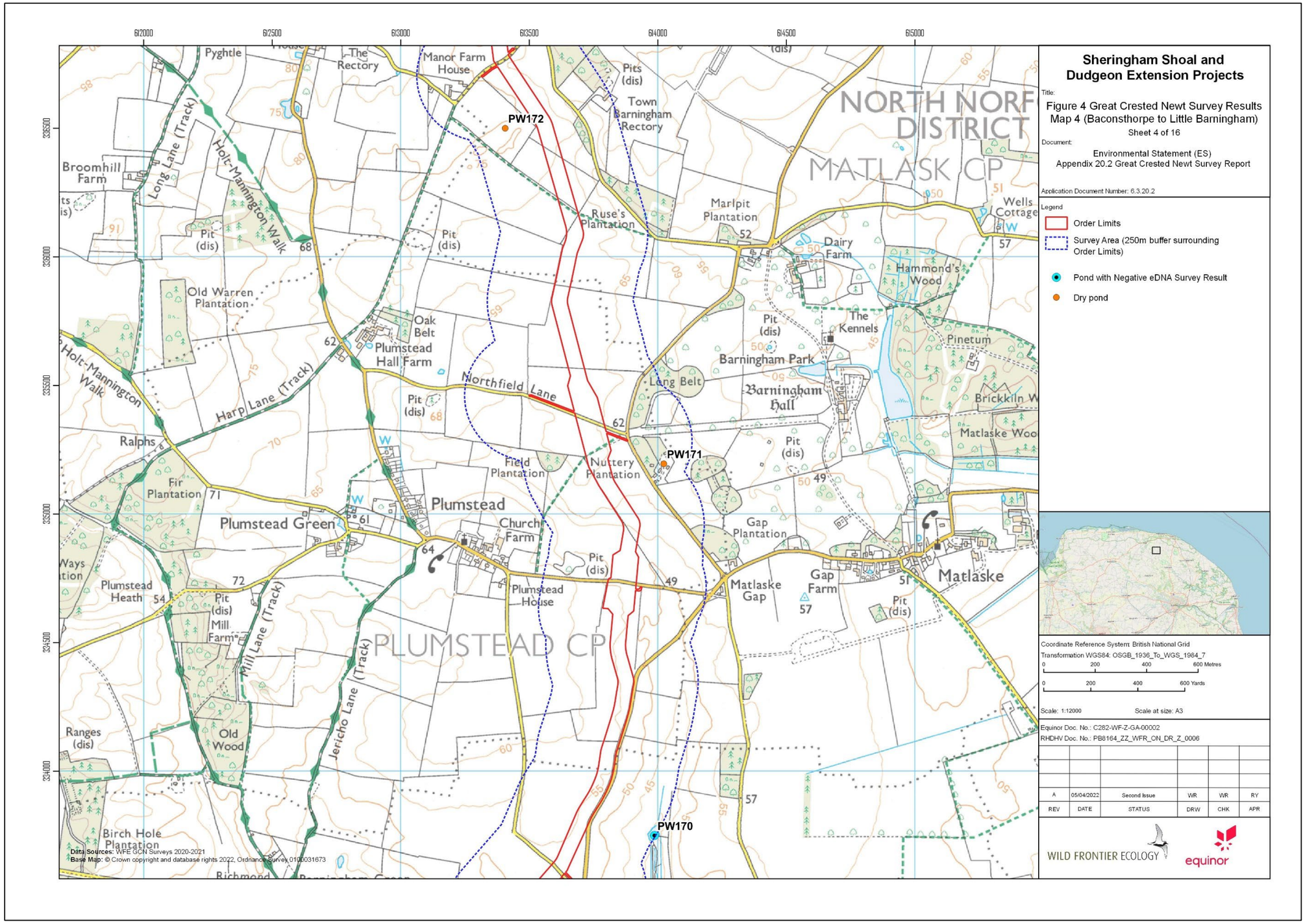


Figure 5: Great Crested Newt Survey Results Map 5 (Little Barningham to Saxthorpe)

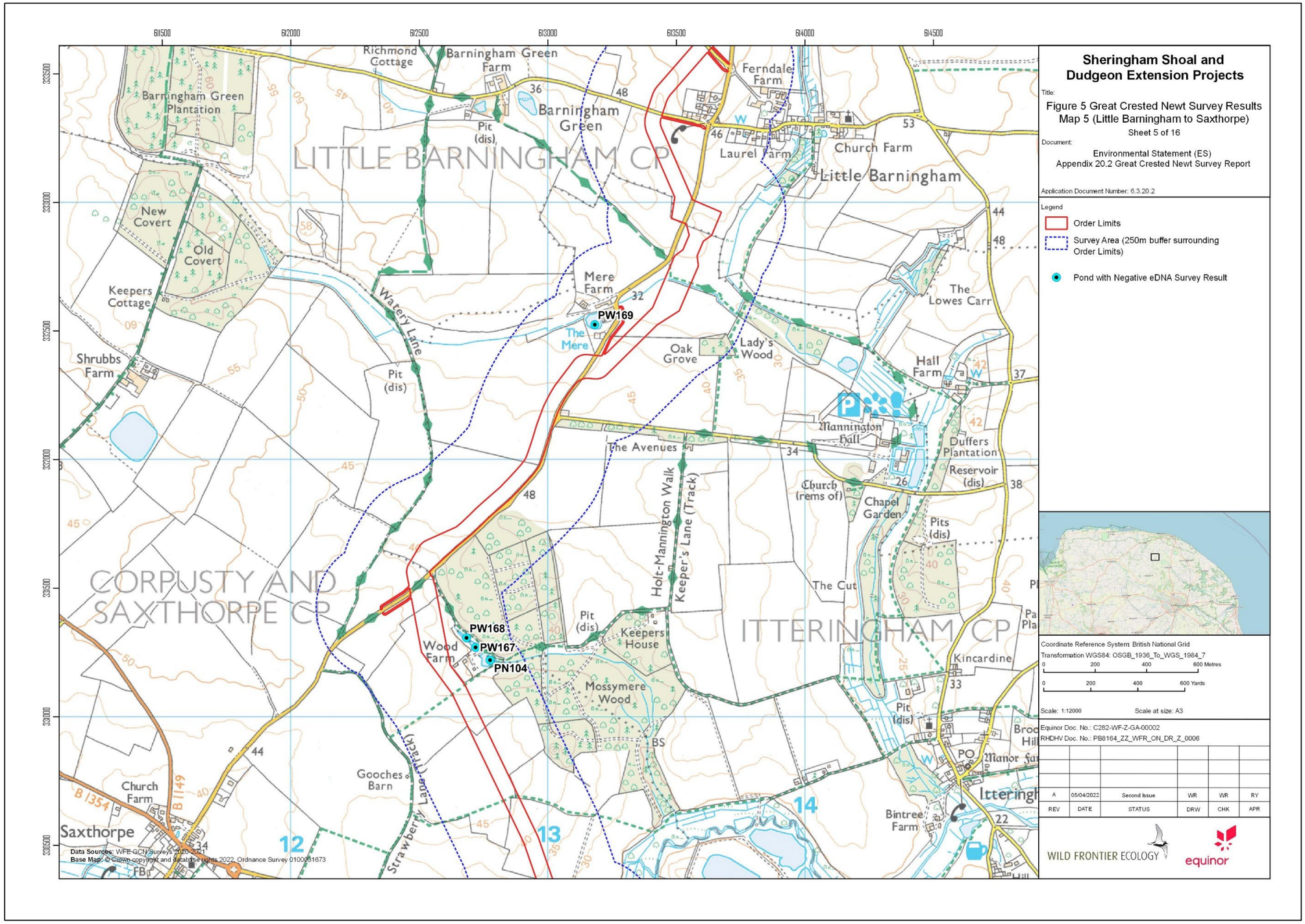


Figure 6: Great Crested Newt Survey Results Map 6 (Saxthorpe to Oulton Street)

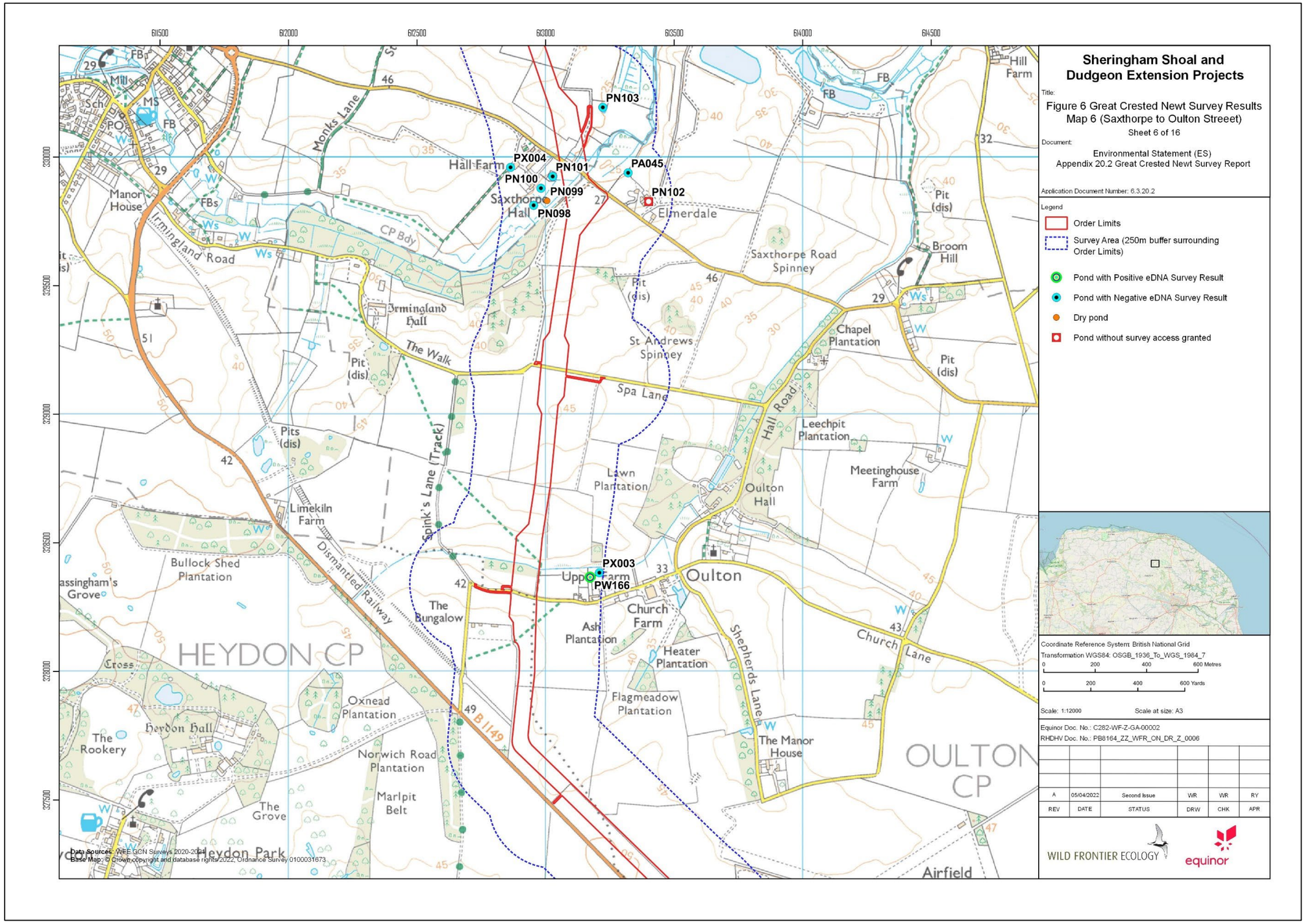


Figure 7: Great Crested Newt Survey Results Map 7 (Oulton Street to Cawston)

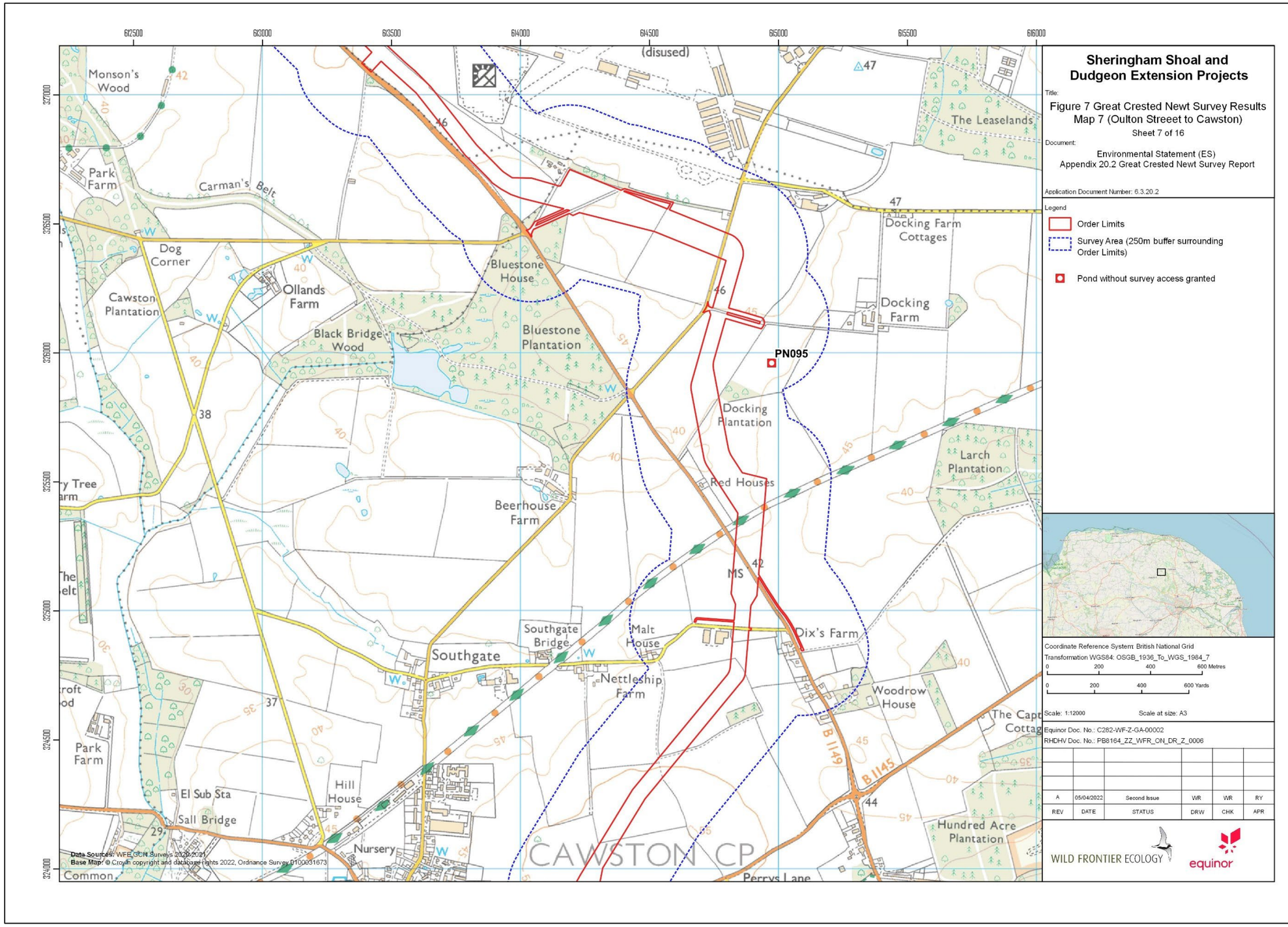


Figure 8: Great Crested Newt Survey Results Map 8 (Cawston to Brandiston)

