



P e l l F r i s c h m a n n

**Hoo Station GRIP 3 – Reopening of
the Grain Branch Line for Passenger
Services**

Great Crested Newt Survey Report

Date: October 2020

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1 Introduction

Pell Frischmann have been commissioned by Medway Council to undertake great crested newt (*Triturus cristatus*) (GCN) surveys for the reopening of the Grain Branch Line for passenger rail services.

The Hoo Peninsula is currently accessed via a single main highway route which links to other roads in the Chatham and Strood area. There is a proposal to increase housing on the peninsula by 12,100 homes, all of which would need to be served by this highway. The resulting significant increase in traffic would exceed the road network's capacity, even with the planned highway upgrades so a shift to rail usage is being sought.

The existing Network Rail lines in the area are the London to Higham / Strood main line and the Grain Line (also known as the Hoo branch line) a freight line to the Isle of Grain. An investigation is underway as to the viability of running a passenger service on the Grain Line.

The GRIP 2 study investigated the feasibility of the scenarios raised by the GRIP 1 report. This focused on providing a passenger service to Sharnal Street (Phase 1), and also considers the passive provision for doubling of the line by Network Rail (Phase 2) to provide for future expansion of passenger and freight services.

This GRIP 3 Study now considers the options in more detail with a view to recommending options to be taken forward This report forms part of that analysis.

The GRIP 3 study has been divided into the following main elements -

- the enabling works to the existing line including any environmental mitigation and structural upgrades to the existing infrastructure
- permanent way modifications including the creation of passing places
- the creation of a new route south to Higham, Strood and the Medway Towns
- civils works for the replacement of existing at-grade crossings,
- the creation of a new station at Sharnal Street and associated infrastructure.

1.1 Survey Scope

The scope of this assessment is to identify:

- the presence or likely absence of GCN within the Site;
- the presence or likely absence of GCN within a 500m radius of the Site.

GCN are afforded full protection under European and British law, which makes it an offence to deliberately kill or injure individuals, damage their breeding or resting places, and/or obstruct access to their breeding or resting places.

Sufficient ecological information is required to fully inform the Scheme design and the proposed construction works. This report aims to enable the project to satisfy current UK and European legal wildlife requirements, as well as national and local planning regulations. Public bodies

have statutory obligations under the Natural Environment and Rural Communities Act 2006 to conserve and enhance biodiversity.

The scheme should aim to minimise impacts to key ecological receptors wherever possible. The ecological zone of influence includes the land within the Network Rail boundaries and the adjoining land. The study area is set out in Section 1.2 below.

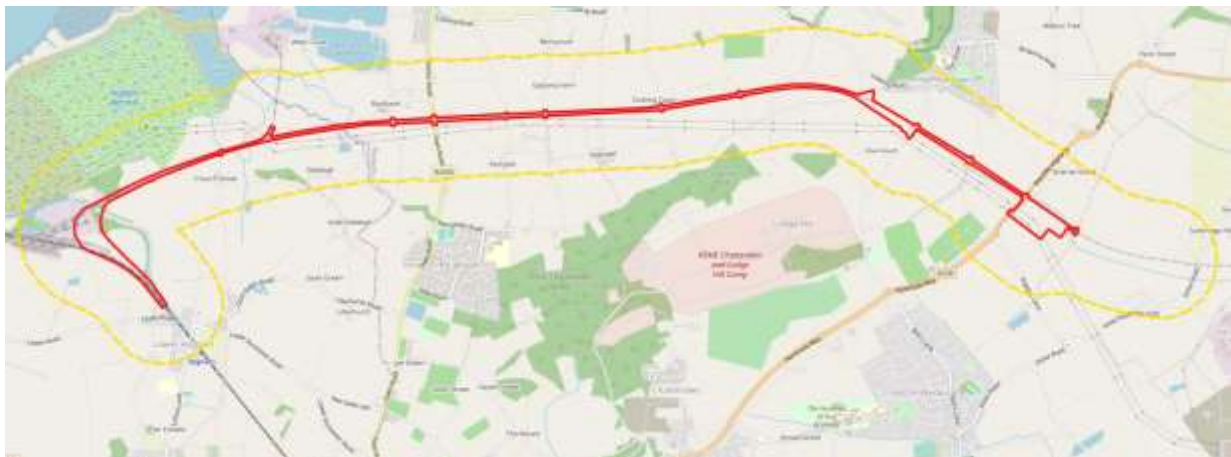
1.2 Study Area and Proposed Development

The study area for this assessment includes the Proposed Scheme (hereafter referred to as ‘the Site’), described above, and the surrounding land within 500m. This 500m buffer represents the ecological zone of influence for GCN and is presented below in Figure 1.

The land adjacent to the Site is generally characterised by arable fields, with wet and dry ditches. The western section of the Site is adjacent to marshy grassland and a number of designated sites of importance for nature conservation including Thames Estuary and Marshes (Ramsar and Special Protection Area (SPA) and the South Thames Estuary and Marshes Site of Special Scientific Interest (SSSI).

A total of 38 ponds were identified within the study area using online maps and aerial photography. The location of these ponds is presented in Appendix A.

Figure 1 Site Red Line Boundary



Map Data Contains © OpenStreetMap Contributors

2 Legislation

GCN are a European protected species and are safeguarded by both the Wildlife and Countryside Act 1981 (as amended) and the Habitat Directive (transposed into UK law as the Conservation of Habitats and Species (Amendment) (EU Exit) Regulations 2019). Legislation extends protection to the animals, their eggs, their breeding sites and resting places and is a means by which sites can be protected from undesirable change.

Actions that would cause an offence under UK law include:

- The deliberate capture, killing, disturbance or injury of GCN;
- Damaging or destroying a breeding or resting place;
- Obstructing access to their resting or sheltering places (deliberately or by not taking enough care);
- Possessing, selling, controlling or transporting live or dead newts, or parts of them; and
- Taking GCN eggs.

The legislation covers each of the life stages including eggs, tadpoles and adult equally.

In most cases, a European Protected Species (EPS) licence may be required from Natural England if the disturbance or damaging of their habitats (ponds and the land around ponds) cannot be avoided. An application can only be made once planning permission for the scheme has been approved. In order to obtain a protected species licence, the client must demonstrate that the work cannot be avoided and that there are no alternatives.

The protected species licence will also require a suitable protection and mitigation method to be adopted.

An alternative to the EPS Licence is the District Level Licence which has been developed by Natural England and is supported within Kent. This approach enables a simpler site-based mitigation approach and allows a landscape or whole local authority area scale compensation approach to development impacts. Overall this approach aims to benefit hunting and breeding grounds for GCN and lead to an increase in population.

For this scheme the District Level Licence approach has been used and is therefore referenced throughout the remainder of this report.

3 Assessment Methodology

GCN surveys were undertaken between 15th June and 26th June 2020 using environmental DNA (eDNA) methods as described below.

eDNA surveys were undertaken on 24 of the 38 ponds identified within 500m of the Site. The ponds not surveyed are detailed in Section 3.6 and were unable to be or unsuitable for survey due to a mix of health and safety constraints, lack of water or lack of land access.

3.1 Desktop Study

To accurately assess the potential ecological impacts of the scheme, a desktop study was undertaken to identify the presence of sensitive ecological receptors at the site and within the surrounding area.

The Kent and Medway Biological Records Centre (KMBRC) were commissioned to undertake a data search for protected and notable species and sites of conservation importance within a 2km radius of the central point of the site.

In addition, the National Biodiversity Network (NBN) database was searched for local records for notable and protected species from 2km of the site boundary within the last 20 years.

Full details of the desktop study (including relevant legal and policy issues) can be found within the Preliminary Ecological Appraisal (PEA) report (103223-PEL-G3-H01-REP-EVV-0001) and details relevant to this great crested newt report have been summarised in Section 4.1 of this report.

3.2 Habitat Suitability Index (HSI)

A Habitat Suitability Index (HSI) assessment for great crested newts was undertaken on waterbodies within 500m of the Site (where access permission was granted). This assessment was undertaken to establish the likelihood of GCN either breeding on the site or dispersing to the Site in their terrestrial phase.

The HSI is a standard assessment method developed specifically to evaluate the habitat suitability for this species. A series of factors must be considered. Each factor is assessed along suitability guidelines and allocated a value of between 0.01 (highly unsuitable) to 1.0 (highly suitable). The geometric mean of these values provides an overall suitability score for each waterbody. HSI can be useful in evaluating the general suitability of a sample of ponds for great crested newt; comparing general suitability of ponds across different areas; and evaluating the suitability of receptor ponds in a proposed mitigation scheme.

HSI is limited by being insufficiently precise to allow one to draw conclusions that a pond with a high score will support Great Crested Newts nor that a pond with a low score will not do so. The results do not allow conclusions on newt populations to be reached. Therefore, an HSI assessment is not a substitute for further great crested newt surveys.

3.3 Great Crested Newts eDNA surveys

The methodology used for surveying GCN by eDNA techniques is approved by Natural England following Defra funded research into the suitability of using this protocol to determine presence/likely absence of GCN. eDNA kits were supplied and analysed by SureScreen Scientifics.

The eDNA survey included taking 20 water samples from each of the ponds; these samples were spread out evenly around the pond edge. The samples were taken from both open water and vegetated areas, avoiding water less than 10cm deep. The water column was mixed gently avoiding stirring up sediment from the bottom.

For each pond, water samples were collected in a Whirl-Pak bag and shaken for 10 seconds to mix evenly. Using a clear plastic pipette, 15ml of water was taken from the Whirl-Pak bag, and transferred into each of six conical tubes containing 35ml preserving fluid (i.e. filling the tube to the 50ml mark). The samples were then transported to the laboratory for analysis.

The SureScreen lab results are attached in Appendix C.

3.4 Surveyors

GCN surveys were completed by C.Gilby from Pell Frischmann accredited under the licence of S.Pagett (Natural England Class Licence 2015-18996-CLS-CLS).

3.5 Accurate Lifespan of Ecological Data

The majority of ecological data remains valid for only short periods due to the inherently transient nature of the subject. The survey results contained in this report are considered accurate for approximately 2 years, beyond which updated surveys may be required to ascertain if the status of the Site with respect to GCN.

3.6 Ecological Survey Constraints and Limitations

Of the 38 ponds identified within 500m of the Site via online and aerial maps, 14 could not be surveyed for the following reasons –

- Ponds 1, 10, 28 and 31 were dry and therefore unsuitable for eDNA surveys as no water samples could be taken. The landowner for Pond 31 identified that this pond is usually wet but dry this year due to the prolonged dry weather during May and early June prior to the undertaking of the eDNA surveys.
- Pond 8 was a large attenuation tank with vertical concrete sides with a drop that made water sample collection unsafe. Despite the visual presence of coot and common frog within this pond, it is considered unsuitable for GCN due to lack of vegetation and inability to leave to travel to terrestrial habitat. The HSI score for this pond was 0.47, 'poor'.
- Aerial imagery indicated the locations of Ponds 12 and 14, however they could not be located within dense bramble and nettle vegetation of the woodland where mapping had indicated they were located. Due to a lack of evidence of ponds in the area they were therefore considered to be dry.

