Norwich Western Link

Environmental Statement Chapter 10: Biodiversity Appendix 10.23: Great Crested Newt eDNA Survey Report 2021

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Contents

1 Introduction

1.1.1 WSP UK Ltd was commissioned by Norfolk County Council to complete GCN surveys, with the following objectives:

- Complete a Habitat Suitability Index (HSI) assessment of water bodies within the Scheme boundary and within 500m of the Scheme boundary to assess their suitability as aquatic habitat for great crested newts.
- Complete a GCN eDNA survey to determine the presence or likely absence of this species from water bodies within the Scheme boundary and within 500m of the Scheme boundary.
- Present the findings of the survey in a baseline report.

1.1.2 The survey findings will be used to inform the impact assessment and proposed mitigation for GCN and other amphibian species present across the Scheme. Details of the impact assessment and mitigation will be included within the Biodiversity Chapter of the Environmental Statement for the Scheme.

1.1.3 We have included a summary of key information shown in this document in an accessible format. However, some users may not be able to access all technical details. If you require this document in a more accessible format please contact norwichwesternlink@norfolk.gov.uk

Norfolk County Council

NORWICH WESTERN LINK ROAD

Great Crested Newt eDNA Survey Report

70061370-09-07
JUNE 2021

PUBLIC

Norfolk County Council

NORWICH WESTERN LINK ROAD

Great Crested Newt eDNA Survey Report

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Norfolk County Council

NORWICH WESTERN LINK ROAD

Great Crested Newt eDNA Survey Report

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APPENDICES

1. INTRODUCTION

1.1. PROJECT BACKGROUND

1.1.1. The Norwich Western Link Road (NWL) is a highway scheme linking the A1270 Broadland Northway from its junction with the A1067 Fakenham Road to the A47 trunk road near Honingham.

1.1.2. The NWL, hereafter referred to as the Scheme, will comprise the following listed below.

- Dualling the A1067 Fakenham Road westwards from its existing junction with the A1270 to a new roundabout located approximately 400m to the north west.
- Construction of a new roundabout.
- Constructing a dual carriageway link from the new roundabout to a new junction with the A47 near Honingham.

1.1.3. As part of a separate planned scheme, Highways England proposes to realign and dual the A47 from the existing roundabout at Easton to join the existing dual carriageway section at North Tuddenham. If that scheme proceeds, it is expected that Highways England will construct the Honingham junction and the Norwich Western Link will connect to the north-eastern side of that junction.

1.1.4. The Scheme will cross the River Wensum and its floodplain by means of a viaduct. The Scheme will also cross four minor roads by means of overpass or underpass bridges. The Scheme will include ancillary works such as provision for non-motorised users, necessary realignment of the local road network and the provision of environmental mitigation measures.

1.2. ECOLOGICAL BACKGROUND

1.2.1. A Phase 1 Habitat Survey (WSP UK Ltd., 2020), undertaken in 2020, identified suitable aquatic and terrestrial habitat which could support Great Crested Newt *Triturus cristatus* (GCN). Habitats included numerous water bodies and terrestrial habitat such as tussocky grassland, woodland, scrub, wetland, field margins and other boundary features such as ditches and hedgerows. It was therefore recommended that a GCN environmental DNA (eDNA) survey be undertaken to establish a sufficient baseline to inform impact assessment.

1.3. BRIEF AND OBJECTIVES

1.3.1. WSP UK Ltd was commissioned by Norfolk County Council to complete GCN surveys, with the following objectives:

- Complete a Habitat Suitability Index (HSI) assessment of water bodies within the Scheme boundary and within 500m of the Scheme boundary to assess their suitability as aquatic habitat for great crested newts.
- Complete a GCN eDNA survey to determine the presence or likely absence of this species from water bodies within the Scheme boundary and within 500m of the Scheme boundary.
- Present the findings of the survey in a baseline report.

1.3.2. The survey findings will be used to inform the impact assessment and proposed mitigation for GCN and other amphibian species present across the Scheme. Details of the impact assessment and mitigation will be included within the Biodiversity Chapter of the Environmental Statement for the Scheme.

1.4. STUDY AND SURVEY AREA

1.4.1. An ecological Desk Study was completed in March 2020 to include recent data relevant to the Route. The Desk Study Area for this was defined as a 2km radius of the Scheme boundary (drawing 70061370-09-07-0001, Appendix A).