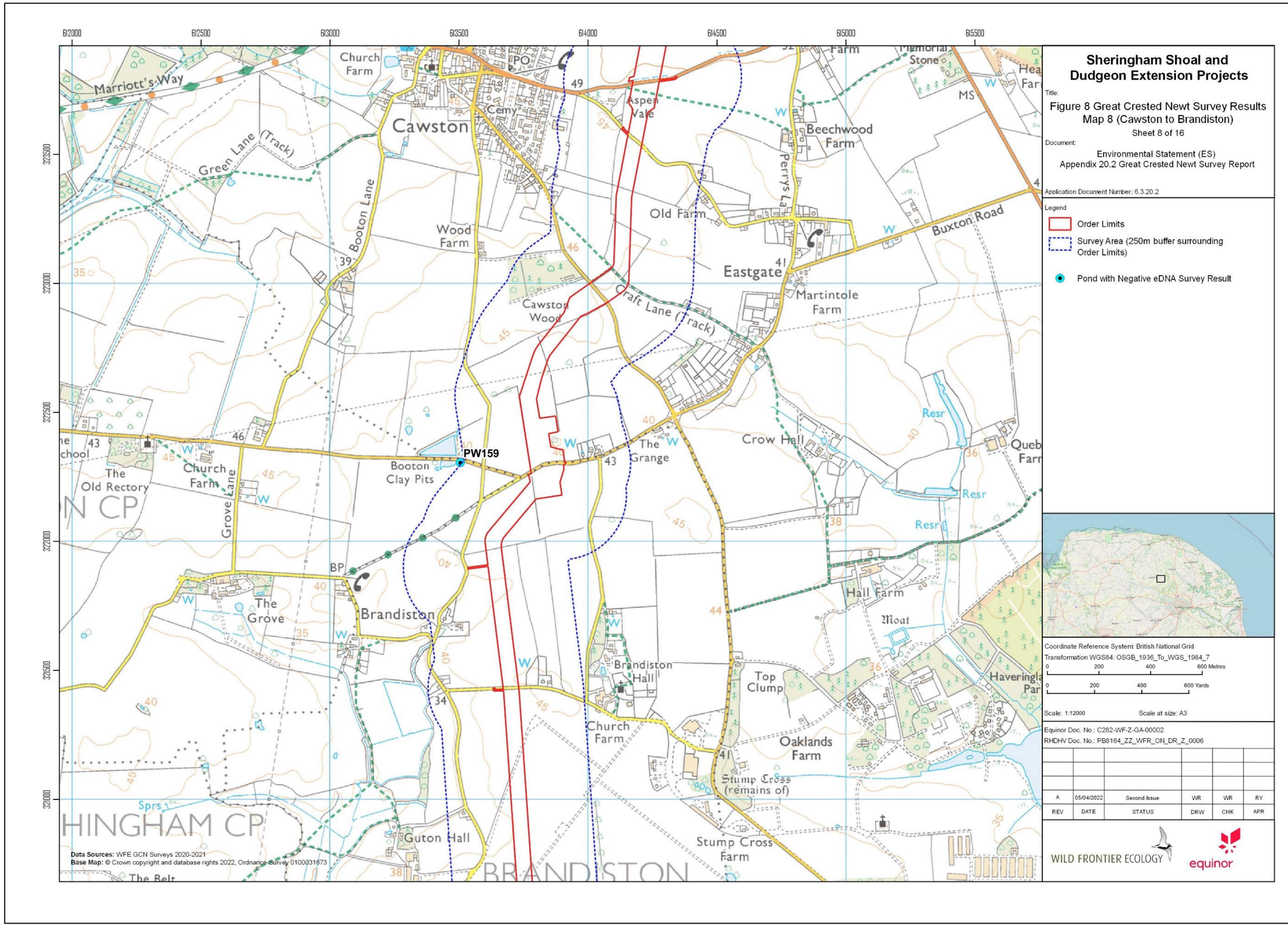


Figure 9: Great Crested Newt Survey Results Map 9 (Brandiston to Attlebridge)

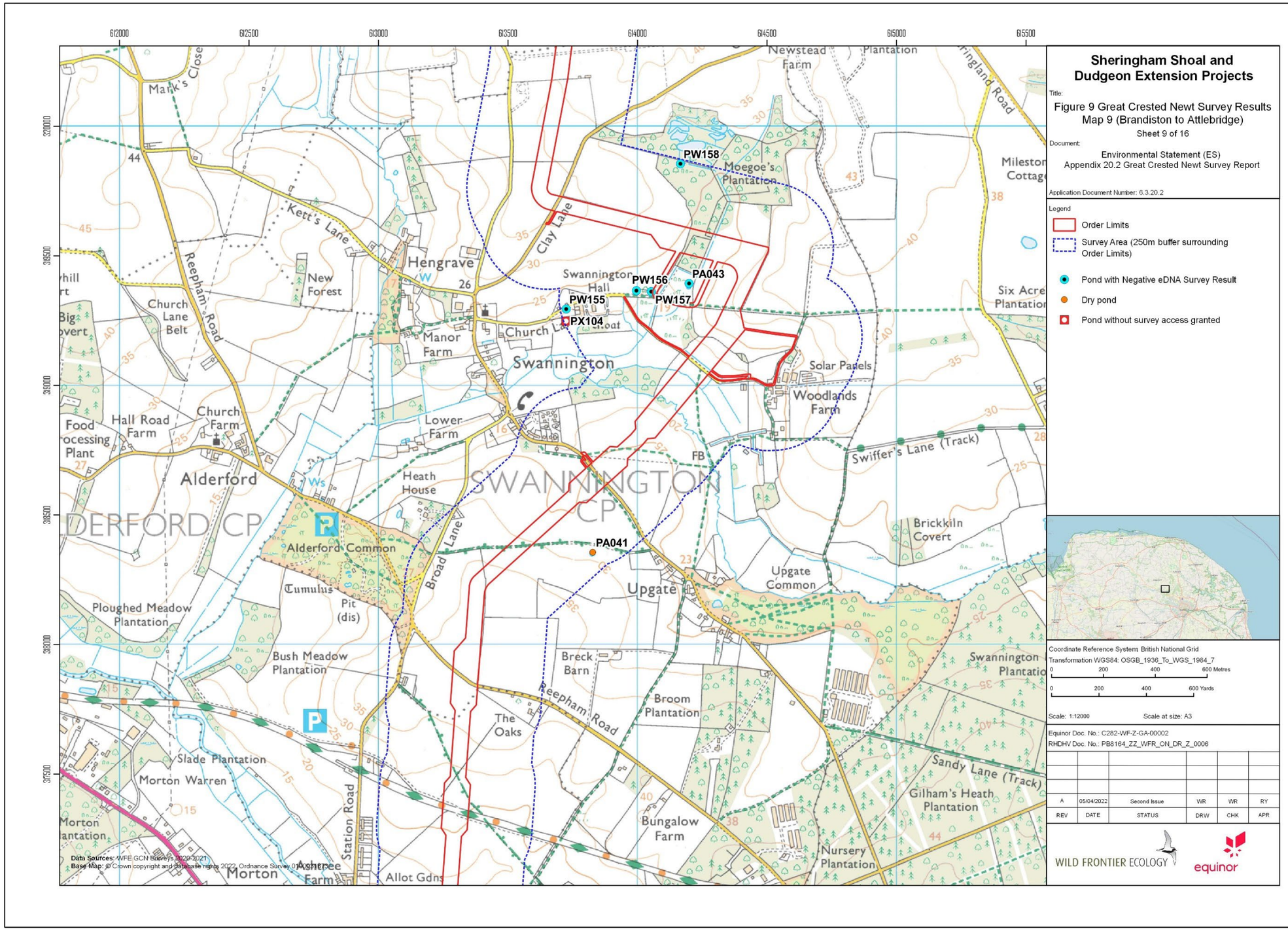


Figure 10: Great Crested Newt Survey Results Map 10 (Attlebridge to Ringland)

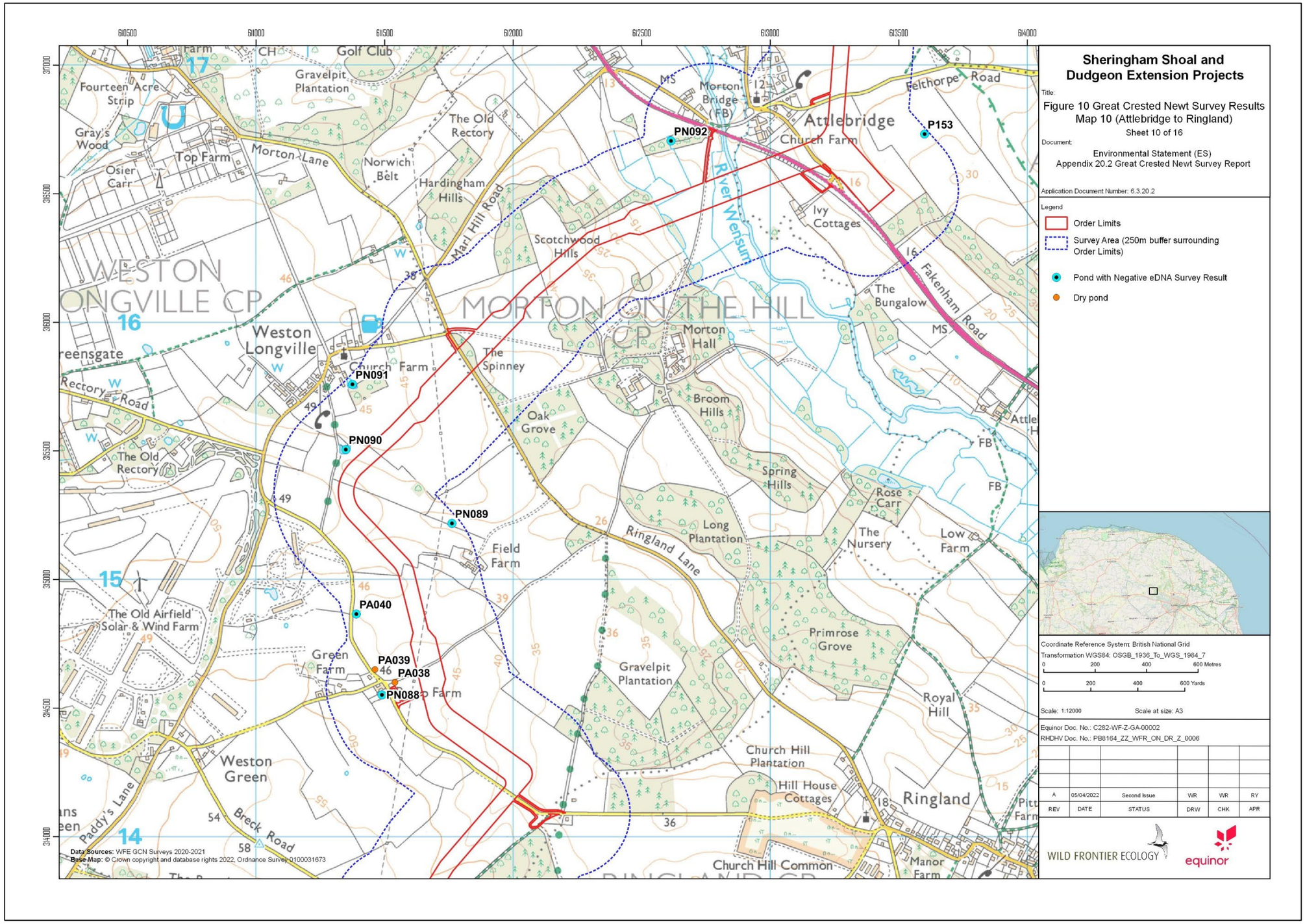


Figure 11: Great Crested Newt Survey Results Map 11 (Ringland to Easton)