- The landowner of Pond 27 denied access for the survey.
- Ponds 32 and 33 were reservoirs with steep sides and pond liners with a total lack of any vegetation. These ponds could not be surveyed due to safety concerns.
- Pond 34 was identified as a covered tank and therefore not a pond. As such it was not subject to further survey.
- Pond 36 could not be accessed due to stockproof bramble surrounding the pond and a locked fence with no access provided.
- Ponds 37 and 38 were advised to be stocked fishing ponds by the landowner of the asbestos works area within which they were present. No access to these ponds was available and as such no HSI could be completed on these ponds.

While every effort was made to assess all ponds within 500m of the Site, it is recognised that mapping is likely to be out of date and as such some some unmapped ponds may not have been subject to survey.

4 Great Crested Newt Survey Results

4.1 Data Records Search

A total of 78 records for GCN have been returned within 2km of the Site, with 45 of these being since 1999. The closest of these records were approximately 720m and 800m to the south of the Site, with further records approximately 1.5km to the south.

A search using NBN has also identified the presence of GCN within 1km of the Site. These records are located to the north of the Site at Cooling and High Halstow, and to the south of the Site near Lodge Hill with the nearest record being approximately 400m to the south.

Granted European Protected Species Licenses (EPSL) for GCN have been identified through MAGIC within 2km of the Site. It should be noted that these records have not been updated since 2016.

4.2 eDNA Survey Results

In total 24 ponds were tested using eDNA survey methods. The results of the eDNA testing were 'Positive' in 7 of these ponds including Ponds 15, 16, 23, 25, 26 which are within 250m of the Site and Ponds 29, 30 which are over 250m from the Site. The presence of GCN within these ponds indicates that they are breeding ponds for the species, though due to the time of year at which the eDNA surveys were undertaken a full population assessment survey could not be undertaken.

The results of the eDNA testing were 'Negative' in Ponds 2, 3, 4, 5, 6, 7, 9, 11, 13, 17, 18, 19, 20, 21, 22, 24 and 35 and GCN are therefore assumed to be absent from these ponds.

The remaining ponds were not surveyed, with the reasoning for the lack of survey set out in Table 1 below and fully detailed in Section 3.6. Full lab results from Surescreen are presented in Appendix C.

Table 1 Summary of results for Ponds within 500m of the Site. Green boxes correlate to the presence of great crested newt DNA within that pond.

Pond Number	Surveyed (Yes/No)	HSI Score	eDNA survey result – GCN present (Yes/No)
1	No – pond dry	-	-
2	Yes	0.62 – average	No
3	Yes	0.71 – good	No
4	Yes	0.85 – excellent	No
5	Yes	0.55 – below average	No
6	Yes	0.85 – excellent	No
7	Yes	0.76 – average	No

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Pond Number	Surveyed (Yes/No)	HSI Score	eDNA survey result – GCN present (Yes/No)
8	No – tank with vertical sides could not be safely sampled.	0.47 – poor	-
9	Yes	0.69 – average	No
10	No – pond dry	0.44 – poor	-
11	Yes	0.72 – good	No
12	No – assumed dry as could not be located	-	-
13	Yes	0.72 – good	No
14	No – assumed dry as could not be located	-	-
15	Yes	0.80 – excellent	Yes
16	Yes	0.84 – excellent	Yes
17	Yes	0.73 – good	No
18	Yes	0.73 – good	No
19	Yes	0.70 – average	No
20	Yes	0.70 – average	No
21	Yes	0.74 – good	No
22	Yes	0.81 – excellent	No
23	Yes	0.79 – good	Yes
24	Yes	0.36 – poor	No
25	Yes	0.80 – excellent	Yes
26	Yes	0.79 – good	Yes
27	No – no access		-
28	No - dry	0.31 – poor	-
29	Yes	0.75 – good	Yes
30	Yes	0.61 – average	Yes
31	No - dry	0.58 – below average	-
32	No – reservoir with pond liner	0.71 – good	-
33	No – reservoir with pond liner	0.71 – good	-
34	No – covered tank	-	-
35	Yes	0.75 - good	No
36	No – could not access	-	-

Pond Number	Surveyed (Yes/No)	HSI Score	eDNA survey result – GCN present (Yes/No)
37	No – could not access. Advised by landowner it is a fishing pond with heavy presence of asbestos	-	-
38	No – could not access. Advised by landowner it is a fishing pond with heavy presence of asbestos	-	-

5 Summary of Impacts

The GCN survey findings, detailed in Section 4, identify the presence of GCN within 7 ponds close to the Site.

No direct impacts to ponds where GCN are present are expected during the construction phase.

Construction works will occur within 250m of Ponds 15, 16, 23, 25 and 26 (all of which tested positive for the presence of GCN) and therefore impacts to GCN are considered likely through temporary disturbance and loss of terrestrial habitat including woodland and scrub; which was considered to offer opportunities for refuge and foraging to GCN as well as connectivity to suitable habitats for the species in the wider area.

These impacts have been summarised in Table 2 below and also shown on the constraints map presented in Appendix D.

Table 2 Likely Impacts of works within 250m of ponds which tested positive for the presence of great crested newts.

Pond ID	Grid Reference	Likely Impact
15	TQ 71119 74225	No direct impact to pond likely. Pond is approx. 35m north of the edge of the existing railway track. It is considered that the GCN population present in this pond is likely to use areas of suitable terrestrial habitat along the railway corridor for refuge, foraging and commuting. Potential impact to GCN through removal of terrestrial habitat due to track dualling.
16	TQ 71527 74137	No direct impact to pond. Pond is approx. 195m south of the edge of the existing railway track. It is considered that the GCN population present in this pond may use areas of suitable terrestrial habitat along the railway corridor for refuge, foraging and commuting. Potential impact to GCN through removal of terrestrial habitat due to track dualling
23	TQ 74511 75078	No direct impact to pond. Pond is approx. 220m north of the edge of the existing railway track. It is considered that the GCN population present in this pond would use the available terrestrial habitat that is immediately to the pond in preference to commuting across the wider landscape as there is little connectivity between the pond and the railway corridor.
25	TQ 75527 75053	No direct impact to pond likely. Pond is approx. 120m north of the edge of the existing railway track. It is considered that the GCN population present in this pond is likely to use areas of suitable terrestrial habitat along the railway corridor as it is well connected, and they have also been recorded in the nearby Pond 26. Potential impact to GCN through removal of terrestrial habitat due to track dualling

Pond ID	Grid Reference	Likely Impact
26	TQ 75584 75009	<p>No direct impact to pond likely.</p> <p>Pond is approx. 65m to the north of the edge of the existing railway track. It is considered that the GCN population present in this pond is likely to use areas of suitable terrestrial habitat along the railway corridor as it is well connected, and they have also been recorded in Pond 25 opposite.</p> <p>Potential impact to GCN through removal of terrestrial habitat due to track dualling</p>
29	TQ 77718 74597	<p>No direct impact to pond.</p> <p>Pond is approx. 275m south of the edge of the existing railway track. It is considered that the GCN population present in this pond would use the available terrestrial habitat that is immediately to the pond in preference to commuting across the wider landscape as there is little connectivity between the pond and the railway corridor.</p>
30	TQ 77672 74515	<p>No direct impact to pond.</p> <p>Pond is approx. 375m south of the edge of the existing railway track. It is considered that the GCN population present in this pond would use the available terrestrial habitat that is immediately to the pond in preference to commuting across the wider landscape as there is little connectivity between the pond and the railway corridor.</p>

5.1 Rapid Risk Assessment

The Rapid Risk Assessment Tool has been used to assess the potential impacts associated with the scheme. The results of the Rapid Risk Assessment are presented in Table 3 below. The results show that there is an Amber risk and without mitigation an offence is LIKELY to occur.

This has been calculated due to the proposals involving the damage/loss of terrestrial habitat alongside the existing railway track on land between within 100m of pond 15, 16, 25 and 26, and loss of further habitat within 250m of these ponds as well as pond 23.

Mitigation to reduce these impacts will be required and has been outlined in 6 below.

Table 3 Likely effect of the proposals upon GCN without mitigation

Component	Likely effect (select one for each component; select the most harmful option if more than one is likely; lists are in order of harm, top to bottom)	Notional offence probability score
Great crested newt breeding pond(s)	No effect	0
Land within 100m of any breeding pond(s)	0.01 - 0.1 ha lost or damaged	0.3
Land 100-250m from any breeding pond(s)	0.01 - 0.1 ha lost or damaged	0.01
Land >250m from any breeding pond(s)	No effect	0
Individual great crested newts	Minor disturbance of newts	0.5
	Maximum:	0.5
Rapid risk assessment result:	AMBER: OFFENCE LIKELY	

6 Provisional Mitigation and Enhancement Measures

The ecological impact hierarchy requires that all steps are taken to avoid adverse impacts to habitats and species. Only where impacts cannot be avoided, steps should be taken to mitigate for losses within the scheme boundary. In cases where options for on-site mitigation have been exhausted, offsite compensation measures can be considered.

Due to the confirmed presence of great crested newts within 7 ponds it is recommended that suitable terrestrial habitat located within 250m of Ponds 15, 16, 23, 25, 26, 29, 30 should be retained and protected during the extent of the works where possible.

In the case that this is not possible, Natural England's view is that:

- A licence **is** needed if the consultant ecologist, on the basis of survey information and specialist knowledge of the species concerned, considers that on balance the proposed activity **is reasonably likely** to result in an offence under Regulation 41 (animals) or 45 (plants); or
- If the consultant ecologist, on the basis of survey information and specialist knowledge of the species concerned, considers that on balance the proposed activity is **reasonably unlikely** to result in an offence under regulation 41 or 45 then no licence is required. However, in these circumstances Natural England would urge that reasonable precautions be taken to avoid affecting EPS during works. If EPS are found, then work should cease until you have assessed (in consultation with a consultant ecologist) whether you can proceed without committing an offence. A licence should be applied for if offences are unavoidable and the work should not be re-started until a licence is obtained (Natural England, 2013).

As there are likely to be construction impacts (including habitat removal) to terrestrial habitat located within 30 - 250m of Ponds 15, 16, 23, 25 and 26, it is considered reasonably likely to result in an offence under Regulation 41.