1.4.2. The Survey Area in relation to GCN comprised a 500m buffer of the Scheme. All suitable water bodies, where access was permitted, identified as having potential to support GCN populations were surveyed. The Scheme and Survey Area are shown on drawing 70061370-09-07-0001, Appendix A).

1.4.3. This report will be updated with the results of further great crested newt surveys to be undertaken in 2021 to complete the baseline. This will include further population size class estimate surveys on waterbodies which returned positive results for great crested newt eDNA, as well as further eDNA surveys of ponds which could not be surveyed in 2020 due to access or being dry, or where the result was classed as inconclusive/indeterminate.

2. RELEVANT LEGISLATION

2.1. LEGAL COMPLIANCE

2.1.1. GCN are afforded a high level of protection under the Conservation of Habitats and Species Regulations 2017 (the 'Habitats Regulations'), the legislation means that it is an offence to;

- deliberately capture, injure or kill a wild great crested newt;
- deliberately disturb wild great crested newts; '*disturbance of animals includes in particular any disturbance which is likely:*
  - *(a) to impair their ability —*
    - *(i) to survive, to breed or reproduce, or to rear or nurture their young; or*
    - *(ii) in the case of animals of a hibernating or migratory species, to hibernate or migrate; or*
  - *(b) to affect significantly the local distribution or abundance of the species to which they belong.'*
- damage or destroy a breeding Site or resting place used by this species.

2.1.2. Protection is also afforded under the Wildlife and Countryside Act 1981 (as amended) with respect to disturbance of animals when using places of shelter, and obstruction of access to places of shelter.

2.1.3. Due to the high level of protection afforded to GCN and their habitat, mitigation for this species is governed by a strict licensing procedure administered by Natural England (normally, planning permission must be obtained before a licence can be sought. However, works which do not require planning permission must still adhere to licensing requirements).

2.1.4. Licencing is subject to three tests, as defined under the Habitats Regulations, these must also be applied by a planning authority before granting permission for activities affecting GCN. For permission to be granted the following criteria must be satisfied;

- the proposal is necessary '*to preserve public health or public safety or other imperative reasons of overriding public interest including those of a social or economic nature and beneficial consequences of primary importance for the environment*';
- '*there is no satisfactory alternative*'; and
- the proposals '*will not be detrimental to the maintenance of the population of the species concerned at a favourable conservation status in their natural range*'.

2.1.5. GCN are also listed as a Species of Principal Importance (SPI) for the Conservation of Biodiversity in England in accordance with Section 41 of the Natural Environment and Rural Communities (NERC) Act 2006. Under Section 40 of the NERC Act (2006) public bodies (including local planning authorities) have a duty to have regard for the conservation of SPI when carrying out their functions, including determining planning applications.

3. METHODS

3.1. OVERVIEW

3.1.1. In total, 49 water bodies were identified. Of these, 39 were visited as part of an HSI assessment and 24 subject to an eDNA survey.

3.1.2. Each water body identified, access permitting, was subject to an HSI assessment. Water bodies that were found to be suitable for GCN were then subject to water sampling for eDNA testing. The surveys took place within in the eDNA testing season on the 15th, 19th and 20th May and 30th June 2020. Only one water body, 23, was subject to surveys outside the GCN eDNA testing season on 14th July 2020.

3.2. DESK STUDY

3.2.1. An ecological desk study was completed in March 2020 to include recent data relevant to the Scheme. Records of any notable or legally protected species, including GCN and other amphibians, from within the Study Area were requested from Norfolk Biodiversity Information Service (NBIS). Freely downloadable datasets (available from Multi Agency Geographic Information for the Countryside (MAGIC), Department for Environment, Food and Rural Affairs) were consulted for information regarding records of European Protected Species Mitigation Licence (EPSML) and survey class licence returns within the Desk Study Area.

3.3. HABITAT SUITABILITY INDEX (HSI) ASSESSMENT

3.3.1. All water bodies within the Survey Area to which access was possible were assessed for their suitability to support great crested newts using the standard HSI assessment method (ARG UK, 2010) and Oldham *et al.* 2000.Water bodies were identified using 1:25,000 OS mapping; this was also cross referenced against aerial photography.

3.3.2. Water bodies were assessed and scored on ten key variables which are known to influence breeding populations of great crested newts, in accordance with standard methods (ARG UK, 2010). These variables are;

- geographic location;
- water body area;
- water body permanence;
- water quality;
- water body shading;
- impact of waterfowl;
- fish stocks;
- number of water bodies within 1km;
- terrestrial habitat around the water body; and
- macrophyte cover of the water body.