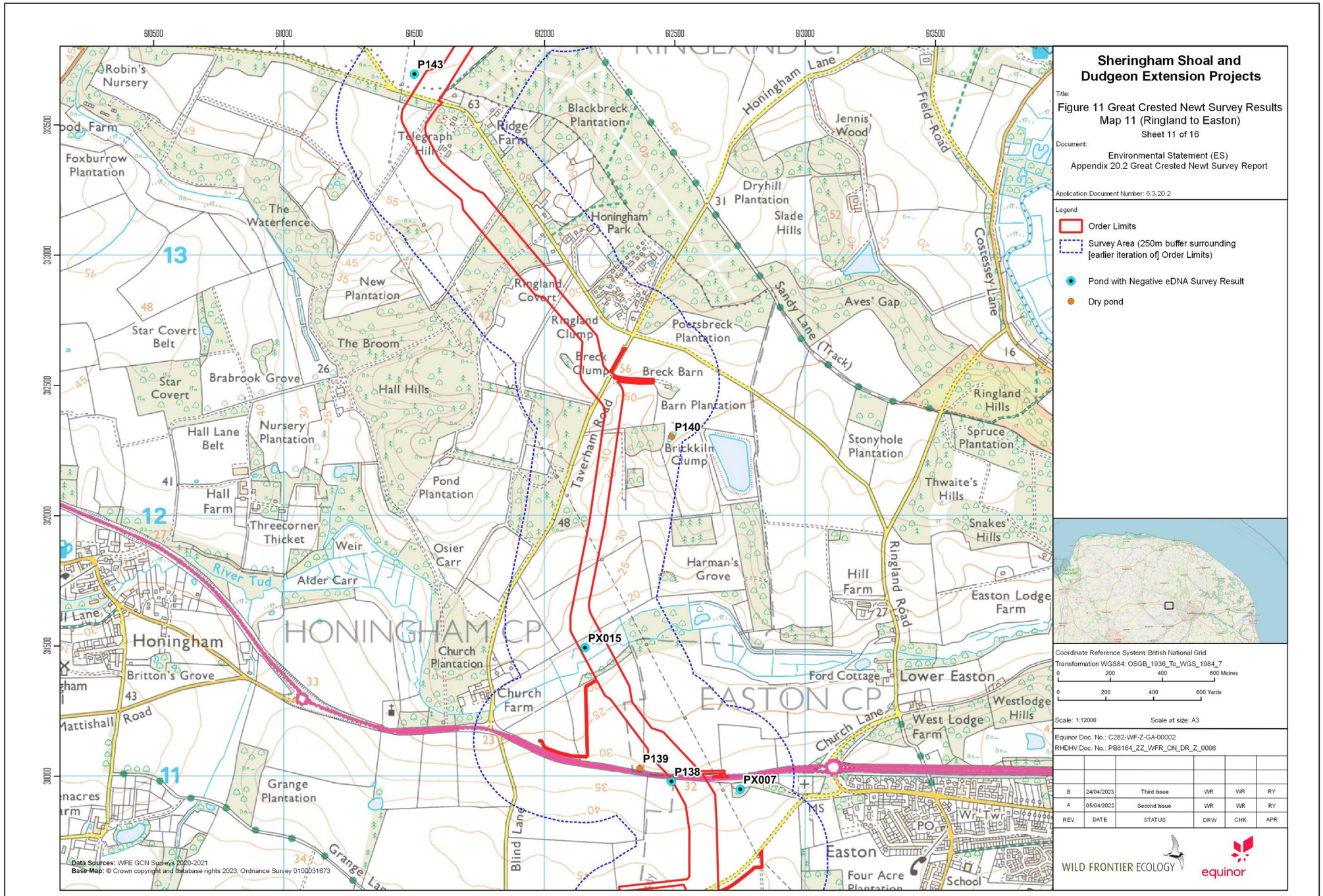


Figure 12: Great Crested Newt Survey Results Map 12 (Easton to Barford)

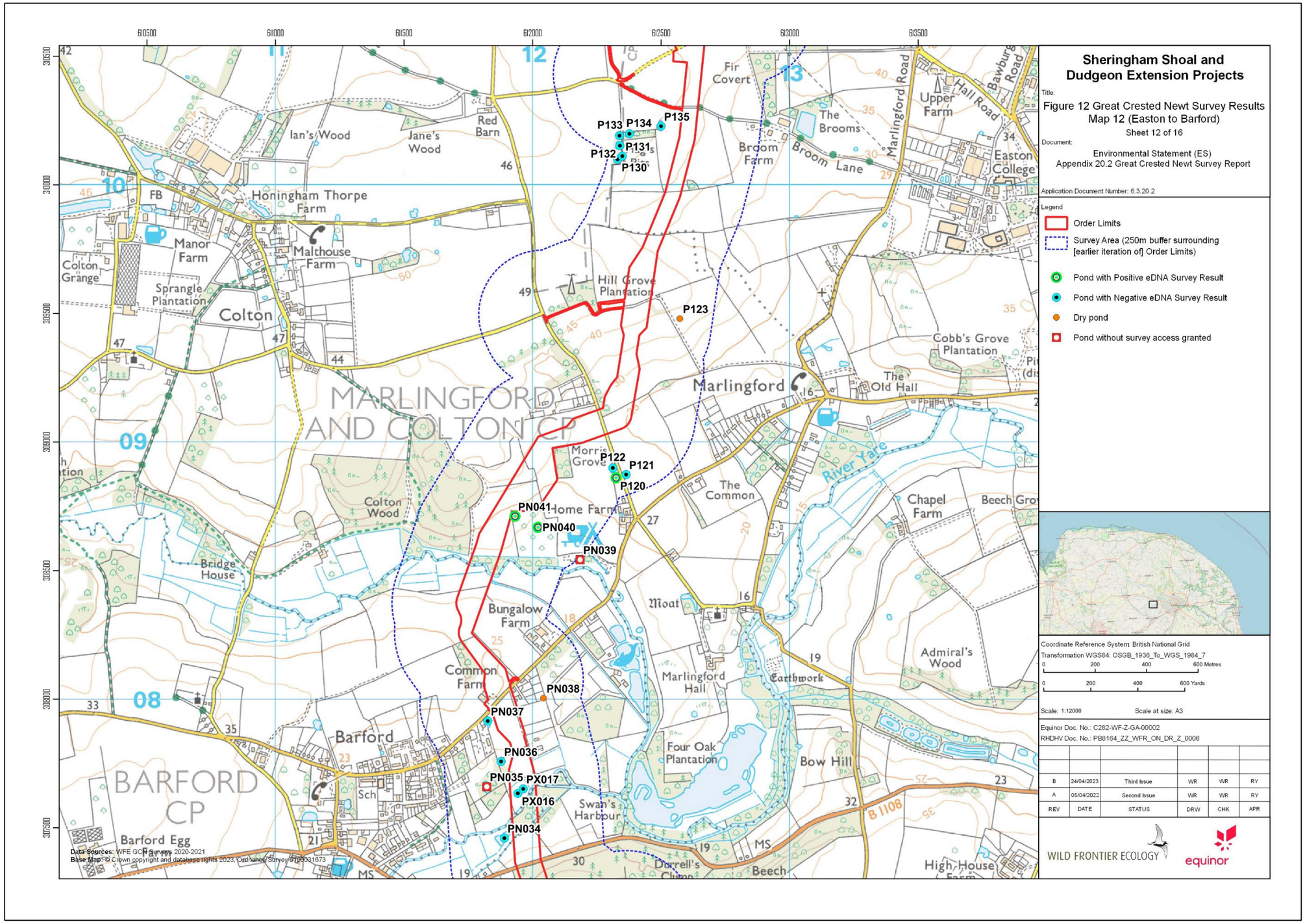


Figure 13: Great Crested Newt Survey Results Map 13 (Barford to Wymondham)

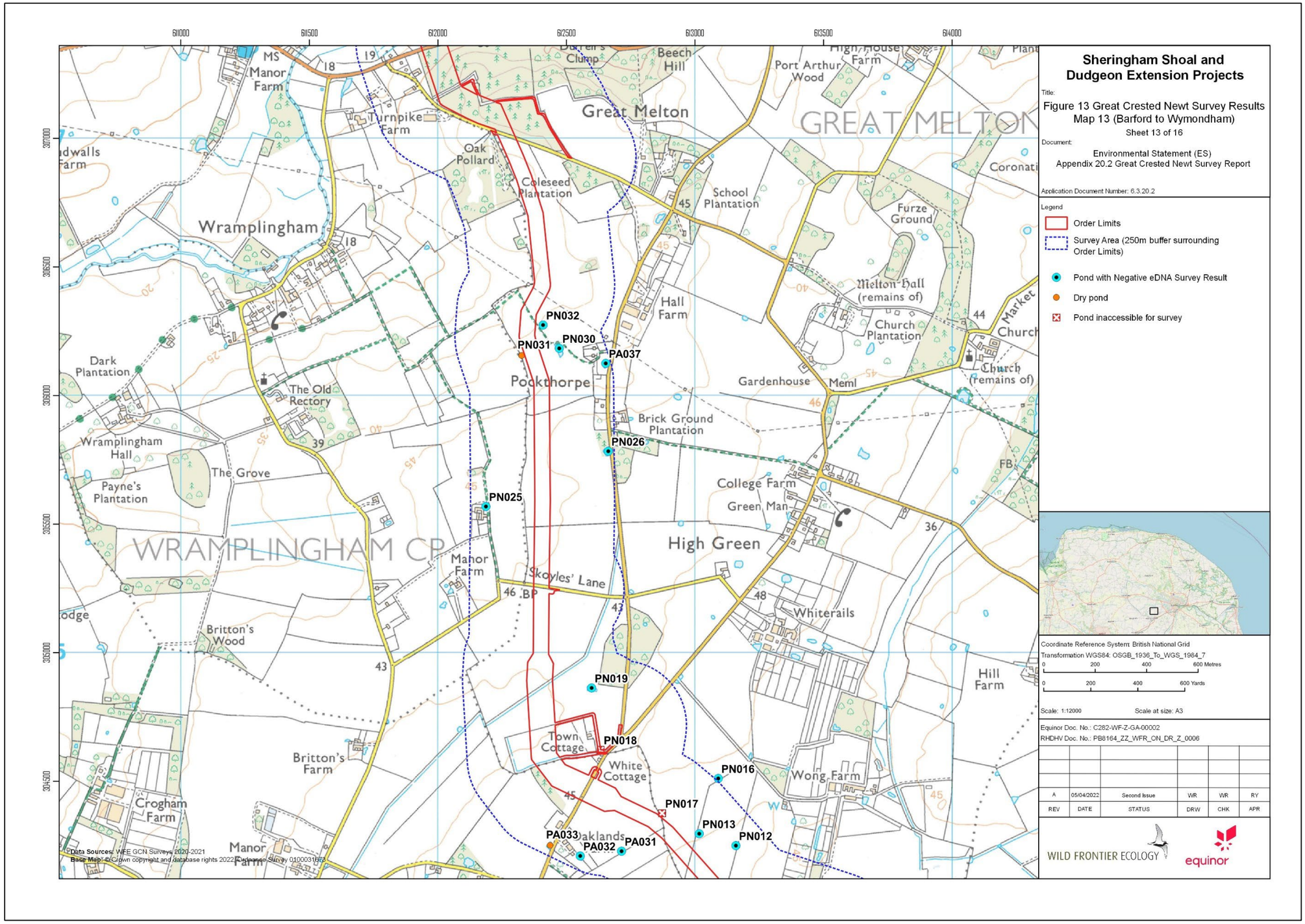


Figure 14: Great Crested Newt Survey Results Map 14 (Wymondham to Ketteringham)

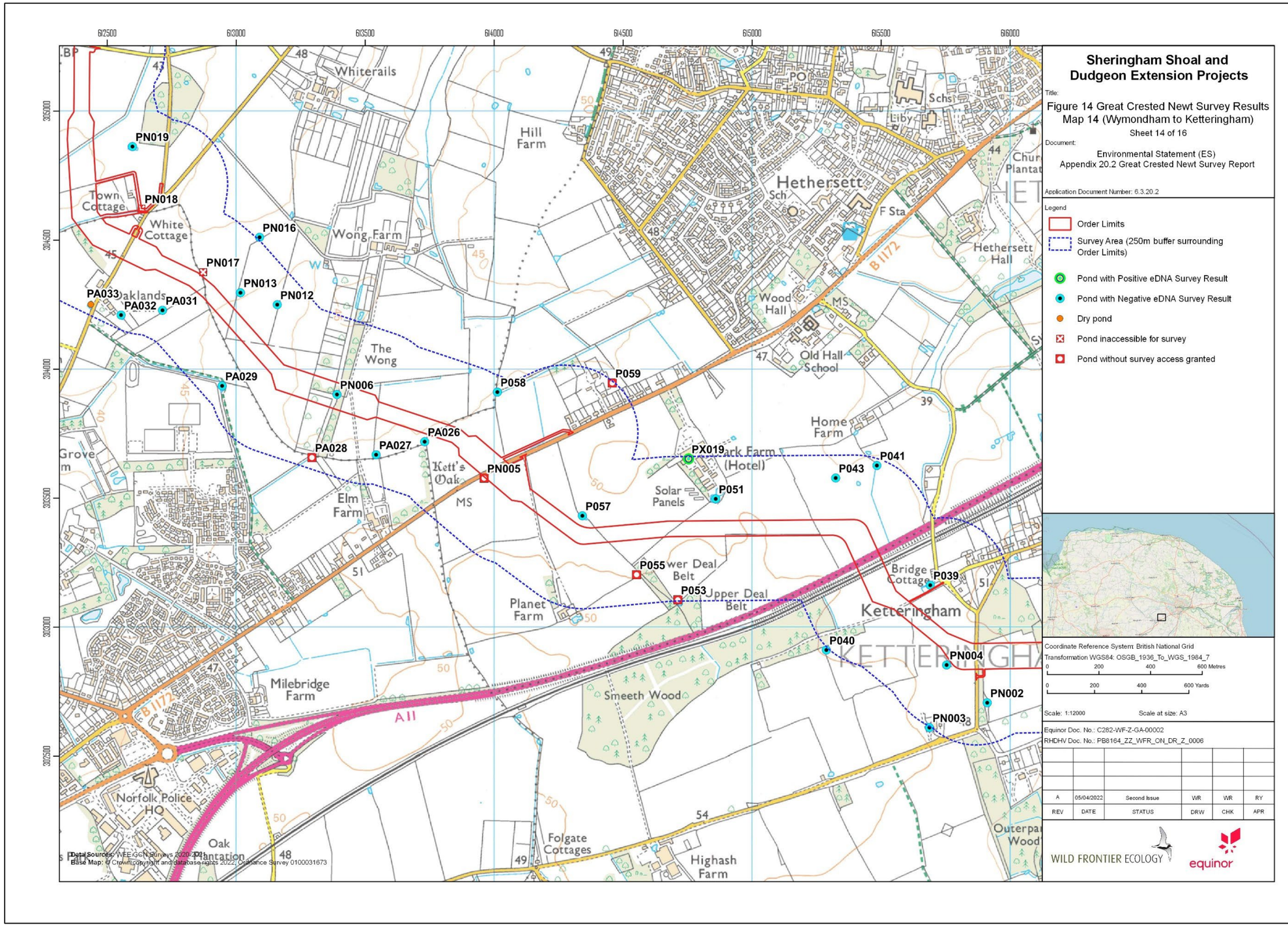


Figure 15: Great Crested Newt Survey Results Map 15 (Ketteringham to Swardeston)