Therefore, in the case that habitat(s) deemed suitable for great crested newt are unable to be retained during the extent of the works in relation to the proposed new curve and the dualling works at Cooling Street, a mitigation licence from Natural England will be required.

6.1 Natural England District Licence

Due to the scale and location of the scheme, the option of a Natural England District Level Licence has been agreed in advance with the client.

Before construction works commence, the District Licence must be obtained from Natural England as the construction works have the potential to impact upon GCN migration, resting, hibernation and foraging areas. No impacts upon breeding ponds are anticipated from the proposed works.

The District Licence application can only be made once planning permission for the scheme has been approved. However, it is recommended that the enquiry form is submitted as early as possible to determine associated costs to the project.

This licence option negates the requirement for full population surveys and further land access requirements. This licence option also negates the need for specific mitigation such as fencing and translocation.

Due to the known presence of GCN, from an animal welfare perspective and to comply with the National Planning Policy Framework (NPPF) (2019), the below options should be considered within the scheme design to provide enhancement for GCN.

6.2 Habitat Enhancement

6.2.1 Vegetation Removal

Re-planting of vegetation removed during construction should be considered to replace GCN habitat lost during construction (such as scrub and woodland). This will also minimise the loss of breeding bird and reptile habitat.

Where possible replacement planting should include locally sourced native species including hawthorn and hazel (*Corylus avellane*) with small quantities of holly (*Ilex aquifolium*), guelder rose (*Viburnum opulus*), dog rose (*Rosa canina*) and dogwood (*Cornus sanguinea*).

6.2.2 Pond Creation

Where there is scope to create new attenuation ponds, such as in the proximity of the new station, pond creation and planting of these ponds could increase the availability of GCN breeding ponds. Planting of these ponds with native aquatic species, and the banks with native shrub planting, will provide enhanced habitat value for the scheme. Ponds or swales would also support large numbers of invertebrates which in turn will further support bats and birds.

Where possible ponds or swales should be created within 250m of an existing breeding pond. Ponds less than approximately 25 square metres surface area tend to have insufficient depth and volume to support many GCN larvae, so the size of the pond should be considered carefully. Larger, deeper ponds tend to be less likely to freeze, with ponds in excess of 1m tending to maintain warmer temperatures at the bottom which are high enough for newt activity throughout the year. For new ponds a surface area between 100 and 800 square metres and depth between 1 and 2 metres deep is recommended.

7 Summary

GCN surveys have been undertaken using eDNA survey techniques to determine the presence/absence of GCN for the reopening of the Grain Branch Line for passenger rail services.

GCN have been identified as being present using eDNA analysis in Ponds 15, 16, 23, 25, 26, 29, 30.

No direct impacts to ponds are expected during the construction phase. However, construction works will occur within 250m of Ponds 15, 16, 23, 25 and 26 (which tested positive for GCN) and therefore impacts to GCN are considered likely through temporary disturbance and loss of terrestrial habitat including woodland and scrub.

Due to the scale and location of the scheme, it is recommended that a Natural England District Licence is obtained for this scheme. Before construction works commence, the District Licence must be obtained from Natural England as the construction works have the potential to impact upon GCN migration, resting, hibernation and foraging areas.

The District Licence application can only be made once planning permission for the scheme has been approved. However, it is recommended that the enquiry form is submitted as early as possible to determine associated costs to the project.

To comply with the National Planning Policy Framework (NPPF) (2019) further measures have also been outlined to enhance the Site for GCN.

8 Ecological Report Limitations

The information reported herein is based only on the interpretation of data collected during the protected species survey visits. This work pertains specifically to the identification of protected species on the proposed site. Information provided to Pell Frischmann has been accepted as being accurate and valid.

This report has been prepared by Pell Frischmann with reasonable skill, care and diligence, and taking account of the manpower and resources devoted to it by agreement with the client.

The evaluation and conclusions do not preclude the existence of other protected species, which could not reasonably have been revealed by the comprehensive desk studies, site visit and protected species surveys. Hence, this report should be used for information purposes only and should not be construed as a comprehensive characterisation of all site habitats.

In addition, this report details only the conditions on site, at the time of reporting. The dynamic nature of the natural environment will result in changes to the surrounding environment as seasons change. No responsibility is taken by Pell Frischmann to the existence of additional species identified on this site at a later date.

This report has been prepared solely for the use of Medway Council and may not be relied upon by other parties without written consent from Pell Frischmann. In addition, it must be understood that this report does not constitute legal advice.

Pell Frischmann disclaims any responsibility to the client and others in respect of any matters outside the agreed scope of the work.

9 References

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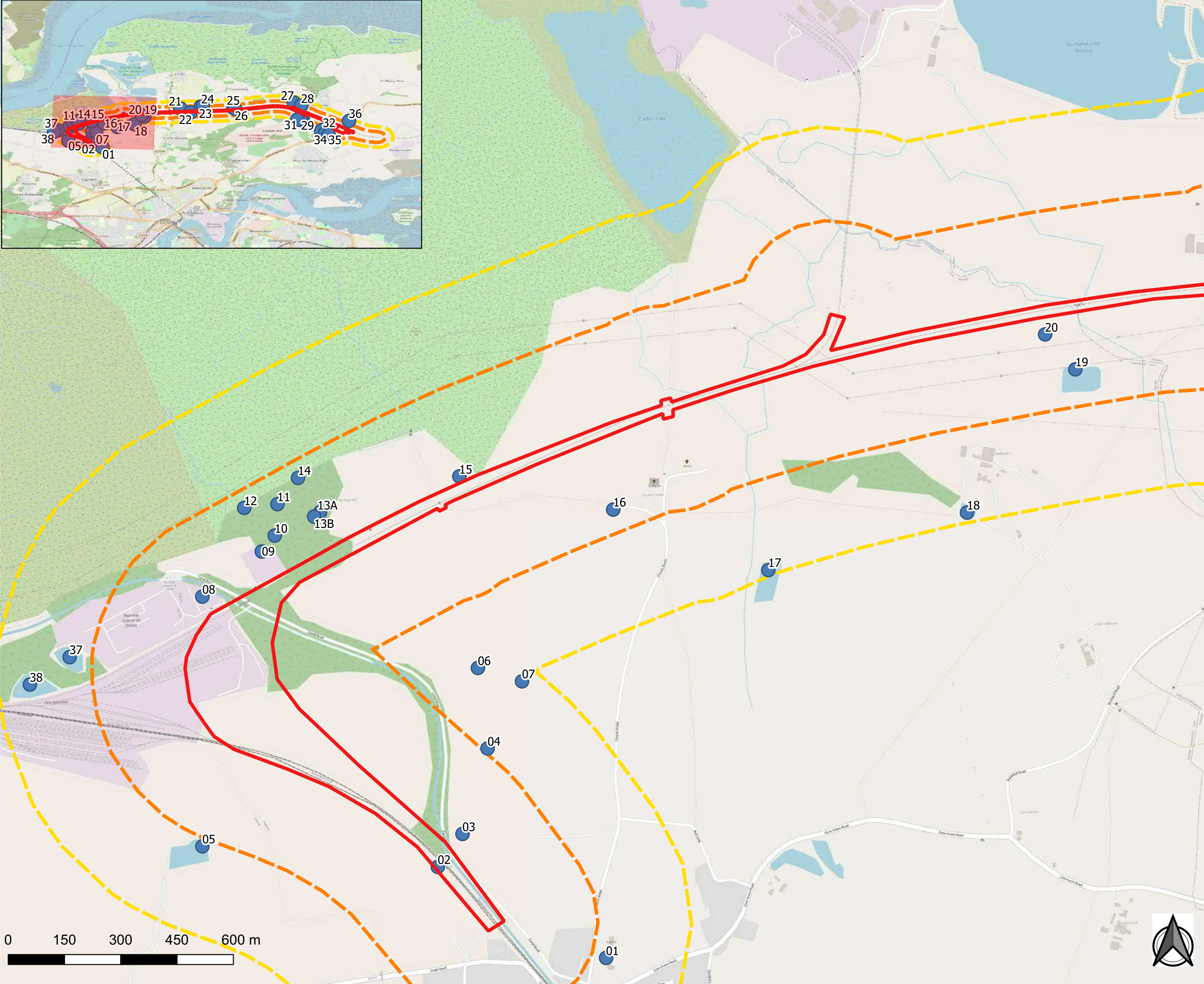
Available: http://www.searchnbn.net/index_homepage/index.jsp

Appendix A

Location of Ponds Surveyed for GCN

Legend

- Site Red Line Boundary
- Pond Locations and ID
- 250m Buffer from Site Boundary
- 500m Buffer from Site Boundary



Hoo Station GRIP 3 -
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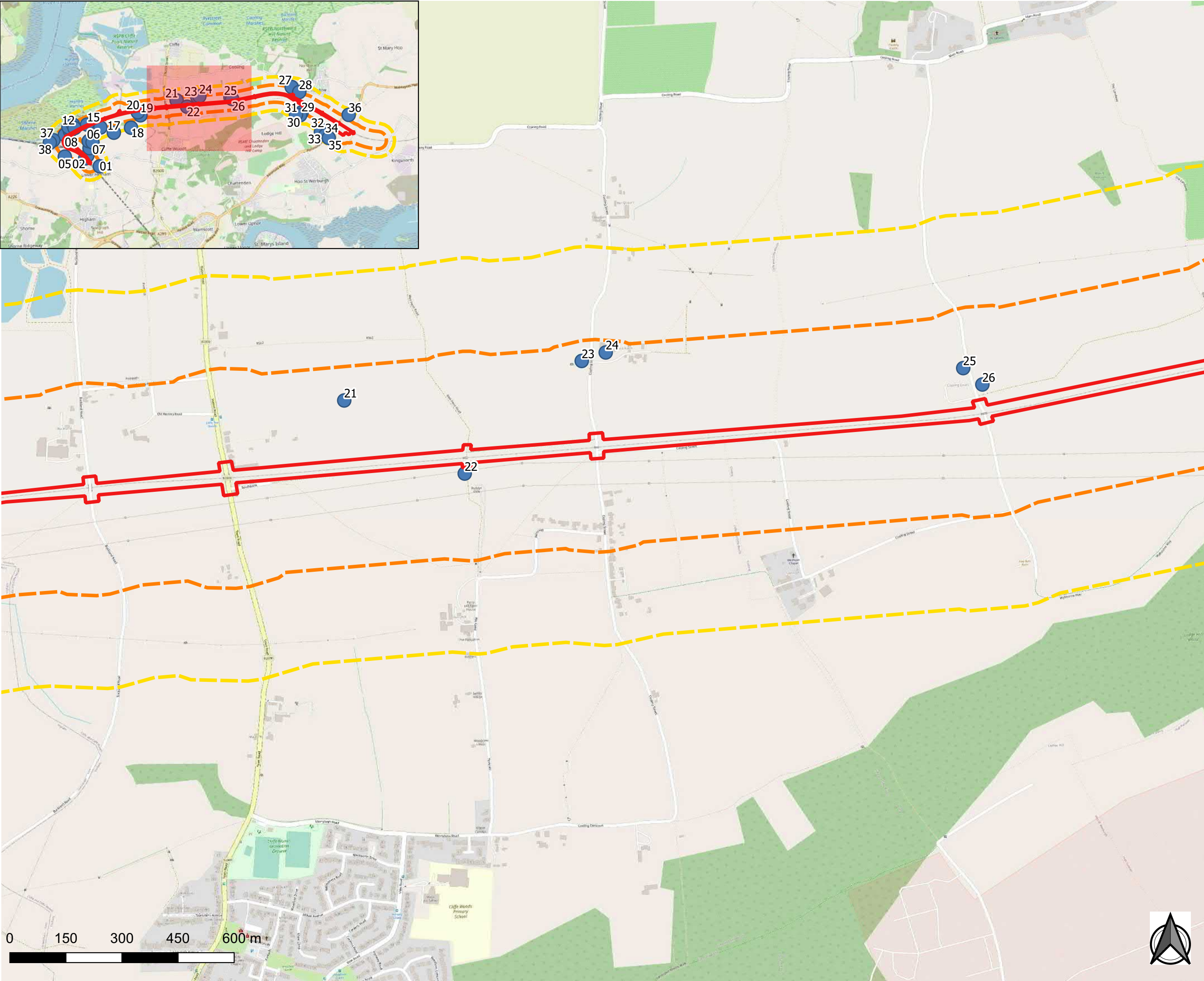
Pond Location Map -
(Sheet 1 of 3)