3.3.3. Scores for each of the above variables were used to calculate an overall HSI value for each water body. This was then cross referenced with the guidelines (ARG UK, 2010) to assign the pond to one of five categories, poor, below average, average, good or excellent, as shown in Table 3-1. Index calculation is not a failsafe method of identifying whether a water body supports great crested newts

or not; therefore, professional judgement and availability of records of GCN in the locality has also been used to inform the requirement for further survey.

Table 3-1 – Pond suitability categorisation based upon HSI score

HSI score	Pond suitability
<0.5	Poor
0.5 – 0.59	Below average
0.6 – 0.69	Average
0.7 – 0.79	Good
>0.8	Excellent

3.4. eDNA WATER SAMPLING

3.4.1. All water bodies found to provide suitable habitat for GCN, e.g. those ranging from poor to excellent suitability (see Table 3-1 above), to which access was possible, were subject to further survey to determine the presence or likely absence of this species. A small number of water bodies though were excluded from the eDNA survey effort. Their exclusion was based on professional judgement and where the habitat was considered completely unsuitable for GCN due to the size, depth and nature of the water body (for example, a concrete well with no features present to support GCN). Water bodies isolated from the Scheme by significant barriers to dispersal such as busy main roads were also discounted. The survey comprised eDNA water sampling. Sampling of eDNA was undertaken concurrently with the HSI survey. Professional judgement gained from previous experience and knowledge of GCN ecology, was exercised in selecting water bodies appropriate for sampling.

3.4.2. Research published in 2013 established a technique for reliably detecting newt eDNA in water bodies, and Natural England subsequently approved a protocol for this to become a survey method. The surveys were undertaken following survey techniques described in Biggs et al. (Biggs *et al*., 2014):

- A single visit to each target water body was made between mid-April and late-June, during the newt breeding season. One water body, pond 23, was subject to a visit in July, outside the newt breeding season.
- Twenty sub-samples of water were taken from each water body using sterile sampling equipment provided by the laboratory (ADAS).
- The locations of the 20 sub-samples were spaced as evenly as possible around the water body margin, and where possible targeted areas of vegetation which could be used as egg laying substrate and open water areas which newts could use for displaying.
- The sub-samples were mixed and pipetted into six sample tubes containing an alcohol and pH buffer solution.
- The samples were sent to ADAS for laboratory testing using real time polymerase chain reaction (PCR) to amplify part of the cytochrome 1 gene found in mitochondrial DNA.

- The water samples from each water body were assigned a positive or negative result as well as a score between 0 and 12 representing the number of positive replicates from a series of 12.

3.4.3. A positive eDNA result concludes that GCN DNA is present in the water sample, whilst a negative result concludes that the presence of GCN is considered unlikely within that water body. Negative eDNA results cannot conclusively say that a GCN are not present within the water body, rather that DNA from the species was not detected. GCN expel DNA into the ponds in which they live when they deposit; skin cells, faeces, mucus, sperm or eggs into the water. The DNA in this material can persist, and be detected, in the water for several weeks.

3.5. DATES OF SURVEY AND PERSONNEL

3.5.1. Lead surveyors were competent and experienced in conducting these surveys and both hold a Natural England survey licence for this species (licence numbers can be made available on request).

3.5.2. The date for each survey visit is displayed in Table 3-2 beneath.

Table 3-2 – Survey Dates

Water Body Ref.	Date of HSI	Date of eDNA
1	15/05/2020	N/A
2	15/05/2020	15/05/2020
3	15/05/2020	15/05/2020
4	15/05/2020	15/05/2020
5	15/05/2020	15/05/2020
6	30/06/2020	30/06/2020
7	15/05/2020	15/05/2020
8	15/05/2020	N/A
9	15/05/2020	15/05/2020
10	15/05/2020	15/05/2020
11	N/A	N/A
12	19/05/2020	19/05/2020
13	19/05/2020	19/05/2020
14	19/05/2020	19/05/2020
15	19/05/2020	19/05/2020
16	19/05/2020	19/05/2020
17	19/05/2020	19/05/2020
18	19/05/2020	19/05/2020