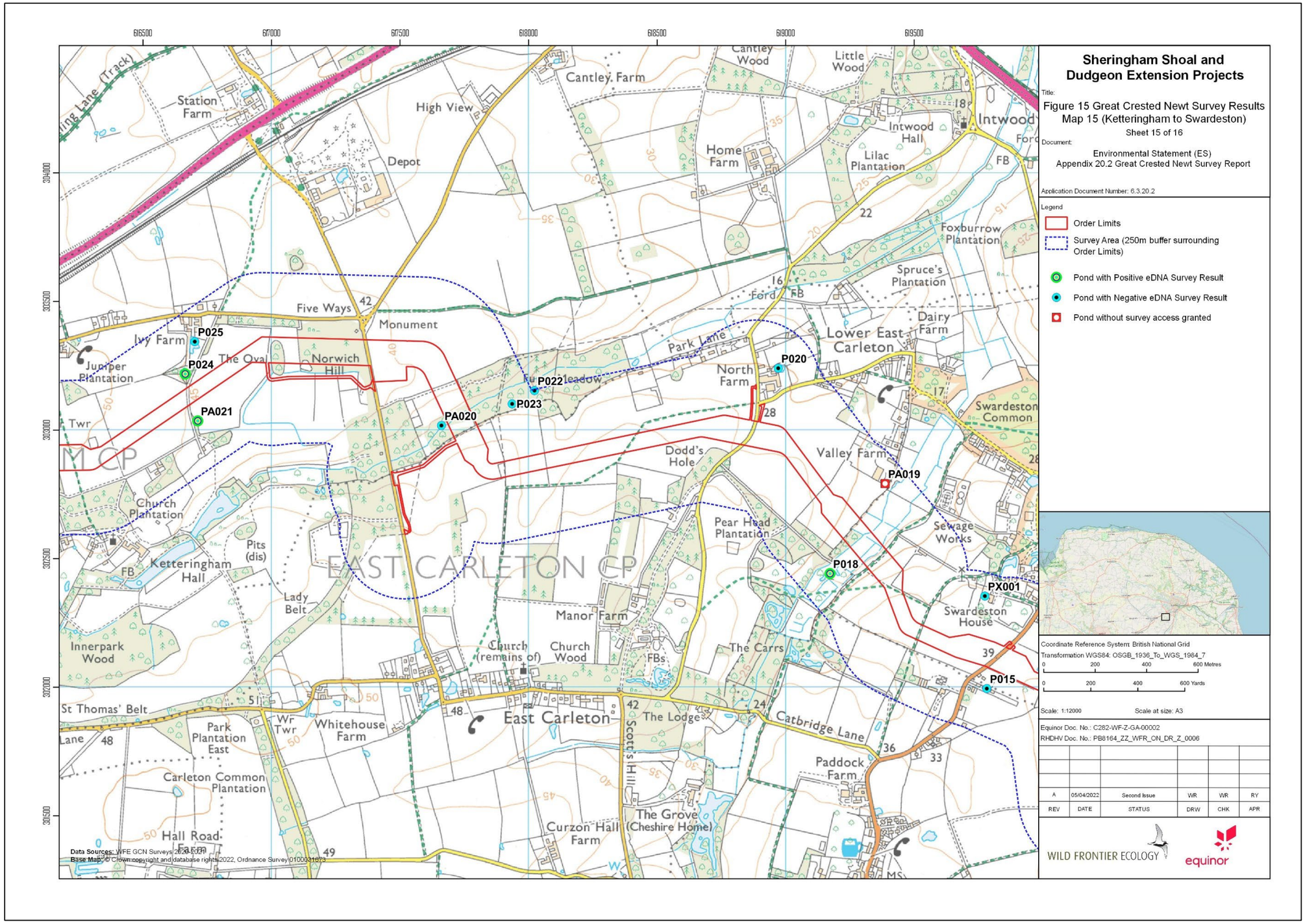
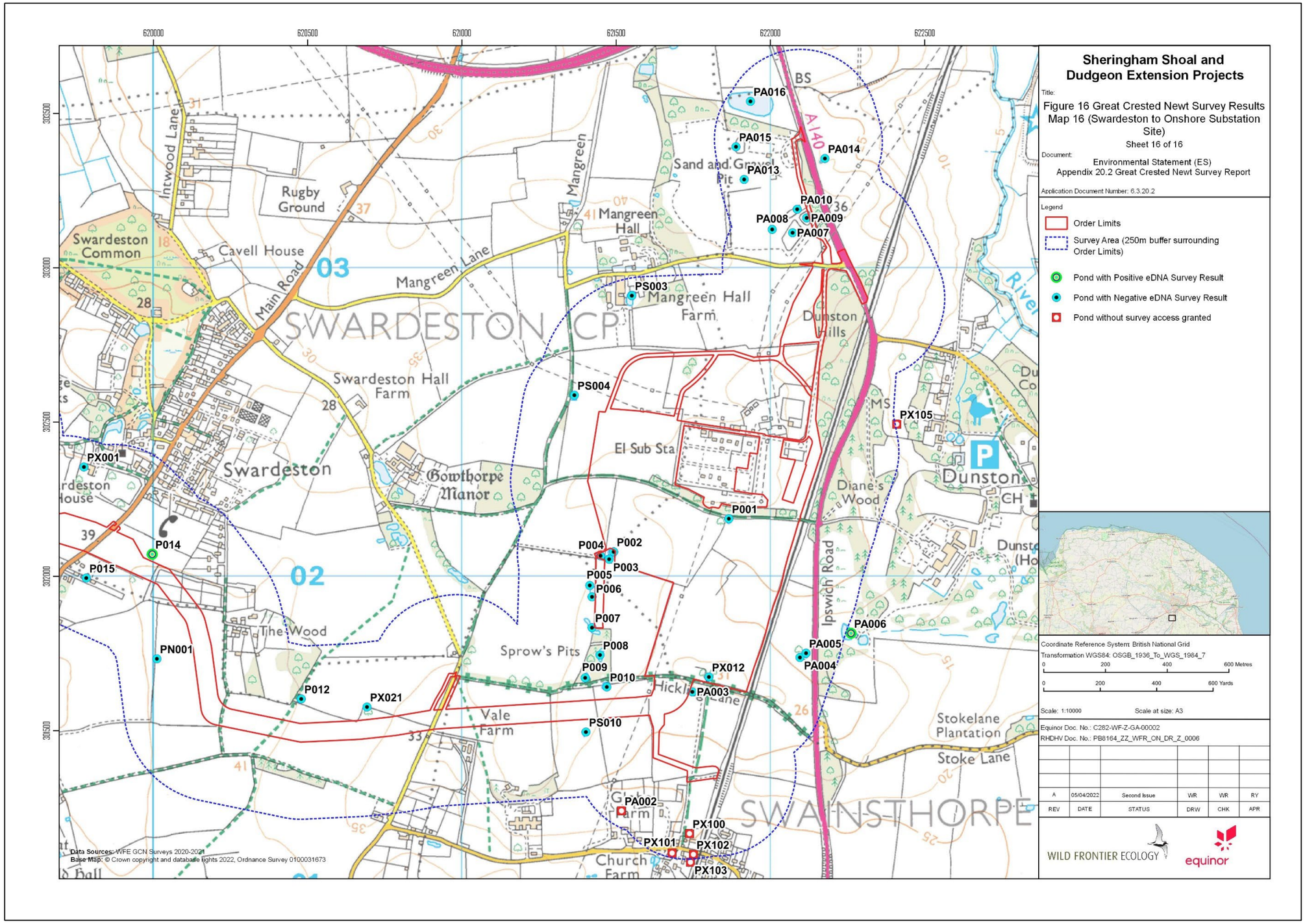


Figure 16: Great Crested Newt Survey Results Map 16 (Onshore Substation Site)



4.3. Constraints and Limitations of Survey

The main constraint to the 2020 and 2021 GCN HSI and eDNA surveys related to the limited landowner access at the times of the surveys; this prevented surveys of 15 ponds.

Four ponds were not physically accessible due to issues such as dangerously steep/unstable banks, impenetrable vegetation around the pond or nesting birds protected under Schedule 1 of the Wildlife and Countryside Act, 1981 (as amended) using the pond (which meant the pond could not be accessed due to the risk of disturbing the birds). Two of these ponds could be HSI appraised from a sufficient distance but none of the four ponds were accessible for eDNA surveys.

Fourteen ponds were found to be dry so were unable to be surveyed and therefore not considered any further in the 2020 or 2021 survey effort. However, this is not considered a significant constraint because any such ponds are likely unsuitable for use as breeding ponds by GCN if dry during the breeding season (March to June), especially when found to be dry in consecutive years (2020 and 2021).

There are inherent constraints to the eDNA surveys, such as potential natural contamination, such as from birds or other wildlife transferring eDNA between ponds, which could lead to false Positives. Conversely, there is also the potential for false Negatives for various reasons. For example, access for eDNA sampling at some ponds was severely restricted such as by steep banks, unstable ground, dense vegetation, fences etc., meaning the sampling may have not collected water from parts of the pond used by GCN. This has been acknowledged as a potential constraint partly because WFE has previously surveyed a number of relevant ponds within the survey area (but for other development proposals in the past) which have confirmed GCN presence, yet eDNA sampling in 2020 and 2021 has returned Negative results. It is possible that GCN are no longer present in some such ponds, but equally it should be noted that eDNA surveys could have returned false Negatives.

SSL data returned three incorrect pond references; in these instances, they are acknowledged in **Table 1**. As each eDNA sample kit has a unique 4-digit reference, inconsistencies in pond referencing could be readily corrected because surveyors recorded which kit was used at each pond.

These constraints are not considered to have had a substantial impact on the reliability of the survey results and therefore the results are considered to be sufficiently accurate and reliable to inform the ecological impact assessment and any mitigation requirements for GCNs.

4.4. Further Survey Requirements and Expiry Dates

Government guidelines[7] state that "*Survey data provided by the developer should be less than 2-4 survey seasons old, depending on the extent of the effects.*" For SEP and DEP, which would largely have temporary construction impacts and not require the removal of any ponds, it is considered that four survey seasons would be an acceptable period of validity of the collected data. The HSI and eDNA survey results should therefore be regarded as valid for up to four years from the dates the surveys were undertaken, meaning the data will begin to 'expire' from mid-April 2024, and by the

[7] https://www.gov.uk/guidance/great-crested-newts-surveys-and-mitigation-for-development-projects

end of June 2025 all the data (from both survey years) will likely be considered invalid in terms of suitability to support a DCO application. However, as the DCO application is due to be submitted in summer 2022, the data will be valid at that time, meaning no updates to the survey would be necessary to support this application.

SEP and DEP is pursuing a DLL to address potential impacts to GCN. DLL involves providing a Conservation Payment to fund a net increase in habitat for GCN across the landscape (at a county scale), rather than specifically within and around the DCO boundary, as is involved in conventional European Protected Species Mitigation Licensing. DLL does not necessarily require GCN survey data to inform the Conservation Payment calculation, but data, where available, (such as from the eDNA surveys completed between 2020 and 2021) can be used to refine the Conservation Payment calculation.

Natural England (which runs DLL in Norfolk) has approved a DLL application for SEP and DEP and an initial Conservation Payment has been made to the DLL scheme (see Annex 3, Appendix 1 of the Planning Statement (document reference 9.1). In the future, when onshore works associated with SEP and DEP are scheduled to commence, an updated DLL application can be submitted to Natural England, using data from the 2020-2021 surveys (or from updated surveys, if completed in the intervening period). Upon settlement of the remaining Conservation Payment to Natural England (and issuing of relevant paperwork), SEP and DEP will have discharged its mitigation obligations under the DLL scheme and works can proceed without any legal obligation to enact on-site mitigation measures for GCN.

However, while there is no legal obligation to mitigate impacts to GCN beyond the Conservation Payment made under DLL, best-practice animal welfare considerations are still advised during construction. Appropriate measures in respect to GCNs are presented in the **Outline Ecological Management Plan** (document reference 9.19).