0 150 300 450 600 m



Legend

- Site Red Line Boundary
- Pond Locations and ID
- 250m Buffer from Site Boundary
- 500m Buffer from Site Boundary

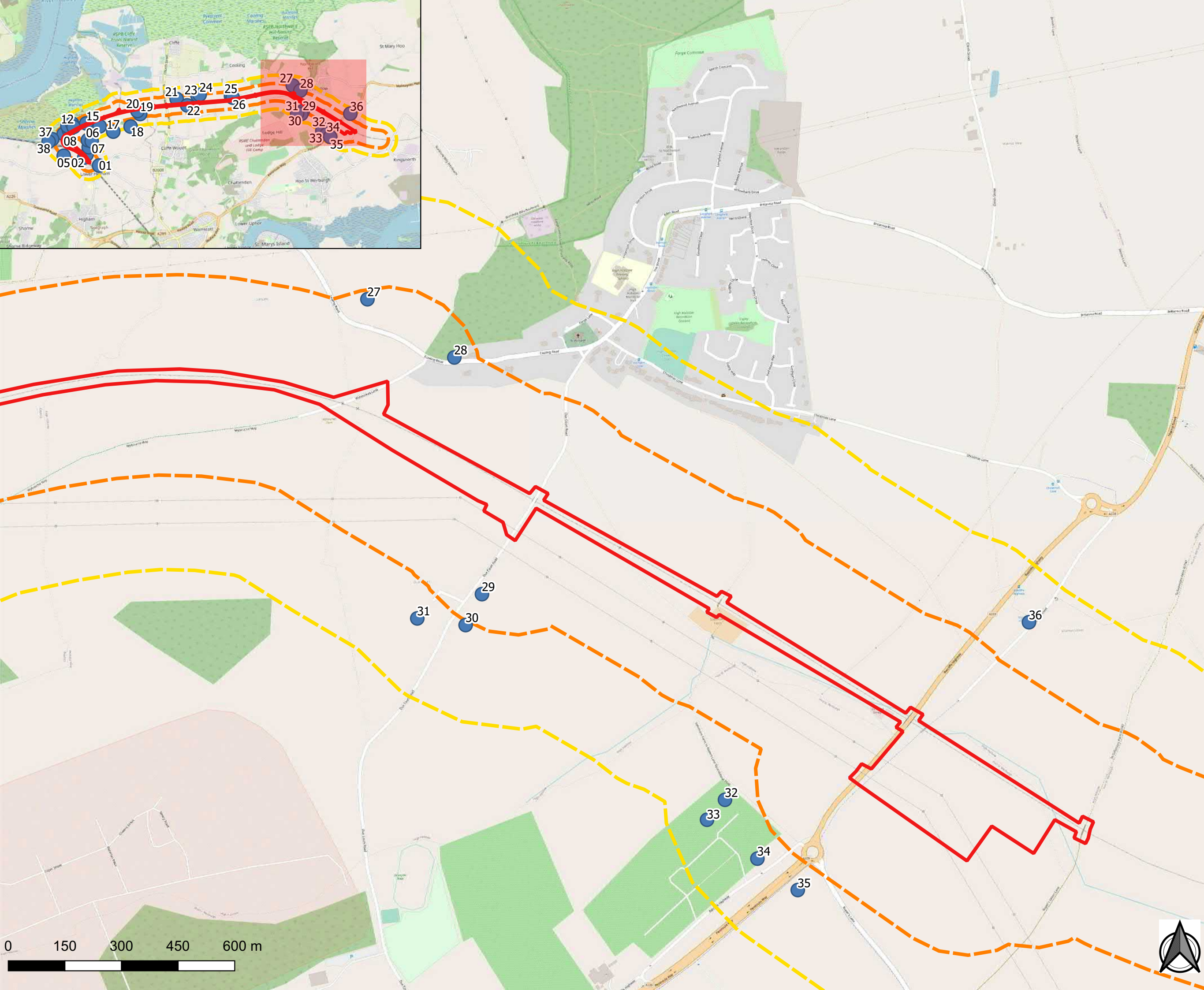


Hoo Station GRIP 3 -
Reopening of the Grain
Branch Line for
Passenger Services

Pond Location Map -
(Sheet 2 of 3)

Legend

- Site Red Line Boundary
- Pond Locations and ID
- 250m Buffer from Site Boundary
- 500m Buffer from Site Boundary



Hoo Station GRIP 3 -
Reopening of the Grain
Branch Line for
Passenger Services

Pond Location Map -
(Sheet 3 of 3)

0 150 300 450 600 m



Appendix B

Habitat Suitability Index

Hoo Station GRIP 3 Reopening of the Grain Branch Line for Passenger Services
 103223-PEL-G3-H01-REP-EVV-0007 Great Crested Newt Survey Report

Pond ID	Geographical Point Score	Area of Pond Score	Water permanence	Water Quality Score	Shade % Score	Waterfowl Presence Score	Fish Presence Score	Pond Count Score	Terrestrial Habitat Score	Macrophyte Score	Overall HSI Score
2	1	0.2	0.5	0.33	0.6	1	0.67	1	0.67	1	0.62 – average
3	1	0.9	0.9	0.33	1	1	0.67	0.9	0.67	0.3	0.71 – good
4	1	0.8	0.9	0.67	1	1	0.67	1	1	0.6	0.85 – excellent
5	1	-	0.9	0.67	1	1	0.67	1	0.01	0.6	0.55 – below average
6	1	0.8	0.9	0.67	1	1	0.67	1	1	0.6	0.85 – excellent
7	1	0.4	1	0.67	0.6	1	0.67	1	1	0.6	0.76 – average
8	1	0.9	1	0.33	1	0.67	0.67	1	0.01	0.4	0.47 – poor
9	1	1	1	0.33	0.6	0.67	0.67	1	0.67	0.4	0.69 – average
10	1	0.2	0.1	0.33	0.2	1	0.67	1	1	0.3	0.44 – poor
11	1	-	1	0.33	0.6	0.67	0.67	1	1	0.4	0.72 – good
13	1	-	1	0.33	0.6	0.67	0.67	1	1	0.4	0.72 – good
15	1	0.4	0.9	0.67	1	1	0.67	1	1	0.7	0.80 – excellent
16	1	0.9	0.5	0.67	1	1	0.67	1	1	0.9	0.84 – excellent
17	1	-	0.9	0.33	1	0.67	0.67	0.8	1	0.4	0.73 – good
18	1	0.9	0.9	0.67	1	0.67	0.67	0.7	0.67	0.4	0.73 – good
19	1	-	0.9	0.33	1	0.67	0.67	0.7	1	0.3	0.70 – average
20	1	-	0.9	0.33	1	0.67	0.67	0.7	1	0.3	0.70 – average
21	1	-	1	0.67	1	1	0.67	0.7	0.33	0.5	0.74 – good
22	1	0.8	1	0.67	1	1	0.67	0.7	0.67	0.7	0.81 – excellent

Hoo Station GRIP 3 Reopening of the Grain Branch Line for Passenger Services
 103223-PEL-G3-H01-REP-EVV-0007 Great Crested Newt Survey Report

Pond ID	Geographical Point Score	Area of Pond Score	Water permanence	Water Quality Score	Shade % Score	Waterfowl Presence Score	Fish Presence Score	Pond Count Score	Terrestrial Habitat Score	Macrophyte Score	Overall HSI Score
23	1	1	1	0.67	0.9	0.67	0.67	0.7	0.67	0.7	0.79 – good
24	1	1	0.9	0.33	0.6	0.01	0.33	0.7	0.33	0.3	0.36 – poor
25	1	0.8	1	0.67	1	0.67	0.67	0.7	0.67	1	0.80 – excellent
26	1	1	0.5	0.67	1	1	0.67	0.7	0.67	0.9	0.79 – good
28	1	0.2	0.1	0.33	1	1	0.67	0.7	0.01	0.3	0.31 – poor
29	1	0.4	1	0.67	0.8	1	0.67	0.8	0.67	0.7	0.75 – good
30	1	0.2	1	0.67	0.2	1	0.67	0.8	0.67	0.7	0.61 – average
31	1	0.4	0.5	0.33	0.2	1	0.67	0.8	0.67	0.9	0.58 – below average
32	1	-	0.9	0.67	1	1	0.67	0.8	0.33	0.3	0.71 – good
33	1	-	0.9	0.67	1	1	0.67	0.8	0.33	0.3	0.71 – good
35	1	0.8	1	0.67	1	0.67	0.67	0.7	0.33	1	0.75 - good

* the following ponds were not assessed using HSI score due to lack of access or online mapping being and there are no longer ponds in that location 1, 12, 14, 27, 34, 36 and 38.

**HSI scores for Ponds 5,11, 13, 17, 19, 20, 21, 32, and 33 used the ‘large waterbody’ calculation and omitted the ‘area of pond’ score.