Water Body Ref.	Date of HSI	Date of eDNA
19	19/05/2020	19/05/2020
20	30/06/2020	N/A
21	30/06/2020	N/A
22	N/A	N/A
23	14/07/2020	14/07/2020
24	30/06/2020	N/A
25	30/06/2020	30/06/2020
26	30/06/2020	N/A
27	20/05/2020	N/A
28	20/05/2020	20/05/2020
29	20/05/2020	20/05/2020
30	20/05/2020	20/05/2020
31	30/06/2020	N/A
32	20/05/2020	20/05/2020
33	N/A	N/A
34	30/06/2020	30/06/2020
35	20/05/2020	20/05/2020
36	19/05/2020	N/A
37	N/A	N/A
38	N/A	N/A
39	N/A	N/A
40	19/05/2020	N/A
41	N/A	N/A
42	N/A	N/A
43	N/A	N/A
44	N/A	N/A
45	20/05/2020	N/A
46	20/05/2020	N/A
47	20/05/2020	N/A
48	N/A	N/A

Water Body Ref.	Date of HSI	Date of eDNA
49	30/06/2020	N/A

3.6. NOTES AND LIMITATIONS

3.6.1. One water body, 23, was sampled for eDNA testing outside the recommended survey period (mid-April to late-June). As the water body returned a negative result, further survey will be required in the 2021 survey season as this year's result cannot be used as evidence of likely absence of GCN.

3.6.2. Seven water bodies were dry at the time of survey. Sampling took place within the recommended period (mid-April – late June), however dry, warm weather prior to the surveys in May and June may have caused water bodies to dry prematurely and therefore may still be suitable for GCN breeding in other years. Therefore, these water bodies should be subject to a walkover during the next suite of GCN surveys and should be sampled for GCN eDNA if found to be holding water.

3.6.3. Due to access restrictions water samples were unable to be taken from two ponds. The status of these ponds and likelihood that they could support GCN will be reviewed after further survey has taken place.

3.6.4. Survey data is considered to be out of date after three years (CIEEM, 2019). As such, this conclusion of likely absence can be considered valid until 2023, after which time an ecologist should be consulted as to the need for updated GCN surveys.

4. RESULTS

4.1. DESK STUDY

4.1.1. The data search returned by NBIS did not contain any records of amphibians, including GCN, within 2km of the Scheme used for the data search. The Scheme and Study Area is included within Appendix A.

4.1.2. A review of freely available data from MAGIC (Defra) returned a record of a GCN EPSML mitigation licence approximately 100m from the Scheme boundary. However, a freedom of information (FOI) request found that this licence was in relation to construction of the Broadland Northway. The junction of the Broadland Northway (west) with the A1067 lies within the Study Area, however the water bodies covered by the EPSML are outside the 2km Desk Study Area, with the nearest water body being approximately 3km from the Scheme.

4.1.3. A further five records for GCN class survey licence returns were found on MAGIC within 2km, the nearest being approximately 0.8km south of the Scheme.

4.2. HABITAT SUITABILITY INDEX (HSI) ASSESSMENT

4.2.1. A summary of the HSI results and location information for the water bodies is included on drawing 70061370-09-07-0003, Appendix C. Water body numbers correspond to those on Appendix B and the HSI calculation is included in Table D-1, Appendix D. Photographs of each water body are included in Appendix E.

4.2.2. A total of 49 water bodies were identified as part of the aerial mapping, prior to the HSI surveys, one water body, 48, was ruled out as it was determined that the A47 provided a sufficient barrier to GCN movement. Nine water bodies were not subject to HSI survey due to access restrictions.

4.2.3. As a result, a total of 39 water bodies were visited as part of an HSI assessment. Of these water bodies, three were no longer present, seven were dry and two were classed as unsuitable because they lacked the appropriate habitat to support GCN. Therefore, 27 water bodies were able to be assessed for GCN suitability. The water bodies in each category is as follows;

- **poor** – five water bodies (3, 6, 7, 18 and 30)
- **below average** – six water bodies (2, 5, 15, 21, 32 and 34)
- **average** – seven water bodies (12, 13, 17, 23, 29, 36, and 40)
- **good** – seven water bodies (4, 9, 10, 14, 16, 25 and 35); and
- **excellent** – two water bodies (19 and 28).

4.3. eDNA WATER SAMPLING

4.3.1. A summary of the results is provided alongside the HSI scores in Table 4-1 and shown on drawing 70061370-09-07-0003, Appendix C. Full laboratory results are available in Appendix F.