5. CONCLUSIONS

The 2020 and 2021 GCN eDNA surveys have confirmed that GCN are present in localised parts of the survey area. Although the 2020 and 2021 HSI and eDNA survey data does not provide full coverage of all ponds (due mainly to restricted landowner access at the time of 2020 or 2021 survey effort), the survey results obtained have revealed a number of apparent clusters of ponds supporting GCN, which likely indicate the presence of metapopulations in these areas. From a review of the spatial distribution of ponds with Positive eDNA results and other records from NBIS and the UCLPRRG, these clusters are located in the following general areas:

- South of Bodham: ponds PW180, PW185, PW186 and PN113. Ponds PW175, PW181, PW182 and PW183 also have various records of GCN presence according to NBIS and UCLPRRG data; these ponds are also likely to support the same GCN metapopulation.
- Between Colton and Marlingford: ponds PN040, PN041 and P120.
- Between Hethersett, Ketteringham and Swardeston: ponds P014, P018, P024 and PA021, plus NBIS records of GCN in this area.

There may also be a metapopulation around Oulton and Saxthorpe. A positive eDNA result was returned for pond PW166 (and there is a NBIS biological record of GCN presence for this pond), and NBIS returned a record of GCN presence at pond PN103, located approximately 1.8km north of PW166.

There are also two isolated Positive results at ponds PA006 (south-east of the onshore substation site) and PW195 (north of Weybourne Woods near the landfall location), although this latter record could feasibly be associated with a wider metapopulation around Bodham.

The survey data have been and will continue to be used to inform DLL to mitigate potential impacts of SEP and DEP on GCN and ensure preservation of the favourable conservation status of the local GCN populations.

6. REFERENCES

ARG UK. (2010). *ARG UK Advice Note 5, Great Crested Newt Habitat Suitability Index*. Amphibian and Reptile Groups of the United Kingdom

Freshwater Habitats Trust (2015) *Pondnet: How to collect an eDNA sample*. Available online at [redacted]

https://www.gov.uk/guidance/great-crested-newts-surveys-and-mitigation-for-development-projects, accessed on 20/04/21

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, accessed on 20/04/21

Oldham R., Keeble J., Swan M. and Jeffcote, M. (2000). Evaluating the suitability of Habitat for Great Crested Newt (*Triturus cristatus*). Herpetological Journal 10: 143-155.

Sayer C. (2020). *Threats to pond networks associated with the Equinor cable – Information provided by Carl Sayer and the Norfolk Ponds Project.* Unpublished report.

Wild Frontier Ecology Ltd. (2020). *Equinor Dudgeon and Sheringham Shoal Offshore Wind Farm Extensions Onshore Grid Connection: Great Crested Newt 2020 Survey Report, August 2020*. Wild Frontier Ecology Ltd. Fakenham, Norfolk.

Annex 1: SureScreen Scientifics Ltd. Reports

SureScreen Scientifics

Folio No: E7133
Report No: 1
Purchase Order: 2020/08
Client: WILD FRONTIER ECOLOGY
Contact: Will Riddett

TECHNICAL REPORT

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

SUMMARY

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

RESULTS

Date sample received at Laboratory: 04/05/2020
Date Reported: 13/05/2020
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
0676	Equinor PW181, Pond Farm, Bodham		Pass	Pass	Pass	Negative	0
0683	Equinor Pond 14, Swardestone Pond 14		Pass	Pass	Pass	Positive	12
0687	Equinor PO111, Waiton Equinor		Pass	Pass	Pass	Negative	0
0693	Equinor PW175, Pond Farm, Bodham		Pass	Pass	Pass	Negative	0

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

SureScreen Scientifics

0694	Equinor PX1, Waring Swardestone		Pass	Pass	Pass	Negative	0
0699	Equinor PW180, Bodham Pond Farm		Pass	Pass	Pass	Positive	1
0700	Equinor Pond 17, Swardestone Pond 17		Pass	Pass	Pass	Negative	0
0704	Equinor PO19, Mr Cooke, Old Nursery, Swardestone		Pass	Pass	Pass	Negative	0
0705	Equinor PX5, Old Nursery, Swardestone		Pass	Pass	Pass	Negative	0

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

Reported by: Chris Troth **Approved by:** Sarah Evans

METHODOLOGY

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

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

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

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

INTERPRETATION OF RESULTS

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

SureScreen Scientifics

SIC: **Sample Integrity Check** [Pass/Fail]
When samples are received in the laboratory, they are inspected for any tube leakage, suitability of sample (not too much mud or weed etc.) and absence of any factors that could potentially lead to inconclusive results.

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

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

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

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

*The Methodology and Interpretation of Results sections of the SSL eDNA reports (pages 2 and 3 of the above report) are the same for each of their reports; these pages are not repeatedly provided for each individual report below.

SureScreen Scientifics

Folio No: E7140
Report No: 1
Purchase Order: 2020/08
Client: WILD FRONTIER ECOLOGY
Contact: Will Riddett

TECHNICAL REPORT

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

SUMMARY

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

RESULTS

Date sample received at Laboratory: 04/05/2020
Date Reported: 12/05/2020
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
0677	Equinor Pond 48, Home Farm Ketteringham		Pass	Pass	Pass	Negative	0
0679	Equinor PW183, Bodham Pond Farm		Pass	Pass	Pass	Negative	0

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

Reported by: Sarah Evans **Approved by:** Chris Troth

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

SureScreen Scientifics

Folio No: E7302
Report No: 1
Purchase Order: 2020/08
Client: WILD FRONTIER ECOLOGY
Contact: Will Riddett

TECHNICAL REPORT

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

SUMMARY

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

RESULTS

Date sample received at Laboratory: 12/05/2020
Date Reported: 19/05/2020
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
1282	Equinor PW169, Harris, Matlaske Road		Pass	Pass	Pass	Negative	0
1283	Equinor Pond PW168, Brooks Pond		Pass	Pass	Pass	Negative	0
1300	Equinor Pond 100, Markham		Pass	Pass	Pass	Negative	0
1301	Equinor PO15, Land at Swardestone		Pass	Pass	Pass	Negative	0
1302	Equinor PW167, Brooks,		Pass	Pass	Pass	Negative	0

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

SureScreen Scientifics

Mossymere Wood

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

Reported by: Chris Troth **Approved by:** Sarah Evans

METHODOLOGY

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

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

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

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

INTERPRETATION OF RESULTS

SIC: **Sample Integrity Check** [Pass/Fail]
When samples are received in the laboratory, they are inspected for any tube leakage, suitability of sample (not too much mud or weed etc.) and absence of any factors that could potentially lead to inconclusive results.

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

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

Result: **Presence of GCN eDNA** [Positive/Negative/Inconclusive]
Positive: GCN DNA was identified within the sample, indicative of GCN presence within the sampling location at the time the sample was taken or within the recent past at the sampling location.

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

SureScreen Scientifics

Folio No: E7322
Report No: 1
Purchase Order: 2020/08
Client: WILD FRONTIER ECOLOGY
Contact: Katrina Salmon

TECHNICAL REPORT

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

SUMMARY

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

RESULTS

Date sample received at Laboratory: 13/05/2020
Date Reported: 20/05/2020
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
1280	201, Equinor		Pass	Pass	Pass	Negative	0
1281	Equinor PW198, Preston		Pass	Pass	Pass	Negative	0
1284	PW203 Equinor, Preston		Pass	Pass	Pass	Negative	0
1286	199, Equinor - Preston		Pass	Pass	Pass	Negative	0
1291	PW200, Equinor, Preston		Pass	Pass	Pass	Negative	0

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

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

SureScreen Scientifics

Folio No: E7446
Report No: 1
Purchase Order: 2020/08
Client: WILD FRONTIER ECOLOGY
Contact: Will Riddett

TECHNICAL REPORT

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

SUMMARY

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

RESULTS

Date sample received at Laboratory: 20/05/2020
Date Reported: 26/05/2020
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
1285	Equinor Pond PX4, Equinor Pond House, Saxthorpe		Pass	Pass	Pass	Negative	0
1287	Equinor PX3, Rowe, Oulton		Pass	Pass	Pass	Negative	0
1298	Equinor PW166, Equinor Pond NR Oulton		Pass	Pass	Pass	Positive	1
1303	Equinor P058, Equinor Land at Heathersett		Pass	Pass	Pass	Negative	0

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

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

SureScreen Scientifics

Folio No: E7536
Report No: 1
Purchase Order: 2020/08
Client: WILD FRONTIER ECOLOGY
Contact: Will Riddett, Katrina Salmon

TECHNICAL REPORT

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

SUMMARY

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

RESULTS

Date sample received at Laboratory: 28/05/2020
Date Reported: 08/06/2020
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
1299	Equinor PN131, Equinor Muckleburgh Collection		Pass	Pass	Pass	Negative	0
1333	PN108, Equinor		Pass	Pass	Pass	Positive	11
1335	PN117, Equinor		Pass	Pass	Pass	Positive	12
1337	PN115, Equinor		Pass	Pass	Pass	Negative	0
1339	Equinor PN103, Agnew Saxthorpe		Pass	Pass	Pass	Negative	0
1370	Equinor PW185,		Pass	Pass	Pass	Positive	3

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

SureScreen Scientifics

	Knowles Bodham						
1372	PN116, Equinor		Pass	Pass	Pass	Positive	2
1375	Equinor PN113, Equinor near Bodham		Pass	Pass	Pass	Positive	5

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

Reported by: Chris Troth **Approved by:** Sarah Evans

METHODOLOGY

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

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

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

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

INTERPRETATION OF RESULTS

SIC: **Sample Integrity Check** [Pass/Fail]
When samples are received in the laboratory, they are inspected for any tube leakage, suitability of sample (not too much mud or weed etc.) and absence of any factors that could potentially lead to inconclusive results.

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

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

SureScreen Scientifics

Folio No: E7613
Report No: 1
Purchase Order: 2020/08
Client: WILD FRONTIER ECOLOGY
Contact: Will Riddett

TECHNICAL REPORT

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

SUMMARY

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

RESULTS

Date sample received at Laboratory: 04/06/2020
Date Reported: 11/06/2020
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
1289	Equinor PN050, Alston Honingham		Pass	Pass	Pass	Negative	0
1328	PN060, Equinor		Pass	Pass	Pass	Negative	0
1330	P136, Equinor		Pass	Pass	Pass	Negative	0
1332	PN062, Equinor		Pass	Pass	Pass	Negative	0
1340	PN065, Equinor		Pass	Pass	Pass	Negative	0
1341	Equinor P143, Ebony Weston Green		Pass	Pass	Pass	Negative	0
1342	P124,		Pass	Pass	Pass	Negative	0

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

SureScreen Scientifics

	Equinor					
1343	P125, Equinor	Pass	Pass	Pass	Negative	0
1344	Equinor P129, Equinor Nr Honingham	Pass	Pass	Pass	Negative	0
1345	Equinor PN089, Wales Weston Longville	Pass	Pass	Pass	Negative	0
1346	Equinor PW164, Friend, Cawston	Pass	Pass	Pass	Negative	0
1347	Equinor Pond PW165, Equinor Nr Cawston	Pass	Pass	Pass	Negative	0
1348	P138a, Equinor	Pass	Pass	Pass	Negative	0
1351	P138, Equinor	Pass	Pass	Pass	Negative	0
1364	Equinor P127, Alston Honingham	Pass	Pass	Pass	Positive	2
1366	Equinor, Alston Honingham	Pass	Pass	Pass	Negative	0
1368	Equinor, Alston, Honingham	Pass	Pass	Pass	Negative	0
2867	PN052, Equinor	Pass	Pass	Pass	Negative	0
2868	PN061, Equinor	Pass	Pass	Pass	Negative	0

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

Reported by: Sarah Evans **Approved by:** Chris Troth

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

SureScreen Scientifics

Folio No: E7687
Report No: 1
Purchase Order: 2020/08
Client: WILD FRONTIER ECOLOGY
Contact: Will Riddett

TECHNICAL REPORT

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

SUMMARY

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

RESULTS

Date sample received at Laboratory: 10/06/2020
Date Reported: 17/06/2020
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
1365	Equinor PN001, Swardeston Peddars Pies		Pass	Pass	Pass	Negative	0
2861	Equinor P013, Srokowski Swardeston		Pass	Pass	Pass	Negative	0
2866	Equinor PN067, Honingham		Pass	Pass	Pass	Negative	0
2870	Equinor PN068, Honingham		Pass	Pass	Pass	Negative	0
2878	Equinor PN063, Honingham		Pass	Pass	Pass	Negative	0

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

SureScreen Scientifics

2879	Equinor PN064, Honingham Thorpe		Pass	Pass	Pass	Negative	0
2880	Equinor PN057, Honingham Fishing Lake		Pass	Pass	Pass	Negative	0
2881	Equinor P154, Mutimer Swannington		Pass	Pass	Pass	Negative	0
2882	Equinor PW170, Little Barningham		Pass	Pass	Pass	Negative	0

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

Reported by: Chris Troth **Approved by:** Sarah Evans

METHODOLOGY

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

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

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

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

INTERPRETATION OF RESULTS

SIC: **Sample Integrity Check** [Pass/Fail]
When samples are received in the laboratory, they are inspected for any tube leakage, suitability of sample (not too much mud or weed etc.) and absence of any factors that could potentially lead to

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

SureScreen Scientifics

Folio No: E7782
Report No: 1
Purchase Order: 2020/08
Client: WILD FRONTIER ECOLOGY
Contact: Will Riddett

TECHNICAL REPORT

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

SUMMARY

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

RESULTS

Date sample received at Laboratory: 12/06/2020
Date Reported: 19/06/2020
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
1350	Equinor P042, Day Ketteringham		Pass	Pass	Pass	Negative	0
2839	Equinor P033, Day Ketteringham		Pass	Pass	Pass	Negative	0
2840	Equinor P032, Day Ketteringham		Pass	Pass	Pass	Positive	2
2841	Equinor P035, Day, Heathersett		Pass	Pass	Pass	Negative	0
2844	Equinor P037, NR Heathersett		Pass	Pass	Pass	Negative	0
2845	Equinor P038,		Pass	Pass	Pass	Negative	0