Appendix C

Great Crested Newt Lab Results

Folio No: E7968
 Report No: 1
 Purchase Order: 30004660
 Client: PELL FRISCHMANN
 Contact: Claire Gilby

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: 24/06/2020
Date Reported: 02/07/2020
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
3311	Pond 35, Hoo	TQ 78555 73823	Pass	Pass	Pass	Negative	0
3313	Pond 13A, Hoo	TQ 706677 604063	Pass	Pass	Pass	Negative	0
3317	Pond 15, Hoo	TQ 71119 74225	Pass	Pass	Pass	Positive	12
3320	Pond 29	TQ 77718 74597	Pass	Pass	Pass	Positive	8
3321	Pond 11, Hoo	TQ 70684 74168	Pass	Pass	Pass	Negative	0
3322	Pond 5, Hoo	TQ 70392 73219	Pass	Pass	Pass	Negative	0
3323	Pond 2, Hoo	TQ 71031 73196	Pass	Pass	Pass	Negative	0



3324	Pond 30, Hoo	TQ 77672 74515	Pass	Pass	Pass	Positive	5
3326	Pond 23, Hoo	TQ 74511 75078	Pass	Pass	Pass	Positive	2
3328	Pond 24, Hoo	TQ 74573 75092	Pass	Pass	Pass	Negative	0
3331	Pond 9, Hoo	TQ 70587 74015	Pass	Pass	Pass	Negative	0
3332	Pond 21, Hoo	TQ 73875 74952	Pass	Pass	Pass	Negative	0
3333	Pond 13B, Hoo	TQ 70667 74063	Pass	Pass	Pass	Negative	0
3334	Pond 26, Hoo	TQ 75584 75009	Pass	Pass	Pass	Positive	7

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.

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.



Folio No: E8224
 Report No: 1
 Purchase Order: 30004691
 Client: PELL FRISCHMANN
 Contact: Claire Gilby

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: 01/07/2020
Date Reported: 10/07/2020
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
3312	HOO - Pond 19	TQ 72778 74488	Pass	Pass	Pass	Negative	0
3314	HOO - Pond 25	TQ 75527 75053	Pass	Pass	Pass	Positive	4
3315	HOO - Pond 3	TQ 71129 73272	Pass	Pass	Pass	Negative	0
3316	HOO - Pond 20	TQ 72668 74605	Pass	Pass	Pass	Negative	0
3329	HOO - Pond 17	TQ 71937 73951	Pass	Pass	Pass	Negative	0
3330	HOO - Pond 22	TQ 74186 74773	Pass	Pass	Pass	Negative	0
3339	HOO - Pond 4	TQ 71194 73503	Pass	Pass	Pass	Negative	0



3340	HOO - Pond 6	TQ 71175 73717	Pass	Pass	Pass	Negative	0
3341	HOO - Pond 7	TQ 71286 73716	Pass	Pass	Pass	Negative	0
3342	HOO - Pond 16	TQ 71527 74137	Pass	Pass	Pass	Positive	5
3345	HOO - Pond 18	TQ 72472 74127	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 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.

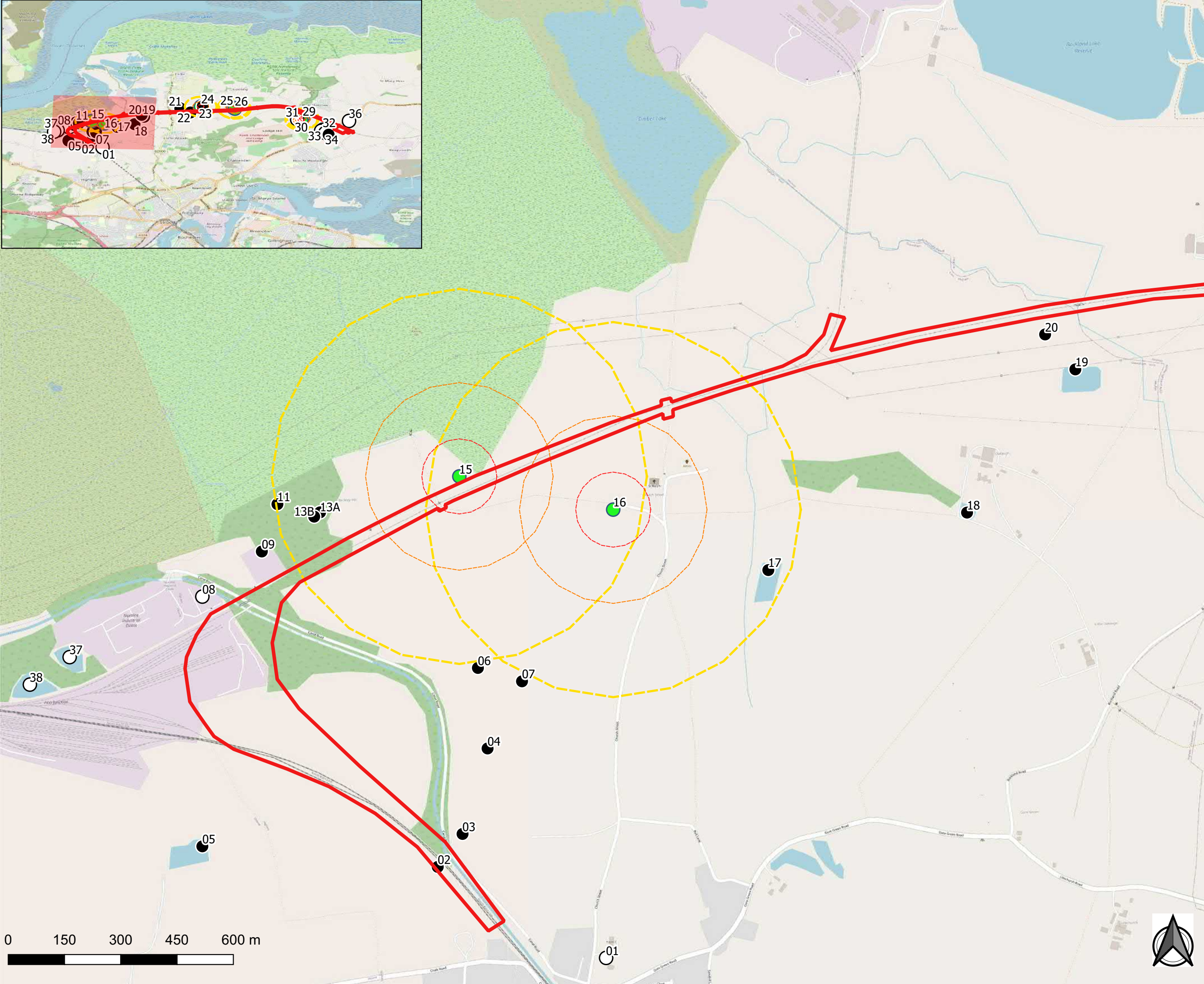


Appendix D

Great Crested Newt Constraints Maps

Legend

-  Site Red Line Boundary
-  100m Buffer of GCN Pond
-  250m Buffer of GCN Pond
-  500m Buffer of GCN Pond
-  GCN Present in Pond
-  GCN Absent in Pond
-  Pond not surveyed



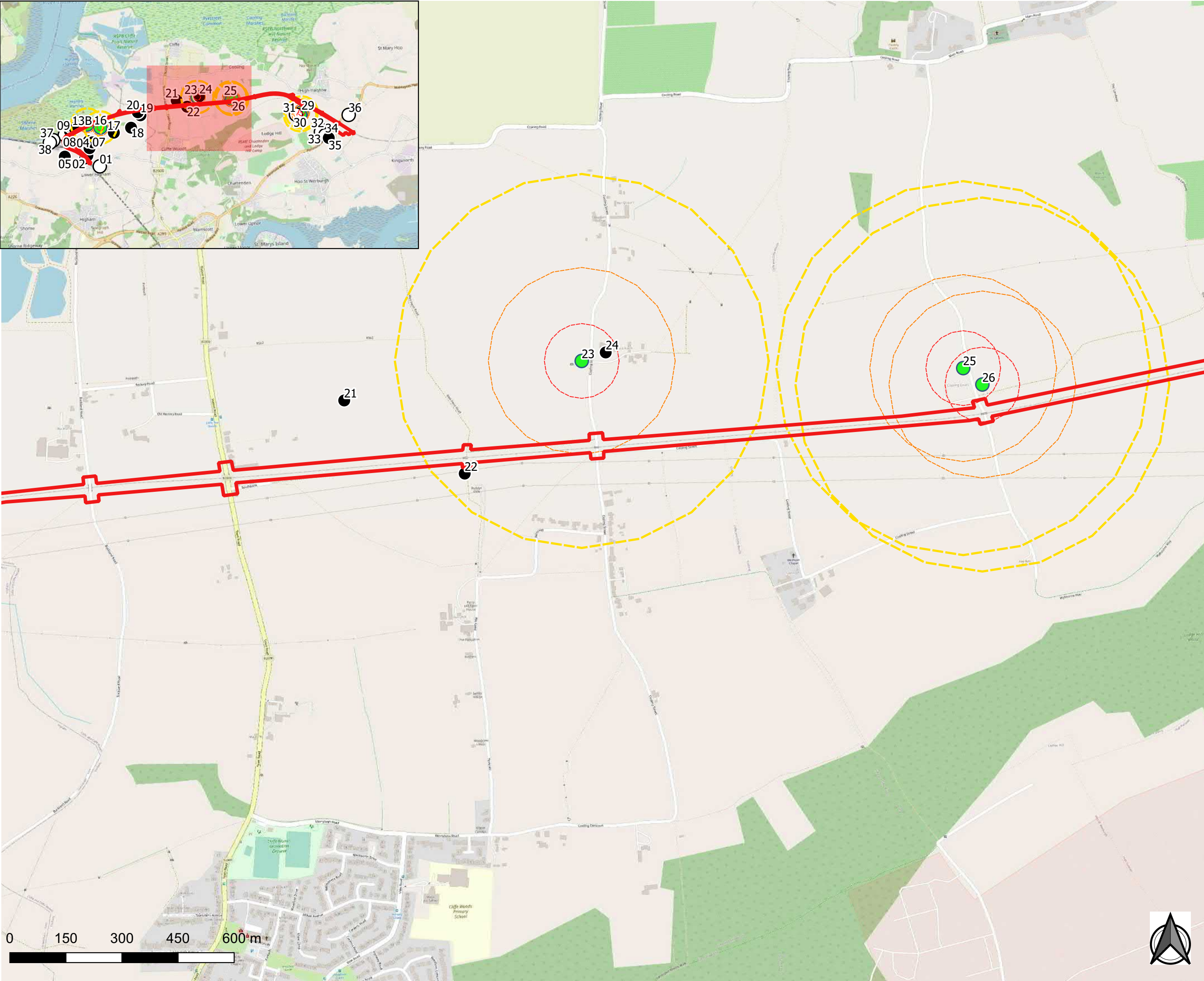
Hoo Station GRIP 3 -
Reopening of the Grain
Branch Line for
Passenger Services