4.3.2. Water sampling for eDNA analysis, where possible, was undertaken immediately following the HSI assessment.

4.3.3. Of the 27 suitable water bodies, 23 were able to be subject to eDNA sampling during the optimal period (mid-April – late-June), with one water body subject to sampling outside this optimal period. The remaining three water bodies could not be sampled due to either low water levels (assessed as dry in the eDNA result) or as being inaccessible for water sampling.

4.3.4. Of the 23 water bodies sampled within the optimal period, two, 15 and 16, returned positive results and the 20 remaining water bodies (2 – 7, 9 and 10, 12 – 14, 17, 19, 25, 28 – 30, 32, 34 and 35) returned negative results. One of the water bodies, 18, returned an indeterminate[1] result and therefore will likely require further survey.

4.3.5. The water body, 23, tested outside the optimal survey season for GCN eDNA returned a negative eDNA result. This though has been classed as inconclusive, as the negative result cannot be used to confirm likely absence of GCN and therefore will require further survey.

4.4. SUMMARY OF HSI AND eDNA RESULTS

4.4.1. The Desk Study did not return any records of amphibians, including GCN, within 2km of the Scheme Boundary used for the data search. A review of freely available data from MAGIC (Defra) returned a record of a GCN EPSL mitigation licence outside the 2km Desk Study Area and a further five GCN class licence returns within the 2km Desk Study Area.

4.4.2. A total of 49 water bodies were identified through aerial mapping. Prior to the surveys, water body 48 was ruled out of surveys as it was determined that the A47 provided a significant barrier to GCN movement. Nine of the water bodies were not subject to surveys due to access restrictions and therefore will require further survey once access is available. Therefore, only 39 water bodies were subject to surveys.

4.4.3. Out of the 39 water bodies that were surveyed, three were found to be no longer present and seven were dry. As a result, 29 waterbodies were assessed for their suitability to support GCN. The results of the HSI assessment were as follows: two ponds were immediately ruled out as unsuitable, five ponds were categorised as poor, six as below average, seven as average, seven as good and two as excellent suitability for GCN.

4.4.4. Of the 27 suitable water bodies, 23 were able to be subject to eDNA sampling during the optimal period (mid-April – late-June), with one water body subject to sampling outside this optimal period. The remaining three water bodies could not be sampled due to either low water levels (assessed as dry in the eDNA result) or as being inaccessible for water sampling.

4.4.5. The results returned two positive results, 20 negative results and one indeterminate result for GCN eDNA. The water body, pond 23, tested outside the optimal survey season for GCN eDNA and returned a negative result. This has been omitted from the above results and classed as inconclusive.

Table 4-1 – Summary of HSI and eDNA results

[1] Results will be recorded as indeterminate if the GCN result is negative and the degradation result is recorded as:
a. evidence of decay - meaning that the degradation control was outside of accepted limits
b. 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 (ADAS, 2020).

Water body Ref.	Grid Reference	Proximity to Scheme (m)	Connectivity to Scheme	HSI Score	HSI Category	eDNA Result
1	TG1037712012	377	Over 250m from Scheme	Dry	Dry	Not suitable for eDNA testing - dry
2	TG1037112032	365	Over 250m from Scheme	0.53	Below Average	Negative
3	TG1019212272	144	Good	0.41	Poor	Negative
4	TG1007812272	25	Good	0.77	Good	Negative
5	TG1036012369	327	Over 250m from Scheme	0.56	Below Average	Negative
6	TG0954712601	123	Isolated within arable field	0.46	Poor	Negative
7	TG1018512635	198	Good	0.43	Poor	Negative
8	TG0997612905	12	Good	Dry	Dry	Not suitable for eDNA testing - dry
9	TG1011913482	69	Good	0.80	Good	Negative
10	TG1022013444	65	Good	0.71	Good	Negative
11	TG1121913288	Not present	N/A	Not present	Not present	Not present
12	TG1151713700	87	Good	0.63	Average	Negative
13	TG1007314111	323	Over 250m from Scheme	0.62	Average	Negative
14	TG1009414218	388	Over 250m from Scheme	0.70	Good	Negative
15	TG1020014165	280	Over 250m from Scheme	0.56	Below Average	Positive
16	TG1038814219	272	Over 250m from Scheme	0.76	Good	Positive
17	TG1033814219	259	Over 250m from Scheme	0.68	Average	Negative
18	TG1135814249	162	Isolated within arable field	0.25	Poor	Indeterminate