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

SureScreen Scientifics

	Day Ketteringham					
2856	Equinor P047, NR Hethersett	Pass	Pass	Pass	Negative	0
2872	Equinor P045, Day Ketteringham	Pass	Pass	Pass	Negative	0
2873	Equinor P041, NR Hethersett	Pass	Pass	Pass	Negative	0
2874	Equinor P043, Day Ketteringham	Pass	Pass	Pass	Negative	0

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

Reported by: Sarah Evans **Approved by:** Chris Troth

METHODOLOGY

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

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

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

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

INTERPRETATION OF RESULTS

SIC: **Sample Integrity Check** [Pass/Fail]
When samples are received in the laboratory, they are inspected for any tube leakage, suitability of sample (not too much mud or weed etc.) and absence of any factors that could potentially lead to

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

SureScreen Scientifics

Folio No: E7823
Report No: 1
Purchase Order: 2020/08
Client: WILD FRONTIER ECOLOGY
Contact: Will Riddett, Katrina Salmon

TECHNICAL REPORT

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

SUMMARY

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

RESULTS

Date sample received at Laboratory: 16/06/2020
Date Reported: 25/06/2020
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
2850	PN012, Wong Farm		Pass	Pass	Pass	Negative	0
2860	PN054, Equinor		Pass	Pass	Pass	Negative	0
2862	Equinor P016, Near Swarestone, Old Rectory		Pass	Pass	Pass	Negative	0
2875	Equinor PX, Poachers Rest, Colston		Pass	Pass	Pass	Negative	0
3529	Equinor PN07, Wong Farm		Pass	Pass	Pass	Positive	3
3530	Equinor PN011, Wong Farm		Pass	Pass	Pass	Positive	4

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

SureScreen Scientifics

3531	PN010, Wong Farm		Pass	Pass	Pass	Negative	0
3534	Equinor PN019, Wong Farm		Pass	Pass	Pass	Negative	0
3537	Equinor PN015, Wong Farm		Pass	Pass	Pass	Negative	0
3538	Equinor PN022, Whiterail Farm		Pass	Pass	Pass	Positive	2
3539	Equinor PN020, Whiterail Farm		Pass	Pass	Pass	Negative	0
3540	Equinor PN013, Wong Farm		Pass	Pass	Pass	Negative	0
3541	Equinor PX10, Whiterail Farm		Pass	Pass	Pass	Negative	0
3542	PN06, Wong Farm		Pass	Pass	Pass	Negative	0
3543	Equinor PX9, Wong Farm		Pass	Pass	Pass	Negative	0
3544	PN016, Wong Farm		Pass	Pass	Pass	Negative	0
3562	PN084, Equinor - Easton Estate		Pass	Pass	Pass	Negative	0
3563	Equinor PN132, Equinor - Easton Estate		Pass	Pass	Pass	Negative	0
3565	PN080, Equinor - Easton Estate		Pass	Pass	Pass	Negative	0
3567	PN079, Equinor - Easton Estate		Pass	Pass	Pass	Negative	0

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

SureScreen Scientifics

Folio No: E7915
Report No: 1
Purchase Order: 2020/08
Client: WILD FRONTIER ECOLOGY
Contact: Will Riddett, Katrina Salmon

TECHNICAL REPORT

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

SUMMARY

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

RESULTS

Date sample received at Laboratory: 23/06/2020
Date Reported: 29/06/2020
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
1309	Equinor PS01, Mangreen PS01 Substation		Pass	Pass	Pass	Negative	0
2838	Equinor PN025, Betts High Green		Pass	Pass	Pass	Negative	0
2846	Equinor PN094, Swannington Weston		Pass	Pass	Pass	Negative	0
2849	Equinor PS04, Mangreen PS04 Susbstation		Pass	Pass	Pass	Negative	0
2863	Equinor P028, Day		Pass	Pass	Pass	Negative	0

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

SureScreen Scientifics

	Ketteringham						
2864	Equinor P010, Mangreen, Hickling Lane		Pass	Pass	Pass	Negative	0
2869	Equinor P060, Hethersett Richardson		Pass	Pass	Pass	Negative	0
2871	Equinor PS03, Mangreen PS03 Substation		Pass	Pass	Pass	Negative	0
2876	P029, Equinor		Pass	Pass	Pass	Negative	0
3527	PN070, Equinor		Pass	Pass	Pass	Negative	0
3528	PN048, Equinor		Pass	Pass	Pass	Negative	0
3532	Equinor PN092, The Lodge, Morton on the Hill		Pass	Pass	Pass	Negative	0
3536	Equinor PN096, Oulton Hall		Pass	Pass	Pass	Negative	0
3545	PW155, Equinor		Pass	Pass	Pass	Negative	0
3564	PN097, Equinor		Pass	Pass	Pass	Negative	0
3568	PN081, Equinor - Easton Estate		Pass	Pass	Pass	Negative	0

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

Reported by: Chris Troth **Approved by:** Sarah Evans

METHODOLOGY

The samples detailed above have been analysed for the presence of GCN eDNA following the protocol stated in DEFRA WC1067 'Analytical and methodological development for improved surveillance of the Great Crested Newt, Appendix 5.' (Biggs et al. 2014). Each of the 6 sub-sample tubes are first centrifuged and pooled together into a single sample which

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

SureScreen Scientifics

Folio No: E8119
Report No: 1
Purchase Order: 2020/08
Client: WILD FRONTIER ECOLOGY
Contact: Will Riddett

TECHNICAL REPORT

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

SUMMARY

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

RESULTS

Date sample received at Laboratory: 29/06/2020
Date Reported: 07/07/2020
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
1304	EQUINOR PS10		Pass	Pass	Pass	Negative	0
1305	P122		Pass	Pass	Pass	Negative	0
1306	PNO26		Pass	Pass	Pass	Negative	0
1307	PNO71		Pass	Pass	Pass	Negative	0
1311	EQUINOR PNO34		Pass	Pass	Pass	Negative	0
1313	PN121		Pass	Pass	Pass	Negative	0
1315	PNO24		Pass	Pass	Pass	Positive	1
1316	PN120		Pass	Pass	Pass	Positive	1
1317	EQUINOR PW156		Pass	Pass	Pass	Negative	0
1318	EQUINOR		Pass	Pass	Pass	Negative	0

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

SureScreen Scientifics

	PW158						
1319	PN072		Pass	Pass	Pass	Negative	0
1320	PN023		Pass	Pass	Pass	Positive	2
1322	EQUINOR PN101		Pass	Pass	Pass	Negative	0
1323	PN029		Pass	Pass	Pass	Negative	0
1325	P119		Pass	Pass	Pass	Negative	0
1327	PN098		Pass	Pass	Pass	Negative	0
2847	PN119		Pass	Pass	Pass	Negative	0
2848	EQUINOR PN119		Pass	Pass	Pass	Negative	0

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

Reported by: Sarah Evans **Approved by:** Chris Troth

METHODOLOGY

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

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

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

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

INTERPRETATION OF RESULTS

SIC: **Sample Integrity Check** [Pass/Fail]
When samples are received in the laboratory, they are inspected for any tube leakage, suitability of sample (not too much mud or weed etc.) and absence of any factors that could potentially lead to

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

SureScreen Scientifics

Folio No: E8453
Report No: 1
Purchase Order: 2020/08
Client: WILD FRONTIER ECOLOGY
Contact: Will Riddett, Alex Lowe

TECHNICAL REPORT

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

SUMMARY

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

RESULTS

Date sample received at Laboratory: 08/07/2020
Date Reported: 22/07/2020
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
3546	Equinor P040, Horner Ketteringham	-	Pass	Pass	Pass	Negative	0
3548	Equinor P022, Moores Ketteringham	-	Pass	Pass	Pass	Negative	0
3549	Equinor P025, Moores, Ketteringham	-	Pass	Pass	Pass	Negative	0
3550	Equinor P026, Moores, Ketteringham	-	Pass	Pass	Pass	Positive	5
3552	Equinor P027, Moores Ketteringham	-	Pass	Pass	Pass	Negative	0
3556	PN125,	-	Pass	Pass	Pass	Negative	0

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

SureScreen Scientifics

	Kelling Health						
3557	Equinor PN088, Weston White	-	Pass	Pass	Pass	Negative	0
3570	Equinor PW186, Thurtle Bodham	-	Pass	Pass	Pass	Positive	12
3571	Equinor PN104, Brooks Saxthorpe	-	Pass	Pass	Pass	Negative	0
3579	Equinor PN053, Colton Curtis	-	Pass	Pass	Pass	Negative	0
3580	Equinor P153, Wensum Dacre	-	Pass	Pass	Pass	Negative	0
3582	Equinor PX11, Scales Colton	-	Pass	Pass	Pass	Negative	0
3583	Equinor PN043, Scales Colton	-	Pass	Pass	Pass	Negative	0
3585	PN003, Horner, Ketteringham	-	Pass	Pass	Pass	Negative	0
3586	PN047 Equinor, Scales, Colton	-	Pass	Pass	Pass	Negative	0
3587	Equinor P024, Moores, Ketteringham	-	Pass	Pass	Pass	Positive	1
3588	Equinor P039, Ketteringham, Horner	-	Pass	Pass	Pass	Negative	0
3589	Equinor P023, Moores Ketteringham	-	Pass	Pass	Pass	Negative	0
3590	Equinor P030, Moores Ketteringham	-	Pass	Pass	Pass	Negative	0

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

SureScreen Scientifics

3593	Equinor PN046, Scales Colton	-	Pass	Pass	Pass	Positive	1
5312	P505, Equinor Substation	-	Pass	Pass	Pass	Negative	0
5313	P012, Equinor Substation	-	Pass	Pass	Pass	Negative	0
5314	P510, Equinor Substation	-	Pass	Pass	Pass	Negative	0
5315	P010, Equinor	-	Pass	Pass	Pass	Negative	0
5316	PN126, Kelling Health	-	Pass	Pass	Pass	Negative	0
5317	P509, Equinor Substation	-	Pass	Pass	Pass	Negative	0
5319	P005, Equinor Substation	-	Pass	Pass	Pass	Negative	0
5322	P002, Equinor Substation	-	Pass	Pass	Pass	Negative	0
5323	P007, Equinor Substation	-	Pass	Pass	Pass	Negative	0
5324	P001, Equinor Substation	-	Pass	Pass	Pass	Negative	0
5326	P006, Equinor Substation	-	Pass	Pass	Pass	Negative	0
5329	PN127, Kelling Health	-	Pass	Pass	Pass	Negative	0

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

Reported by: Chris Troth **Approved by:** Sarah Evans

METHODOLOGY

The samples detailed above have been analysed for the presence of GCN eDNA following the protocol stated in DEFRA WC1067 'Analytical and methodological development for improved surveillance of the Great Crested Newt, Appendix 5.' (Biggs et al. 2014). Each of the 6 sub-sample tubes are first centrifuged and pooled together into a single sample which then undergoes DNA extraction. The extracted sample is then analysed using real time PCR (qPCR), which uses species-

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

SureScreen Scientifics

Folio No: E9649
Report No: 1
Purchase Order: 2021/11
Client: WILD FRONTIER ECOLOGY
Contact: Will Riddett

TECHNICAL REPORT

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

SUMMARY

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

RESULTS

Date sample received at Laboratory: 25/04/2021
Date Reported: 07/05/2021
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
1688	PA040		Pass	Pass	Pass	Negative	0
1697	PA021		Pass	Pass	Pass	Positive	2
1705	POND PA020		Pass	Pass	Pass	Negative	0
1709	PA029	NORFOLK	Pass	Pass	Pass	Negative	0
1711	PA034	NORFOLK	Pass	Pass	Pass	Negative	0
1713	POND A36	NORFOLK	Pass	Pass	Pass	Negative	0
1717	PA026	NORFOLK	Pass	Pass	Pass	Negative	0
1720	PA004		Pass	Pass	Pass	Negative	0
1721	PAO43		Pass	Pass	Pass	Negative	0
1722	PA003		Pass	Pass	Pass	Negative	0
1730	PA005		Pass	Pass	Pass	Negative	0
2980	PA031	NORFOLK	Pass	Pass	Pass	Negative	0

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

SureScreen Scientifics

2981	PA027		Pass	Pass	Pass	Negative	0
2982	PA037		Pass	Pass	Pass	Negative	0

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

Reported by: Chris Troth **Approved by:** Chris Troth

METHODOLOGY

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

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

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

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

INTERPRETATION OF RESULTS

SIC: **Sample Integrity Check** [Pass/Fail]
When samples are received in the laboratory, they are inspected for any tube leakage, suitability of sample (not too much mud or weed etc.) and absence of any factors that could potentially lead to inconclusive results.