GCN Constraints Map
- (Sheet 1 of 3)



Legend








- Site Red Line Boundary
- 100m Buffer of GCN Pond
- 250m Buffer of GCN Pond
- 500m Buffer of GCN Pond
- GCN Present in Pond
- GCN Absent in Pond

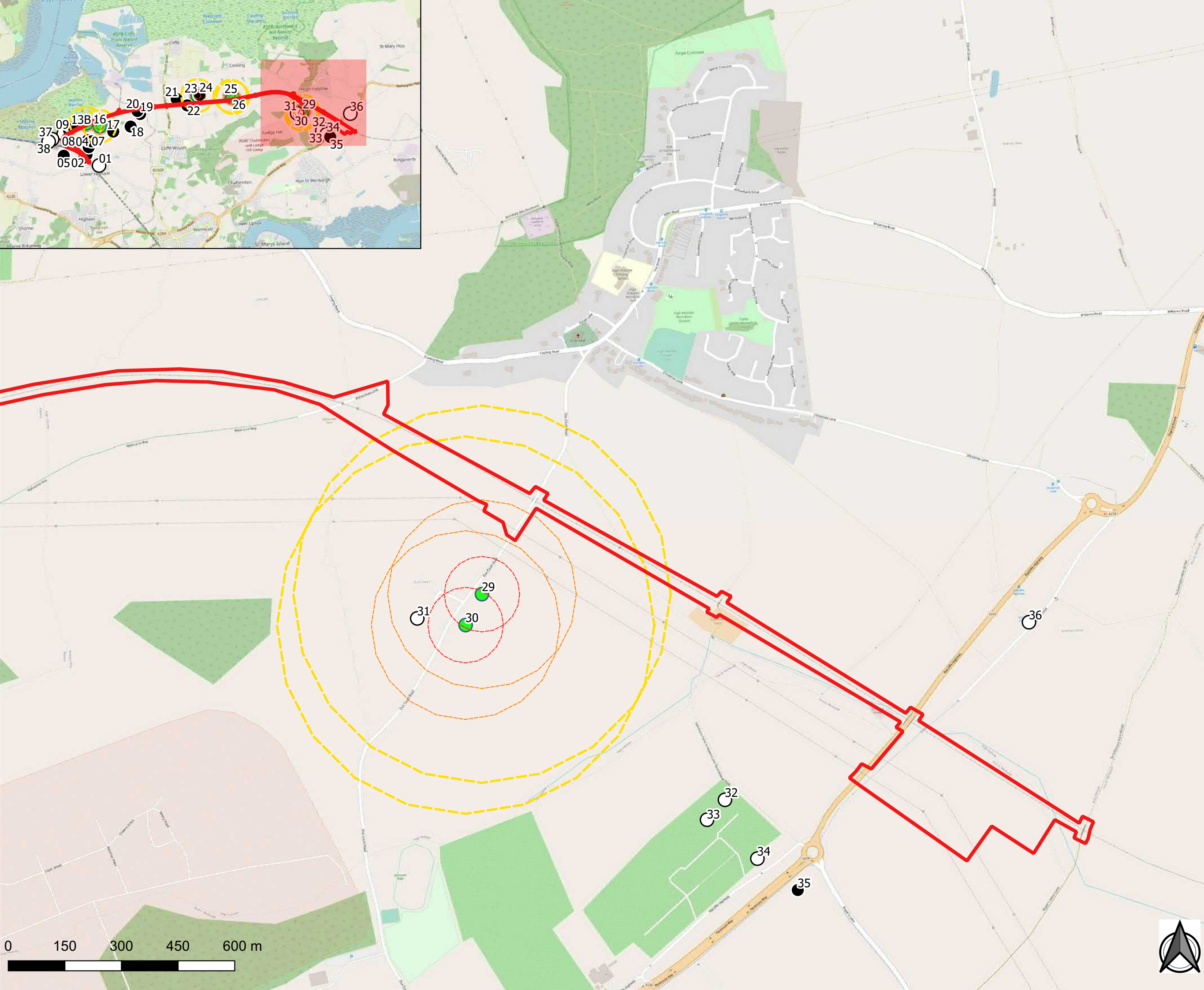


Hoo Station GRIP 3 -
Reopening of the Grain
Branch Line for
Passenger Services

Pond Location Map -
(Sheet 2 of 3)

Legend

-  Site Red Line Boundary
-  100m Buffer of GCN Pond
-  250m Buffer of GCN Pond
-  500m Buffer of GCN Pond
-  GCN Present in Pond
-  GCN Absent in Pond
-  Pond not surveyed



Hoo Station GRIP 3 -
Reopening of the Grain
Branch Line for
Passenger Services

Pond Location Map -
(Sheet 3 of 3)



Appendix E

Survey Photographs

Site Survey Photographs



Pond 8 – could not be surveyed due to H&S concerns.



Pond 15 – eDNA surveys identified GCN present within Pond 15



Pond 22 – eDNA surveyed indicate that GCN are absent



Pond 29 – eDNA surveys identified GCN present in Pond 29



Pond 33 – Could not be surveyed due to H&S concerns. Pond 32 was the same.



Pond 34 – mapping is inaccurate as this is a covered tank with no open water

Great Crested Newt Habitat Suitability and eDNA Sampling

Hoo Peninsular, Kent. July 2020

*This assessment for likely presence / absence of great crested newt (*Triturus cristatus*) has been prepared by CSA Environmental on behalf Project Centre, in relation to land at on the Hoo Peninsular (hereafter the 'Site'). It provides methods and results for a great crested newt Habitat Suitability Index (HSI) assessment and eDNA sampling for six ponds; undertaken in June 2020.*

1.0 Introduction

1.1 CSA Environmental were asked to undertake assessments for presence / likely absence of great crested newts within six ponds (R1 – R6) as identified on the Pond Survey Plans provided in Appendix A.

2.0 Legislation

2.1 Great crested newts are legally protected as European Protected Species (EPS) under Regulation 43 of the Conservation of Habitats and Species Regulations 2017. These Regulations make it an offence to:

- Deliberately capture, injure, kill or capture a great crested newt
- Deliberately disturb great crested newts, impairing their ability to survive, breed, reproduce or rear/nurture their young
- Damage or destroy a breeding site or resting place used by a great crested newt

2.2 Great crested newts are also fully protected under the Wildlife & Countryside Act 1981, making it an offence to:

- Intentionally or recklessly disturb a great crested newt while it is occupying a structure or place of shelter or protection
- Intentionally or recklessly obstruct access to any structure or place of shelter or protection

2.3 Disturbance of great crested newts is covered by both the 2017 Regulations and the 1981 Act. Disturbance that impairs survival or successful reproduction would be covered by the Regulations, while less significant acts of disturbance may only be covered by the Act.

2.4 It is important to note that great crested newts and their habitats (such as breeding ponds) are protected throughout the year, regardless of whether or not newts are present at the time. Great crested newts are also listed as a species of principal importance for the conservation of biodiversity in England, under Section 41 (S41) of the Natural Environment and Rural Communities (NERC) Act 2006. The S41 species list is used to guide decision-makers, including planning authorities, in implementing their duty

under Section 40 of the NERC Act to have regard to the conservation of biodiversity in England, when carrying out their normal functions.

Licensing

- 2.5 Where development is proposed that would result in an offence under the Habitats and Species Regulations, a statutory derogation licence may be granted by Natural England to permit an act that would otherwise be unlawful. To obtain an EPS licence for development, it must be demonstrated that the purpose of the act to be licensed is for:

“preserving public health or public safety or other imperative reasons of overriding public interest including those of social or economic nature and beneficial consequences of primary importance for the environment” (Regulation 55(2)(e))

- 2.6 In addition, Natural England will not grant an EPS licence unless they are satisfied that:

“There is no satisfactory alternative” (Regulation 55(9)(a))

“The action authorised will not be detrimental to the maintenance of the population of the species concerned at a favourable conservation status in their natural range” (Regulation 55(9)(b))

3.0 Methods

- 3.1 Great crested newt survey work was undertaken on 25 June 2020 by Clare Caudwell MCIEEM CEcol (Natural England Survey Class Licence CL08 Registration Number 2015-16920-CLS-CLS) and Aaron White GradCIEEM (Natural England Survey Class Licence CL08 Registration Number 2016-26357-CLS-CLS). Survey conditions were hot and dry.

- 3.2 Access for survey as granted to five of the six ponds identified (R1 – R5); it was not possible to access R6. R1 to R5 were subject to a Habitat Suitability Index (HSI) assessment to determine their suitability to support breeding great crested newt. A standard approach to HSI was used, as set out by Oldham *et al* (2000).

- 3.3 One of the five ponds (R3) subject to HSI was dry at the time of survey; and such eDNA sampling was not possible. eDNA sampling was undertaken for the remaining four ponds (R1, R2, R4, R5) to determine the presence/ likely absence of great crested newts. This method has been shown to be a highly effective in detecting the presence of great crested newts (Biggs *et al.* 2014). Water samples were collected from ponds following the recommended procedure. Appropriate biosecurity measures were taken to avoid cross contamination of great crested newt eDNA. Subsequently the samples were sent to ADAS for DNA analysis.

Limitations

- 3.4 The accepted period for eDNA sampling is 15 April to 30 June; with eDNA sampling being effective at detecting great crested newt presence within the last 21 days prior to sampling taking place. eDNA sampling was undertaken on 25 June, towards the end of the recommended period and following a period of warm and dry weather with little precipitation. As such, where negative results were returned, these only indicate that

great crested newt are unlikely to have been present within the preceding 21 day period, and could potentially have made use of the ponds earlier in the spring.