Water body Ref.	Grid Reference	Proximity to Scheme (m)	Connectivity to Scheme	HSI Score	HSI Category	eDNA Result
19	TG1148314557	14	Within Scheme	0.84	Excellent	Negative
20	TG1139514861	317	Over 250m from Scheme	Dry	Dry	Not suitable for eDNA testing - dry
21	TG1176915218	356	Over 250m from Scheme	0.51	Below Average	Not suitable for eDNA testing - dry
22	TG1139215764	408	Over 250m from Scheme	No access	No access	No access
23	TG1163616380	150	Good	0.68	Average	Inconclusive[2]
24	TG1247015804	445	Over 250m from Scheme	Unsuitable for GCN	Unsuitable for GCN[3]	Unsuitable for GCN
25	TG1283215954	246	Good	0.74	Good	Negative
26	TG1300416367	Not present	N/A	Not present	Not present	Not present
27	TG1329115209	0	Within Scheme	0.51	Unsuitable for GCN[4]	Unsuitable for GCN
28	TG1349015353	0	Within Scheme	0.83	Excellent	Negative
29	TG1354915740	361	Over 250m from Scheme	0.63	Average	Negative
30	TG1372715865	258	Over 250m from Scheme	0.31	Poor	Negative
31	TG1366815516	Not present	N/A	Not present	Not present	Not present
32	TG1380315733	149	Good	0.59	Below Average	Negative

[2] Due to late sampling outside the recommended survey period, this result cannot be used to confirm likely absence of GCN and therefore will be subject to further survey.

[3] Unsuitable for GCN as water body was identified to be a concrete well with no features present to support GCN.

[4] HSI assessment categorised water body as below average suitability, however categorised as unsuitable for GCN due the size, depth and nature of the water body (<1m area, shallow depth and lined).

Water body Ref.	Grid Reference	Proximity to Scheme (m)	Connectivity to Scheme	HSI Score	HSI Category	eDNA Result
33	TG1445716195	480	Over 250m from Scheme	No access	No access	No access
34	TG1384915163	127	Good	0.55	Below Average	Negative
35	TG1406915409	21	Within Scheme	0.71	Good	Negative
36	TG1409715079	326	Over 250m from Scheme	0.70	Average	Not suitable for eDNA testing - inaccessible
37	TG1427615335	187	Good	No access	No access	No access
38	TG1436715263	212	Good	No access	No access	No access
39	TG1435315237	249	Good	No access	No access	No access
40	TG1446415082	365	Over 250m from Scheme	0.70	Average	Not suitable for eDNA testing - inaccessible
41	TG1453514973	425	Over 250m from Scheme	No access	No access	No access
42	TG1457014927	466	Over 250m from Scheme	No access	No access	No access
43	TG1458515014	412	Over 250m from Scheme	No access	No access	No access
44	TG1457014927	485	Over 250m from Scheme	No access	No access	No access
45	TG1481915393	0	Within Scheme	Dry	Dry	Not suitable for eDNA testing - dry
46	TG1481915393	0	Within Scheme	Dry	Dry	Not suitable for eDNA testing - dry
47	TG1481915393	0	Within Scheme	Dry	Dry	Not suitable for eDNA testing - dry

Water body Ref.	Grid Reference	Proximity to Scheme (m)	Connectivity to Scheme	HSI Score	HSI Category	eDNA Result
48	TG0949411924	434	Isolated by A47	Surveys not required[5]	Surveys not required	Surveys not required
49	TG1147814649	87	Good	Dry	Dry	Not suitable for eDNA testing - dry

[5] Surveys were not required for water body 48 as it is considered that the A47 provides a sufficient barrier to prevent GCN moving onto the Scheme.

5. FURTHER SURVEY WORK IN 2021

5.1.1. Recommended survey work to be conducted in 2021 which follows on from the 2020 surveys includes:

- Population size class assessment surveys of ponds 16 and 17 which tested positive for GCN eDNA;
- An update walkover of ponds found to be dry or inaccessible for sampling in 2020, with follow-up eDNA surveys where ponds are found to be accessible or holding water sufficient for sampling;
- Repeat eDNA surveys of ponds which returned an inconclusive (i.e. 23) or indeterminant (i.e. 18) result in 2020.