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

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

Result: **Presence of GCN eDNA** [Positive/Negative/Inconclusive]
Positive: GCN DNA was identified within the sample, indicative of GCN presence within the sampling

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

SureScreen Scientifics

Folio No: E9712
Report No: 1
Purchase Order: 2021/11
Client: WILD FRONTIER ECOLOGY
Contact: Justin Parry

TECHNICAL REPORT

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

SUMMARY

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

RESULTS

Date sample received at Laboratory: 27/04/2021
Date Reported: 09/05/2021
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
1706	EQUINOR PA050	-	Pass	Pass	Pass	Negative	0
1726	EQUINOR PA045	-	Pass	Pass	Pass	Negative	0
1728	EQUINOR PA049	-	Pass	Pass	Pass	Negative	0

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

Reported by: Chris Troth **Approved by:** Chris Troth

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

SureScreen Scientifics

Folio No: E9860
Report No: 1
Purchase Order: 2021/11
Client: WILD FRONTIER ECOLOGY
Contact: Will Riddett, Katrina Salmon, Justin Parry

TECHNICAL REPORT

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

SUMMARY

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

RESULTS

Date sample received at Laboratory: 30/04/2021
Date Reported: 12/05/2021
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
1718	PN037, Equinor		Pass	Pass	Pass	Negative	0
1719	PN100, Equinor		Pass	Pass	Pass	Negative	0
1723	PN036, Equinor		Pass	Pass	Pass	Negative	0
1724	PX016, Equinor		Pass	Pass	Pass	Negative	0
1725	PX017, Equinor		Pass	Pass	Pass	Negative	0

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

Reported by: Chris Troth **Approved by:** Chris Troth

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

SureScreen Scientifics

Folio No: E9909
Report No: 1
Purchase Order: 2021/11
Client: WILD FRONTIER ECOLOGY
Contact: Will Riddett

TECHNICAL REPORT

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

SUMMARY

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

RESULTS

Date sample received at Laboratory: 03/05/2021
Date Reported: 14/05/2021
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
1727	PN009		Pass	Pass	Pass	Negative	0
1729	PN032		Pass	Pass	Pass	Negative	0
1731	PN014		Pass	Pass	Pass	Negative	0
1732	PN008		Pass	Pass	Pass	Negative	0
1733	PN030		Pass	Pass	Pass	Negative	0
2916	PN067		Pass	Pass	Pass	Negative	0
2920	PN068		Pass	Pass	Pass	Negative	0
2925	PN004		Pass	Pass	Pass	Negative	0
2926	PN050		Pass	Pass	Pass	Negative	0
2928	PN049		Pass	Pass	Pass	Negative	0
2931	PN002		Pass	Pass	Pass	Negative	0

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

SureScreen Scientifics

Folio No: E10001
Report No: 1
Purchase Order: 2021/11
Client: WILD FRONTIER ECOLOGY
Contact: Will Riddett

TECHNICAL REPORT

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

SUMMARY

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

RESULTS

Date sample received at Laboratory: 06/05/2021
Date Reported: 18/05/2021
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
2927	Equinor Swardeston Chapman PAO18		Pass	Pass	Pass	Negative	0
2930	River Tud		Pass	Pass	Pass	Negative	0

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

Reported by: Chris Troth **Approved by:** Chris Troth

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

SureScreen Scientifics

Folio No: E10108
Report No: 1
Purchase Order: 2021/11
Client: WILD FRONTIER ECOLOGY
Contact: Will Riddett

TECHNICAL REPORT

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

SUMMARY

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

RESULTS

Date sample received at Laboratory: 09/05/2021
Date Reported: 20/05/2021
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
2902	EQUINOR PW202		Pass	Pass	Pass	Negative	0
2904	PA022		Pass	Pass	Pass	Negative	0
2905	PNO27		Pass	Pass	Pass	Negative	0
2906	P131		Pass	Pass	Pass	Negative	0
2907	EQUINOR PNO90		Pass	Pass	Pass	Negative	0
2908	EQUINOR PX013		Pass	Pass	Pass	Negative	0
2909	EQUINOR PA035		Pass	Pass	Pass	Negative	0
2910	EQUINOR PX014		Pass	Pass	Pass	Negative	0
2911	PX018		Pass	Pass	Pass	Negative	0

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

SureScreen Scientifics

2978	POO8		Pass	Pass	Pass	Negative	0
2888	P003		Pass	Pass	Pass	Negative	0
2894	POO9		Pass	Pass	Pass	Negative	0
2895	P004		Pass	Pass	Pass	Negative	0
2896	P008		Pass	Pass	Pass	Negative	0
2897	PS009		Pass	Pass	Pass	Negative	0
2898	PS007		Pass	Pass	Pass	Negative	0
2899	PS002		Pass	Pass	Pass	Negative	0

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

Reported by: Chris Troth **Approved by:** Chris Troth

METHODOLOGY

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

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

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

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

INTERPRETATION OF RESULTS

SIC: **Sample Integrity Check** [Pass/Fail]
When samples are received in the laboratory, they are inspected for any tube leakage, suitability of sample (not too much mud or weed etc.) and absence of any factors that could potentially lead to inconclusive results.

DC: **Degradation Check** [Pass/Fail]
Analysis of the spiked DNA marker to see if there has been degradation of the kit or sample between the

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

SureScreen Scientifics

Folio No: E10377
Report No: 1
Purchase Order: 2021/11
Client: WILD FRONTIER ECOLOGY
Contact: Will Riddett

TECHNICAL REPORT

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

SUMMARY

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

RESULTS

Date sample received at Laboratory: 17/05/2021
Date Reported: 26/05/2021
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
2884	Equinor PA030, Equinor Wymonham rugby club		Pass	Pass	Pass	Negative	0
2885	Equinor PA032, Equinor Oaklands Farm, Melton		Pass	Pass	Pass	Negative	0
2886	Equinor PN091, Equinir Weston Longville		Pass	Pass	Pass	Negative	0
2887	Equinor PW194,		Pass	Pass	Pass	Negative	0

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

SureScreen Scientifics

	Equinor Weybourne Wood					
2889	Equinor PW159, Equinor NR Cawston	Pass	Pass	Pass	Negative	0
2917	Equinor P057, Equinor	Pass	Pass	Pass	Negative	0
2918	Equinor P051, Park Farm Hotel, Hethersett	Pass	Pass	Pass	Negative	0
2919	Equinor P020, Equinor North Farm	Pass	Pass	Pass	Negative	0
2912	Equinor PX020, Equinor Park Farm Hotel Hethersett	Pass	Pass	Pass	Negative	0
2923	Equinor PA006	Pass	Pass	Pass	Positive	6

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

Reported by: Chris Troth **Approved by:** Chris Troth

METHODOLOGY

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

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

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

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

SureScreen Scientifics

Folio No: E10577
Report No: 1
Purchase Order: 2021/11
Client: WILD FRONTIER ECOLOGY
Contact: Will Riddett

TECHNICAL REPORT

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

SUMMARY

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

RESULTS

Date sample received at Laboratory: 25/05/2021
Date Reported: 03/06/2021
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
2914	PW157, Equinor Swannington		Pass	Pass	Pass	Negative	0
2979	Equinor PA024, Hethersett		Pass	Pass	Pass	Negative	0

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

Reported by: Chris Troth **Approved by:** Chris Troth

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

SureScreen Scientifics

Folio No: E10800
Report No: 1
Purchase Order: 2021/11
Client: WILD FRONTIER ECOLOGY
Contact: Will Riddett

TECHNICAL REPORT

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

SUMMARY

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

RESULTS

Date sample received at Laboratory: 07/06/2021
Date Reported: 11/06/2021
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
2951	Equinor PA016		Pass	Pass	Pass	Negative	0
2955	Equinor PA015		Pass	Pass	Pass	Negative	0
2958	Quarry Equinor PA007		Pass	Pass	Pass	Negative	0
2959	Quarry Equinor PA013		Pass	Pass	Pass	Negative	0
2961	Quarry nr Substation PA009		Pass	Pass	Pass	Negative	0
2962	Quarry nr Substation PA010		Pass	Pass	Pass	Negative	0

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

SureScreen Scientifics

2963	Quarry Equinor PA008		Pass	Pass	Pass	Negative	0
2983	Equinor PA014		Pass	Pass	Pass	Negative	0

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

Reported by: Chris Troth **Approved by:** Chris Troth

METHODOLOGY

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

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

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

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

INTERPRETATION OF RESULTS

SIC: **Sample Integrity Check** [Pass/Fail]
When samples are received in the laboratory, they are inspected for any tube leakage, suitability of sample (not too much mud or weed etc.) and absence of any factors that could potentially lead to inconclusive results.

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

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

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

SureScreen Scientifics

Folio No: E11161
Report No: 1
Purchase Order: 2021/11
Client: WILD FRONTIER ECOLOGY
Contact: Will Riddett

TECHNICAL REPORT

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

SUMMARY

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

RESULTS

Date sample received at Laboratory: 23/06/2021
Date Reported: 05/07/2021
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
6127	PN130 Equinor, Weybourne	Norfolk	Pass	Pass	Pass	Negative	0
6133	PX021 Equinor	Norfolk	Pass	Pass	Pass	Negative	0

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

Reported by: Chris Troth **Approved by:** Chris Troth

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

SureScreen Scientifics

Folio No: E11274
Report No: 1
Purchase Order: 2021/11
Client: WILD FRONTIER ECOLOGY
Contact: Will Riddett

TECHNICAL REPORT

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

SUMMARY

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

RESULTS

Date sample received at Laboratory: 28/06/2021
Date Reported: 07/07/2021
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
6110	PW178 Bodham - Eqvinon		Pass	Pass	Pass	Negative	0
6118	PW176 Bodham - Eqvinon		Pass	Pass	Pass	Negative	0
6120	PW177 Bodham - Eqvinon		Pass	Pass	Pass	Negative	0
6121	PW174 Bodham - Eqvinon		Pass	Pass	Pass	Negative	0
6123	PW179 Baconsthorpe Wood - Eqvinon		Pass	Pass	Pass	Negative	0

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

SureScreen Scientifics

6124	PW182 Baconsthorpe Wood - Eqvinon		Pass	Pass	Pass	Negative	0
6134	PA001 Swainsthorpe - Eqvinon		Pass	Pass	Pass	Negative	0
6136	PN105 Bodham - Eqvinon - Fishing pond		Pass	Pass	Pass	Negative	0

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

Reported by: Chris Troth **Approved by:** Chris Troth

METHODOLOGY

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

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

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

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

INTERPRETATION OF RESULTS

SIC: **Sample Integrity Check** [Pass/Fail]
When samples are received in the laboratory, they are inspected for any tube leakage, suitability of sample (not too much mud or weed etc.) and absence of any factors that could potentially lead to inconclusive results.

DC: **Degradation Check** [Pass/Fail]
Analysis of the spiked DNA marker to see if there has been degradation of the kit or sample between the

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

SureScreen Scientifics

Folio No: E11618
Report No: 1
Purchase Order: 2021/11
Client: WILD FRONTIER ECOLOGY
Contact: Alex Lowe

TECHNICAL REPORT

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

SUMMARY

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

RESULTS

Date sample received at Laboratory: 05/07/2021
Date Reported: 18/07/2021
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
6108	PW195 6718 HAY-SMITH		Pass	Pass	Pass	Positive	12

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

Reported by: Jennifer Higginbottom **Approved by:** Jennifer Higginbottom

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

SureScreen Scientifics

Folio No: E11625
Report No: 1
Purchase Order: 2021/11
Client: WILD FRONTIER ECOLOGY
Contact: Alice Petherick

TECHNICAL REPORT

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

SUMMARY

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

RESULTS

Date sample received at Laboratory: 05/07/2021
Date Reported: 18/07/2021
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
6109	EQUINOR WEYBOURNE PW197		Pass	Pass	Pass	Negative	0

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

Reported by: Jennifer Higginbottom **Approved by:** Jennifer Higginbottom

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

SureScreen Scientifics

Folio No: E11627
Report No: 1
Purchase Order: 2021/11
Client: WILD FRONTIER ECOLOGY
Contact: Alice Petherick

TECHNICAL REPORT

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

SUMMARY

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

RESULTS

Date sample received at Laboratory: 05/07/2021
Date Reported: 19/07/2021
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
6114	EQUINOR BODHAM PW173		Pass	Pass	Pass	Negative	0

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

Reported by: Chris Troth **Approved by:** Chris Troth

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

Annex 2: UCL Pond Restoration Research Group Information

Threats to pond networks associated with the Equnior cable – Information provided by Carl Sayer and the Norfolk Ponds Project

This letter provides some information to inform on the proposed Equinor cable corridor for North Norfolk in terms of its intersection with farmland ponds. The particular focus of this is letter is Great Crested Newt (GCN) and initially data is provided for the Bodham-Baconsthorpe (**Fig. 1, Fig. 2**) and Heydon (**Fig. 3**) areas, both of which are in or close to the proposed cable corridor and which have also been the subject of major landscape-scale pond restoration projects of the Norfolk Ponds Project (NPP). Note that further information is held on many other species groups: plants, invertebrates, other amphibians, birds, bats, fish (including eels) for many of the ponds that can be made available.

Working towards the goals of the NPP a major activity of the UCL Pond Restoration Research Group (PRRG) has been the restoration of farmland ponds with the aim of increasing aquatic and terrestrial biodiversity and aquatic connectivity. A key issue tackled is pond terrestrialisation as, since the 1960s/70s period, with the cessation of traditional pond management practices, Norfolk farmland ponds have become highly overgrown with negative impacts for species due to habitat uniformity (almost all ponds are overgrown presenting a lack of aquatic plant habitat) and because overgrown ponds afford very harsh (anoxic) conditions to aquatic species (Sayer *et al.,* 2012; 2013; Sayer, 2014). Thus the project has re-instated management and undertaken major scrub and sediment removal for networks of ponds. The project started its work on the Bodham area, with Sayer's Black Pit (**Fig. 2**) the first restored pond. The results of the project have been startling which substantial measured gains for aquatic plants, invertebrates, amphibians (including the protected Great Crested Newt), farmland birds and pollinator communities across the ponds (Sayer *et al.,* 2012; 2013; Sayer, 2014; Lewis-Phillips *et al.* 2019, 2020 and see **Fig. 1**).

At Bodham-Baconsthorpe the UCL PRRG has been working on several ponds (with 11 ponds restored since 2011), some 19 of which have recorded Great Crested Newt presence (**Table 1**) and highly diverse communities of plants (e.g. **Fig. 1**), invertebrates, dragonflies, pollinators and farmland birds. Note we have data for all of these groups for several of the ponds. Important pond plants include *Nitella opaca, Nitella flexilis and Tolypella glomerata* and the rare (Norfolk BAP species) crucian carp (*Carassius carassius*) is present in four ponds in this area.