4.0 Results

HSI Assessment

4.1 HSI assessment was completed for ponds R1 – R5; findings are as follows with regards to predicted breeding suitability for great crested newts (full results are provided in Appendix B):

- **R1 (MOD):** A large pond set in semi-improved damp grassland, used as a fishing lake. On-line with stream and fairly deep with steep sides. **Poor suitability (HSI Score = 0.48)**
- **R2 (MOD):** Field pond likely to be spring fed as located on top of a ridge. Low water levels at the time of survey, with *Typha* sp. dominating and self-set *Salix* sp. in the centre. **Average suitability (HSI Score = 0.66)**
- **R3 (Abby Homes):** Large attenuation basin created for new development adjacent; not holding any water at the time of survey (and only likely to hold water during storm events), no aquatic or marginal vegetation. HSI calculation as 'below average suitability' (HSI Score = 0.58), **however this pond is not considered suitable for great crested newt due to lack of permanent water.**
- **R4 (MDC):** Large field pond created (lined) within rough grassland area adjacent to golf course. Possibly created as great crested newt mitigation / wildlife enhancement ponds. Well established with good marginal habitat. **Excellent suitability (HSI Score = 0.88)**
- **R5 (MDC):** Large field pond created (lined) within rough grassland area adjacent to golf course. Possibly created as great crested newt mitigation / wildlife enhancement ponds. Well established with good marginal habitat. **Poor suitability (HSI Score = 0.89)**

eDNA Sampling

4.2 All samples were within degradation control limits. eDNA results for the ponds samples are as follows (full results provided in Appendix C):

- R1 (MOD): Negative (0/12 samples)
- R2 (MOD): **Positive** (12/12 samples)
- R4 (MDC): **Positive** (3/12 samples)
- R5 (MDC): **Positive** (5/12 samples)

4.3 In line with guidance set out within the Great Crested Newt Mitigation Guidelines (English Nature, 2001), proposed development with a 500m radius of ponds where great crested

newt have been confirmed to be present will need to clearly assess potential impacts to this species, both from any proposed aquatic or terrestrial habitat loss and fragmentation of suitable habitats between ponds used by this species (which forms metapopulations; requiring dispersal between breeding ponds to maintain gene flow).

4.4 Should impacts to great crested newt habitat be identified from the proposals, then a suitable mitigation approach will need to be agreed as part of any planning consent and a European Protected Species Licence (EPSL) will be required to permit development to commence. Two EPS licencing routes are currently available in Kent, as follows:

- 'Traditional' EPSL - where mitigation (including impact avoidance and provision of compensatory habitat) is delivered by the applicant, either within the Application Site and/or on off-Site land (using Natural England EPSL Policy 2). Full great crested newt population data would be required to support such an application; which would involve further survey of ponds to be affected (6 x pond surveys required using 3 x survey methods; surveys possible mid-March to mid-June with at least half of the surveys between mid-April and mid-May). Compensation for any loss of aquatic or terrestrial habitat would be required; with an emphasis on the delivery of net gain in terms of habitat provision. The mitigation approach would need to be agreed as part of any planning consent before an EPSL application can be made post-planning consent.
- 'District Level Licencing' - where the applicant would enter into a legal agreement with Natural England to provide financial contributions to strategic great crested newt mitigation provision within Kent. Detailed survey information is not required to support a DLL application; but any existing survey data must be taken into account. A DLL agreement must be secured prior to planning; with a counter signed legal agreement being submitted at the planning stage and secured by way of Condition / Section 106. Once planning consent has been secured; the agreement can be finalised and a DLL licenced issued; financial contributions must then be made within 30 days.

5.0 References

Biggs J, Ewald N, Valentini A, Gaboriaud C, Griffiths RA, Foster J, Wilkinson J, Arnett A, Williams P and Dunn F. 2014. *Analytical and methodological development for improved surveillance of the Great Crested Newt*. Appendix 5. Technical advice note for field and laboratory sampling of great crested newt (*Triturus cristatus*) environmental DNA. Freshwater Habitats Trust, Oxford.

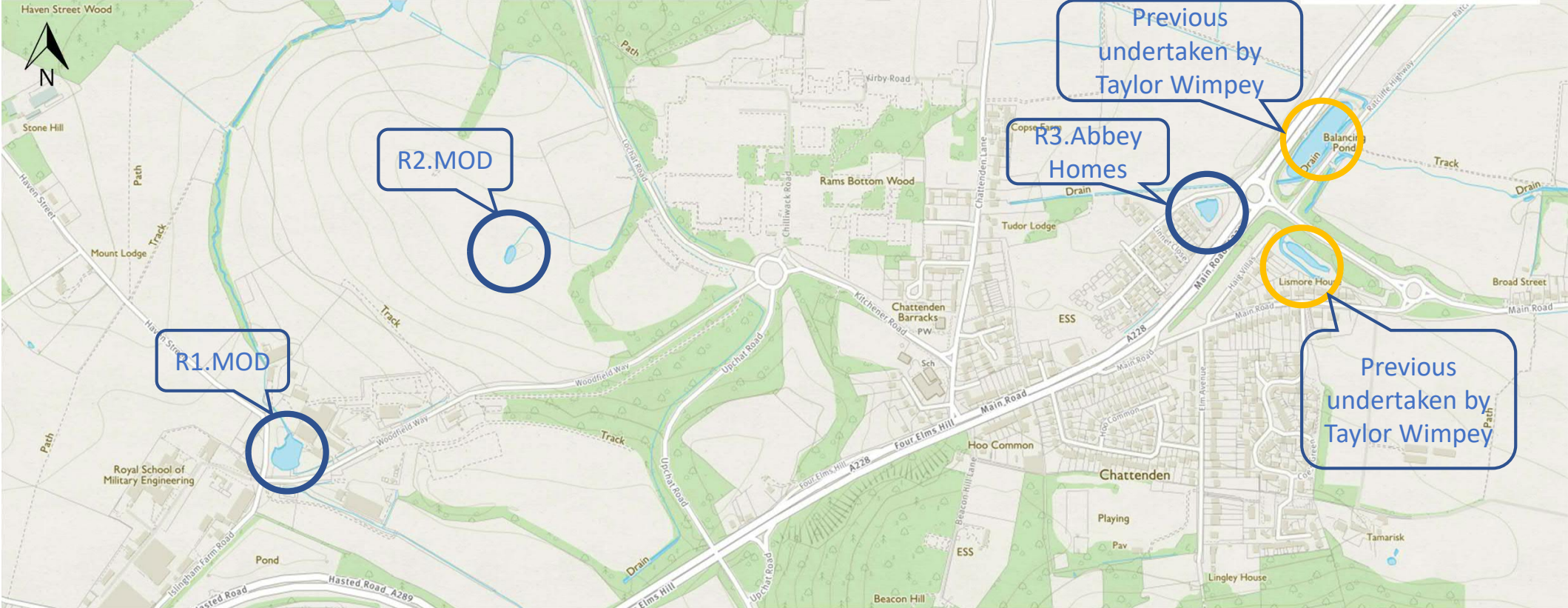
English Nature, 2001. *Great Crested Newt Mitigation Guidelines*. English Nature, Peterborough.

Oldham R.S., Keeble J., Swan M.J.S. & Jeffcote M., 2000. Evaluating the suitability of habitat for the Great Crested Newt (*Triturus cristatus*). *Herpetological Journal* 10(4), 143-155