6. REFERENCES

6.1. PROJECT REFERENCES

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6.2. TECHNICAL REFERENCES

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Appendix A

SURVEY AND DESK STUDY AREA

THIS DRAWING MAY BE USED ONLY FOR THE PURPOSE INTENDED AND ONLY WRITTEN DIMENSIONS SHALL BE USED

Legend:

- 2km Study Area
- March 2020 Scheme Boundary

Revision Details	By / Check	Date	Suffix

Drawing Status: FINAL

Job Title: Norfolk County Council Norwich Western Link

Drawing Title: March 2020 Desk Study

Scale at A1: 1:15,000

Drawn	Stage 1 Check	Stage 2 Check	Originated	Date
UKMCW003	OP	AH	MW	07/10/2020

0 200 400 600 800 1,000 m

wsp

Drawing Number: 70061370-09-07-0001

Path: C:\Users\UKMCW003\Desktop\Reptile survey map.qgz

Appendix B

EDNA RESULTS 2020

Path: \\uk.wspgroup.com\central data\Projects\70041900\70041922 - Norwich Western Link\02 WIP\EC Ecology\06 GIS & Mapping\02 MXD Files\2020 GCN Surveys\Figure 3 - GCN eDNA results.mxd

THIS DRAWING MAY BE USED ONLY FOR THE PURPOSE INTENDED AND ONLY WRITTEN DIMENSIONS SHALL BE USED

Legend

- Scheme Boundary
- Survey Area

eDNA Results

- Positive
- Inconclusive
- Indeterminate
- Negative
- Not suitable for eDNA testing - Dry
- Not suitable for eDNA testing - Inaccessible
- No access
- Not present
- Surveys not required
- Unsuitable for GCN

Revision Details	By / Check	Date	Suffix

Drawing Status: FINAL

Job Title: Norfolk County Council
Norwich Western Link

Drawing Title: GCN eDNA Results

Scale at A3: 1:10,000

Drawn: UKPAL003

Stage 1 check	Stage 2 check	Originated	Date
OP	AH	RD	17/11/2020

0 200 400 600 m

Drawing Number: 70061370-09-07-0002

Appendix C

HSI RESULTS 2020

THIS DRAWING MAY BE USED ONLY FOR THE PURPOSE INTENDED AND ONLY WRITTEN DIMENSIONS SHALL BE USED

Legend

- Scheme Boundary
- Survey Area

HSI Results

- Excellent
- Good
- Average
- Below Average
- Poor
- Dry
- No Access
- Not Present
- Surveys not required
- Unsuitable for GCN

Revision Details	By / Check	Date	Suffix

Drawing Status: FINAL

Job Title: Norfolk County Council
Norwich Western Link

Drawing Title: Habitat Suitability Index (HSI) Assessment Results

Scale at A3: 1:10,000

Drawn: UKPAL003

Stage 1 check	Stage 2 check	Originated	Date
OP	AH	RD	17/11/2020

0 200 400 600 m

Drawing Number: 70061370-09-07-0003

Path: \\uk.wspgroup.com\central data\Projects\70041900\70041922 - Norwich Western Link\02 WIP\EC Ecology\06 GIS & Mapping\02 MXD Files\2020 GCN Surveys\Figure 2 - HSI results.mxd

Appendix D

HSI CALCULATIONS

Table D-1 – Habitat Suitability Indexes

Pond reference	Grid reference	Date of HSI	Geographic location	Pond area	Permanence	Water quality	Shade	Fowl	Fish	Pond count	Terrestrial	Macrophytes	HSI score	HSI category
1	TG1037712012	15/05/2020											N/A	Dry
2	TG1037112032	15/05/2020	1	0.1	0.5	0.33	0.6	1	1	0.85	0.67	0.3	0.53	Below average
3	TG1019212272	15/05/2020	1	0.1	0.1	0.33	0.2	1	1	0.9	0.67	0.3	0.41	Poor
4	TG1007812272	15/05/2020	1	0.6	1	0.67	1	0.67	0.67	0.9	0.67	0.7	0.77	Good
5	TG1036012369	15/05/2020	1	0.2	0.5	0.33	0.6	1	1	0.9	0.33	0.5	0.56	Below average
6	TG0954712601	30/06/2020	1	0.9	0.5	0.33	1	1	1	0.9	0.01	0.35	0.46	Poor
7	TG1018512635	15/05/2020	1	0.4	1	0.33	0.6	1	1	0.95	0.01	0.3	0.43	Poor
8	TG0997612905	15/05/2020											N/A	Dry
9	TG1011913482	15/05/2020	1	0.8	0.9	0.67	1	0.67	1	0.9	1	0.35	0.80	Good
10	TG1022013444	15/05/2020	1	0.8	1	0.33	0.6	0.67	1	0.9	1	0.35	0.71	Good
11	TG1121913288	N/A											N/A	Not present
12	TG1151713700	19/05/2020	1	0.85	1	0.67	0.3	0.67	0.67	0.65	0.67	0.3	0.63	Average