At the Heydon pond landscape (**Fig. 3**) Great Crested Newt has been recorded at some 9 ponds (**Table 2**). Some rare plants have been recorded from these ponds, including *Najas marina* (PYES1), *Hottonia palustris* (HEY97), *Oenanthe aquatica* (several ponds) and *Tolypella glomerata* (several ponds), with one restored pond (COLG3) supporting a remarkable 6 charophytes species. In addition the crucian carp has been recorded at 3 of the Heydon ponds. Thus there are very valuable restored pond landscapes adjacent to and within the cable route that will be compromised.

Given our major interests in Norfolk farmland ponds and in the network of hedges, woodland and meadows that form important corridors and connections between ponds that will be damaged and compromised by the cable works and in particular given the proximity of the Equinor cable route to our two major pond restoration areas at Bodham and Heydon we are obviously very concerned regarding potential impacts.

Yours sincerely

[redacted]

The UCL Pond Restoration Research Group
c/o Dr. Carl Sayer, Department of Geography, University College London, Gower Street London, WC1E 6BT, Tel: 07766717245, e-mail: c.sayer@ucl.ac.uk

Pond name	Pond code	NGR	Date restored by NPP	Great Crested Newt known to be (breeding)
Hart Lane Pond	HART	TG 12952 39304	Not restored	Yes
Beckett's Farm Pond	BECK	TG 1110 3765	Restored Sept. 2014	Yes
Shooting Close Pond	SHOOT	TG 1135 3780	Restored Sept. 2014	Yes
Sayer's Black Pit	SABA	TG 1265 3960	Restored Sept. 2011	Yes
Sayer's New Pond	SAYNE	TG 12561 39892	Restored Sept. 2011	Yes
Bodham Mystery Pit	MYST	TG 1260 3945	Restored Sept. 2011	Yes
Mystery Pit Friend	MYSTF	TG 12430 39444	Not restored	Yes
Church Farm Pond 1	CHFA1	TG 11704 38768	Not restored	No
Church Farm Pond 2	CHFA2	TG 11886 38818	Restored Sept. 2017	Yes
Church Farm Pond 3	CHFA3	TG 11735 38720	Restored Sept. 2017	Yes
Church Farm Pond 4	CHFA4	TG 11874 38908	Not restored	Yes
Baconsthorpe Wood S. Pond	BAWO2	TG 12846 38343	Restored Nov. 2017	Yes
Breck Farm Pond	BRECK	TG 12591 37622	Not restored	Yes
Baconsthorpe Wood N. Pond	BAWO1	TG 12759 38591	Not restored	Yes
New Road Pond	NROAD	TG 12882 37684	Restored Nov. 2017	No
Skylark Pond	SKYLA	TG 11060 38332	Restored Sept. 2017	Yes
Wrong Close Pond	WRONG	TG 1160 3750	Not restored	Yes
Rail Pit	RAIL	TG 1235 3890	Not restored	Yes
Pond Farm Pond 1	POFA1	TG 1315 3860	Not restored	Yes
Pond Farm Pond 2	POFA2	TG 1315 3855	Not restored	Yes
Pond Farm Pond 3	POFA3	TG 1330 3865	Not restored	Yes
Pond Farm Pond 4	POFA4	TG 1325 3815	Restored Sept. 2010	Unknown

Table 1. Key Norfolk Pond Project study ponds within or close to the proposed Equinor cable corridor in the Bodham-Baconsthorpe area detailing known sites for Great Crested Newt (GCN) breeding as recorded by Carl Sayer & Ewan Shilland of the Pond Restoration Research Group at UCL. All sites with a Yes for breeding had GCN eggs.

Pond name	Pond code	NGR	Date restored by NPP	Great Crested Newt known to be (breeding)
Colgreen Field Pond 1	COLG1	TG 10448 26942	Not restored	No
Colgreen Field Pond 2	COLG2	TG 10426 26632	Restored Sept. 2015	Yes
Colgreen Field Pond 3	COLG3	TG 10553 26790	Restored Sept. 2015	No
Colgreen Field Pond 4	COLG4	TG 10462 26806	Restored Sept. 2015	Yes
Heydon Pond 102	HEY102	TG 10706 27106	Restored Sept. 2018	No
Heydon Pond 103	HEY103	TG 10650 27056	Not restored	No
Heydon Pond 97	HEY97	TG 10720 26945	Restored Sept. 2016	No
Heydon Pond 96	HEY96	TG 10786 26862	Restored Sept. 2016	No
Bonfire Field Pond	BONF	TG 1095 126778	Restored Sept. 2015	Yes
Heydon Pond 94	HEY94	TG 11057 26722	Not restored	Yes
Heydon Pond 93	HEY93	TG11363 28269	Restored Sept. 2016	Yes
Bullock Shed Pond 1	BULLS1	TG 11267 28319	Not restored	No
Bullock Shed Pond 2	BULLS2	TG 11108 28326	Not restored	No
Heydon Pond 90	HEY90	TG 11133 28459	Not restored	Yes
Heydon Pond 89	HEY89	TG 11363 28268	Not restored	No
Holly Grove Pond	HOLLY	TG 10707 27940	Not restored	No
Dairy Farm Pond 1	DAIRY1	TG 10501 27652	Not restored	Yes
Dairy Farm Pond 2	DAIRY2	TG 10534 27732	Restored Sept. 2018	Yes
Dairy Farm Pond 3	DAIRY3	TG 10598 27758	Not restored	No
Dairy Farm Pond 4	DAIRY4	TG 10627 27783	Not restored	Yes
Cinders Hill Pond	CIND	TG 10908 28756	Restored Sept. 2012 + managed Feb. 2015	No
Pyes Pit 1	PYES1	TG 1330 2555	Restored Feb. 2015	No
Pyes Pit 2	PYES2	TG 1340 2535	Restored Feb. 2015	Yes

Table 2. Key Norfolk Pond Project study ponds close to the proposed Equinor cable corridor in the Bodham-Baconsthorpe area detailing known sites for Great Crested Newt (GCN) breeding as recorded by Carl Sayer & Ewan Shilland of the Pond Restoration Research Group at UCL. All sites with a Yes for breeding had GCN eggs.

Figure 1. Shooting Close Pond (TG 1135 3780), a small Great Crested Newt supporting farmland pond close to the Orsted cable corridor in the Bodham-Baconsthorpe area before (a), during (b) and two years after restoration (c) by scrub and sediment removal in September 2014.

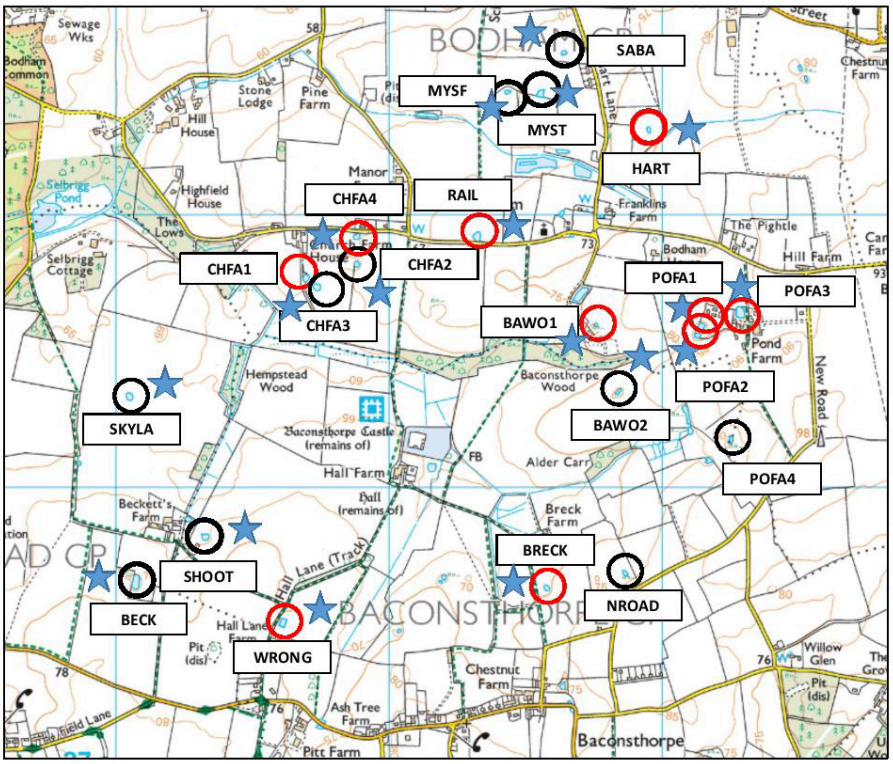


Figure 2. Ponds in the Bodham-Baconsthorpe Norfolk Ponds Project pond landscape as discussed in the text and detailed in Table 1. Ponds circled black have been restored by the NPP and ponds with a blue asterisk next to them are known to support Great Crested Newt as recorded by Carl Sayer & Ewan Shilland of the Pond Restoration Research Group at UCL.

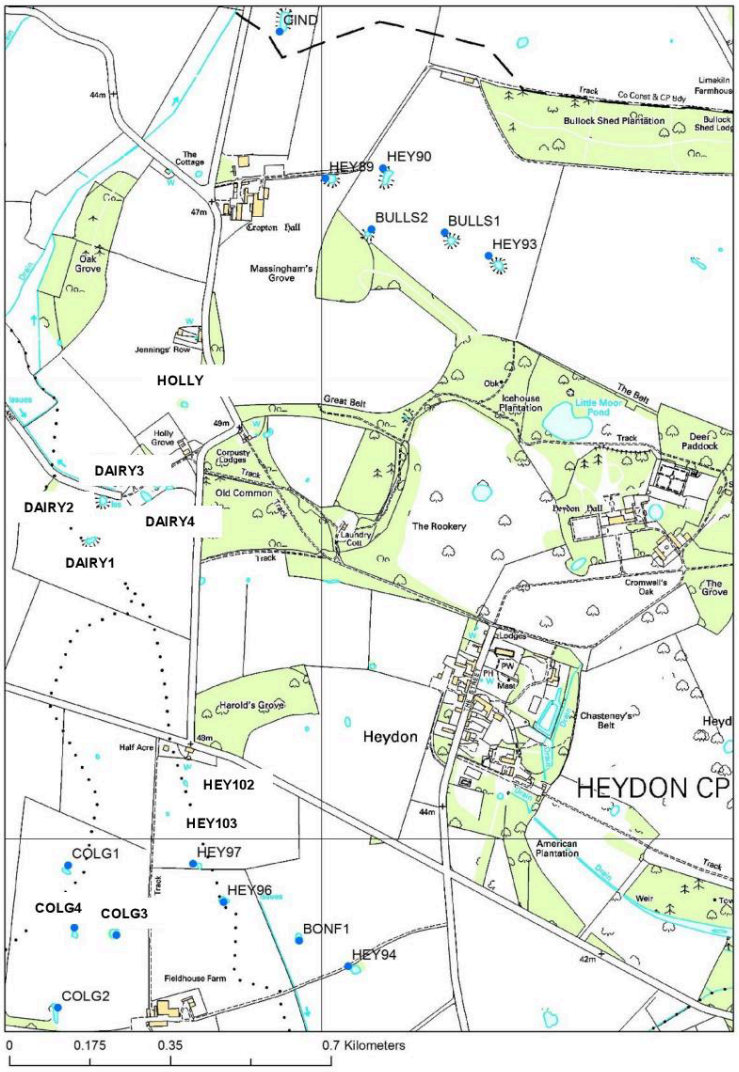


Figure 3. Ponds in the Heydon area Norfolk Ponds Project pond landscape. Note that all of CIND, PYES1, PYES2, COLG1, COLG3, COLG4, HEY97, HEY96, BONF1 and HEY93 have all been restored since 2012. Great Crested Newt breeding is known for ponds BONF, HEY94, COLG3, COLG2, HEY93, DAIRY1, DAIRY4 and PYES2 and we expect the species to spread further throughout the pond network in the coming years. Note the NPP is monitoring the ponds closely in this respect each year.

References

Lewis-Phillips, J., Brooks, S., Sayer, C., McCrea, R., Siriwardena, G., Axmacher, J.C. (2019). Pond management enhances the local abundance and species richness of farmland bird communities. *Agriculture, Ecosystems and Environment*, 273, 130-140.

Lewis-Phillips, J., Brooks, S.J., Sayer, C.D., Patmore, I.R., Hilton, G., Harrison, A., ...Axmacher, J.C. (2020). Ponds as insect chimneys: restoring overgrown farmland ponds benefits birds through elevated productivity of emerging aquatic insects. *Biological Conservation*, 108253,

Sayer, C.D., Copp, G.H., Emson, D., Godard, M.J., Zieba, G., Wesley, K.J. (2011). Towards the conservation of crucian carp *Carassius carassius*: understanding the extent and causes of decline within part of its native English range. *Journal of Fish Biology*, 79 (6), 1608-1624. doi:10.1111/j.1095-8649.2011.03059.x

Sayer, C.D., Andrews, K., Shilland, E., Edmonds, N., Edmonds-Brown, R., Patmore, I., Emson, D. & Axmacher, J.A. (2012). The role of pond management for biodiversity conservation in an agricultural landscape. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 22 (5), 626-638. doi:10.1002/aqc.2254

Sayer, C.D., Shilland, E.M., Greaves, H., Dawson, B., Patmore, I., Emson, D., Alderton, E., Robinson, P., Andrews, K., Axmacher, J.A., Wiik, E. (2013). Managing Britain's ponds - conservation lessons from a Norfolk farm. *British Wildlife* 25(1), 21-28.

Sayer, C.D. (2014). Conservation of aquatic landscapes: ponds, lakes, and rivers as integrated systems. *WIRE's: Water*, 1 (6), 573-585. doi:10.1002/wat2.1045