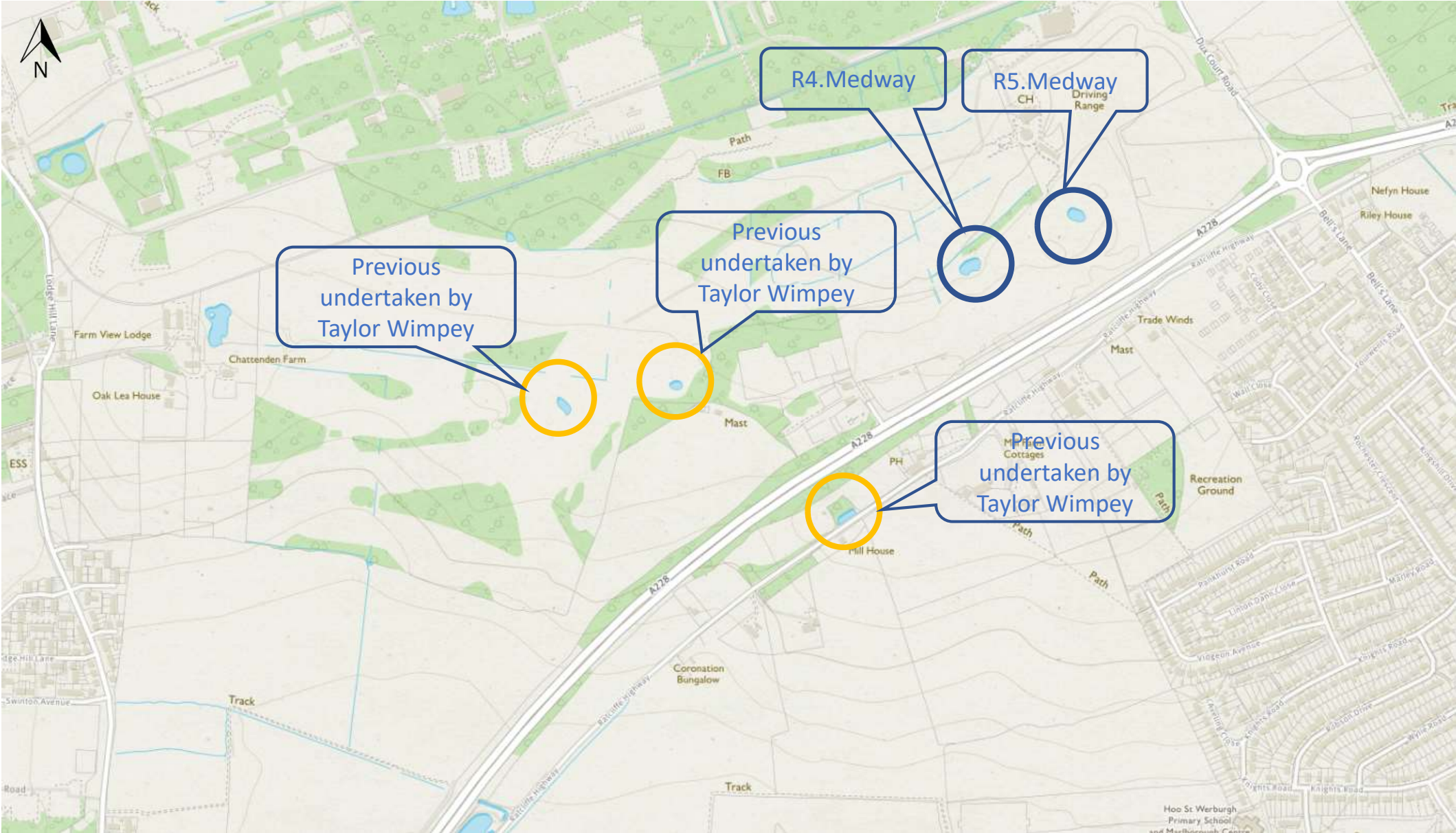
Appendix A

Pond Locations Plans

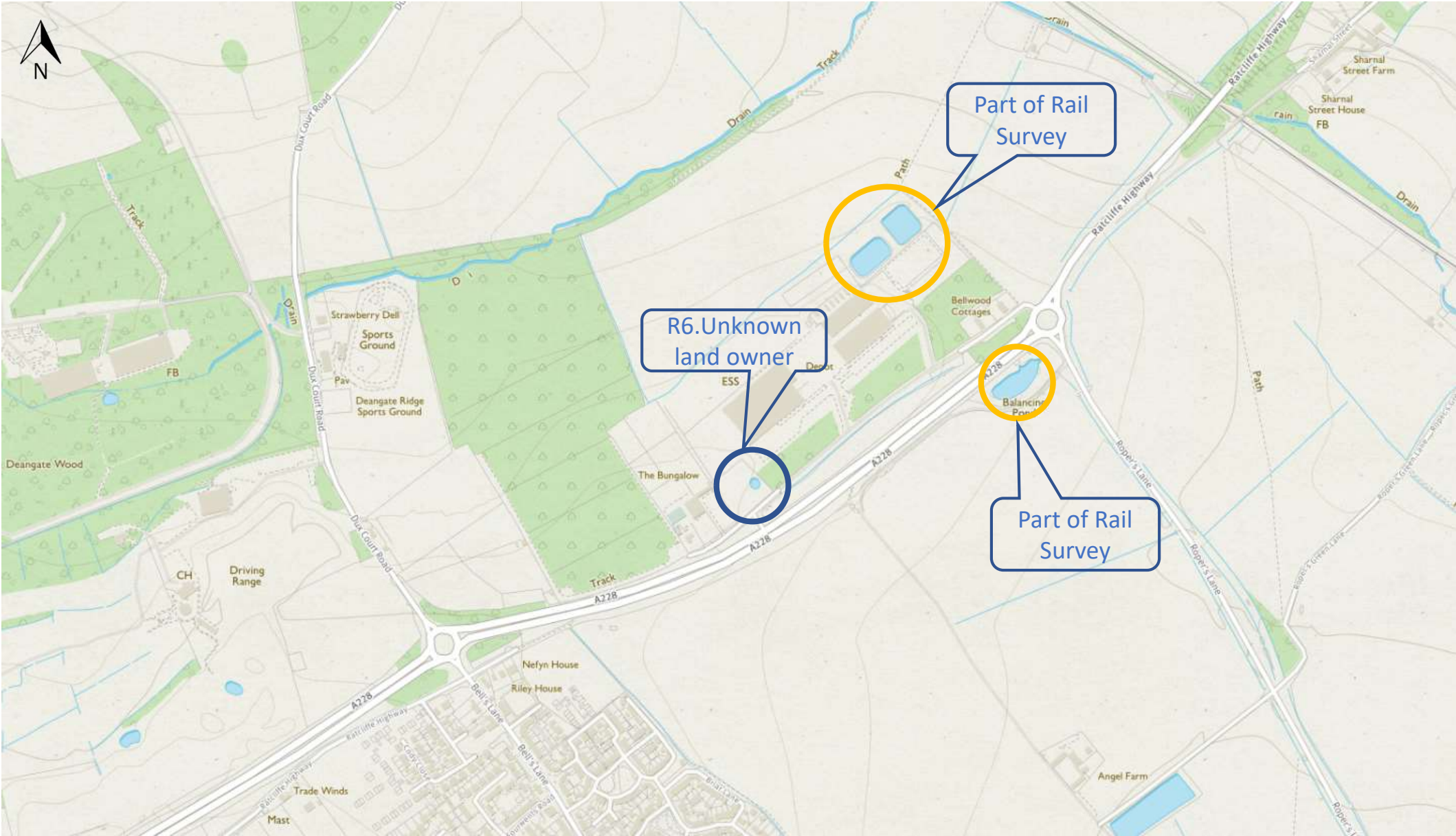
Pond Locations



Pond Locations



Pond Locations



Appendix B

Habitat Suitability Index Results

HSI Pond Assessment

Site Name:	Hoo Peninsular (HIF)
Job Number:	5069
Date:	25.06.2020.
Surveyor:	CC & AW

Notes:

Habitat Suitability Factors:	Pond Number:	R1	R2	R3	R4	R5					
Map location <i>A (optimal), B (marginal) or C (unsuitable)</i>		A	A	A	A	A					
Pond area in m² <i>Estimate</i>		1900	350	825	530	395					
Permanence / Dessication <i>Estimate or ask landowner (never/rarely/sometimes/annually)</i>		Never	Anually	Anually	Never	Rarely					
Water quality <i>(bad/poor/moderate/good)</i>		Moderate	Moderate	-	Good	Good					
Percentage perimeter shade to at least 1m from shore <i>Estimate</i>		10%	5%	0%	0%	0%					
Waterfowl impact (excluding moorhen) <i>(major/minor/absent)</i>		Minor	Absent	Absent	Absent	Absent					
Fish presence <i>(major/possible/minor/absent)</i>		Major	Absent	Absent	Absent	Absent					
Number of ponds within 1km not separated by barriers		2	2	8	10	10					
Terrestrial habitat <i>(none/poor/moderate/good)</i>		Good	Moderate	Moderate	Good	Good					
Percentage of pond surface occupied by aquatic vegetation (March – May)		30%	80%	-	10%	10%					
HSI SCORE:		0.48	0.66	0.58*	0.88	0.89					
<i>Poor (<0.5), Below average (0.5-0.59), Average (0.6-0.69), Good (0.7-0.79), Excellent (>0.8) *R3 = Attenuation Basin not liekly to hold water permanently</i>											

Appendix C

Great Crested Newt eDNA Results

Client: Clare Caudwell
CSA Environmental



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Spring Lodge
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Helsby
WA6 0AR

Tel: 01159 516747
Email: Helen.Rees@adas.co.uk

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Sample ID: 2020-1698 Condition on Receipt: Low Sediment Volume: Passed
Client Identifier: Pond 1 MOD, 5069 Description: pond water samples in preservative
Date of Receipt: 01/07/2020 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	08/07/2020
Degradation Control [§]	Within Limits	Real Time PCR	08/07/2020
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	08/07/2020
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [#]	4 of 4	Real Time PCR	As above for GCN

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

Signed:  Signed: 

Position: Director: Biotechnology Position: MD: Biotechnology

Date of preparation: 14/07/2020 Date of issue: 14/07/2020

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

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

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

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

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

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Sample ID: 2020-1699 Condition on Receipt: White Precipitate Volume: Passed
Client Identifier: Pond 4 (mdc) 5069 Description: pond water samples in preservative
Date of Receipt: 01/07/2020 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	13/07/2020
Degradation Control [§]	Within Limits	Real Time PCR	13/07/2020
Great Crested Newt*	3 of 12 (GCN positive)	Real Time PCR	13/07/2020
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [#]	4 of 4	Real Time PCR	As above for GCN

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

Signed:  Signed: 

Position: Director: Biotechnology Position: MD: Biotechnology

Date of preparation: 14/07/2020 Date of issue: 14/07/2020

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

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

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

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

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

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Sample ID: 2020-1701 Condition on Receipt: Low Sediment Volume: Passed
Client Identifier: p5 (mdc) 5069 Description: pond water samples in preservative
Date of Receipt: 01/07/2020 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	09/07/2020
Degradation Control [§]	Within Limits	Real Time PCR	09/07/2020
Great Crested Newt*	5 of 12 (GCN positive)	Real Time PCR	09/07/2020
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [#]	4 of 4	Real Time PCR	As above for GCN

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

Signed:  Signed: 

Position: Director: Biotechnology Position: MD: Biotechnology

Date of preparation: 14/07/2020 Date of issue: 14/07/2020

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

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

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

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

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

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Sample ID: 2020-1702 Condition on Receipt: Low Sediment Volume: Passed
Client Identifier: Pond 2 MOD 5069 Description: pond water samples in preservative
Date of Receipt: 01/07/2020 Material Tested: eDNA from pond water samples

Determinant	Result	Method	Date of Analysis
Inhibition Control [†]	2 of 2	Real Time PCR	09/07/2020
Degradation Control [§]	Within Limits	Real Time PCR	09/07/2020
Great Crested Newt*	12 of 12 (GCN positive)	Real Time PCR	09/07/2020
Negative PCR Control (Nuclease Free Water)	0 of 4	Real Time PCR	As above for GCN
Positive PCR Control (GCN DNA 10 ⁻⁴ ng/μL) [#]	4 of 4	Real Time PCR	As above for GCN

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

Signed:  Signed: 

Position: Director: Biotechnology Position: MD: Biotechnology

Date of preparation: 14/07/2020 Date of issue: 14/07/2020

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

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

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

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

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

Appendix 1: Interpretation of results

Sample Condition

Upon sample receipt we score your samples according to quality: good, low sediment, medium sediment, high sediment, white precipitate, and presence of algae.

There are three reasons as to why sediment should be avoided:

1. It is possible for DNA to persist within the sediment for longer than it would if it was floating in the water which could lead to a false positive result i.e. in this case GCN not recently present but present a long time ago
2. In some cases sediment can cause inhibition of the PCR analysis used to detect GCN eDNA within samples which could lead to an indeterminate result.
3. In some cases sediment can interfere with the DNA extraction procedure resulting in poor recovery of the eDNA which in turn can lead to an indeterminate result.

Algae can make the DNA extraction more difficult to perform so if it can be avoided then this is helpful.

Sometimes samples contain a white precipitate which we have found makes the recovery of eDNA very difficult. This precipitate can be present in such high amounts that it interferes with the eDNA extraction process meaning that we cannot recover the degradation control (nor most likely the eDNA itself) at sufficient levels for the control to be within the acceptable limits for the assay, therefore we have to classify these type of samples as indeterminate.

What do my results mean?

A positive result means that great crested newts are present in the water or have been present in the water in the recent past (eDNA degrades over around 7-21 days).

A negative result means that DNA from the great crested newt has not been detected in your sample.

On occasion an inconclusive result will be issued. This occurs where the DNA from the great crested newt has not been detected but the controls have indicated that either: the sample has been degraded and/or the eDNA was not fully extracted (poor recovery); or the PCR inhibited in some way. This may be due to the water chemistry or may be due to the presence of high levels of sediment in samples which can interfere with the DNA extraction process. A re-test could be performed but a fresh sample would need to be obtained. We have successfully performed re-tests on samples which have had high sediment content on the first collection and low sediment content (through improved sample collection) on the re-test. If water chemistry was the cause of the indeterminate then a re-test would most likely also return an inconclusive result.

The results will be recorded as indeterminate if the GCN result is negative and the degradation result is recorded as:

1. evidence of decay - meaning that the degradation control was outside of accepted limits
2. evidence of degradation or residual inhibition - meaning that the degradation control was outside of accepted limits but that this could have been due to inhibitors not being removed sufficiently by the dilution of inhibited samples (according to the technical advice note)