13	TG1007314111	19/05/2020	1	1	0.5	0.33	0.2	1	1	0.85	1	0.3	0.62	Average
14	TG1009414218	19/05/2020	1	0.85	0.9	0.33	1	0.67	0.67	0.85	1	0.3	0.70	Good
15	TG1020014165	19/05/2020	1	1	0.5	0.01	1	1	1	0.85	1	0.7	0.56	Below average
16	TG1038814219	19/05/2020	1	0.6	0.9	0.67	1	0.67	1	0.85	0.33	1	0.76	Good
17	TG1033814219	19/05/2020	1	0.8	0.33	0.2	0.67	1	1	0.85	1	0.7	0.68	Average
18	TG1135814249	19/05/2020	1	0.2	0.1	0.01	0.6	1	1	0.85	0.01	1	0.25	Poor
19	TG1148314557	19/05/2020	1	0.95	0.9	1	1	0.67	0.67	0.7	0.67	1	0.84	Excellent
20	TG1139514861	30/06/2020											N/A	Dry
21	TG1176915218	30/06/2020	1	0.2	0.1	0.33	1	1	1	0.65	0.67	0.4	0.51	Below average
22	TG1139215764	N/A											N/A	No access
23	TG1163616380	14/07/2020	1	0.4	0.5	0.67	0.8	0.67	0.67	0.45	1	1	0.68	Average
24	TG1247015804	N/A											N/A	Unsuitable for GCN[6]
25	TG1283215954	30/06/2020	1	0.4	1	0.67	1	1	0.33	0.85	1	0.7	0.74	Good
26	TG1300416367	30/06/2020											N/A	Not present
27	TG1329115209	20/05/2020	1	0.1	0.5	0.33	0.33	1	1	1	0.7	0.3	0.51	Unsuitable for GCN[7]

[6] Unsuitable for GCN as water body was identified to be a concrete well with no features present to support GCN.

[7] HSI assessment categorised water body as below average suitability, however classed as unsuitable for GCN due the size, depth and nature of the water body (<1m area, shallow depth and lined).

28	TG1349015353	20/05/2020	1	0.9	1	0.67	0.7	0.67	0.67	0.95	1	0.9	0.83	Excellent
29	TG1354915740	20/05/2020	1	0.8	0.9	0.67	0.3	0.67	0.33	1	1	0.3	0.63	Average
30	TG1372715865	20/05/2020	1	0.1	0.1	1	0.3	1	1	1	0.01	0.3	0.31	Poor
31	TG1366815516	30/06/2020											N/A	Not present
32	TG1380315733	20/05/2020	1	0.1	0.5	1	1	1	1	1	0.33	0.3	0.59	Below average
33	TG1445716195	N/A											N/A	No access
34	TG1384915163	30/06/2020	1	0.2	1	0.33	1	0.67	0.33	1	0.33	0.5	0.55	Below average
35	TG1406915409	20/05/2020	1	0.8	0.9	0.67	1	0.67	0.33	1	1	0.3	0.71	Good
36	TG1409715079	19/05/2020	1	1	0.1	0.33	1	1	1	1	1	0.8	0.70	Average
37	TG1427615335	N/A											N/A	No access
38	TG1436715263	N/A											N/A	No access
38	TG1435315237	N/A											N/A	No access
40	TG1446415082	19/05/2020	1	0.95	0.1	0.33	1	1	1	1	1	0.85	0.70	Average
41	TG1453514973	N/A											N/A	No access
42	TG1457014927	N/A											N/A	No access
43	TG1458515014	N/A											N/A	No access
44	TG1457014927	N/A											N/A	No access
45	TG1481915393	20/05/2020											N/A	Dry

46	TG1481915393	20/05/2020										N/A	Dry
47	TG1481915393	20/05/2020										N/A	Dry
48	TG0949411924	N/A										N/A	Not subject to survey
49	TG1147814649	30/06/2020										N/A	Dry

Appendix E

PHTOTOGRAPHS

Table E-1 - Photographs of Ponds

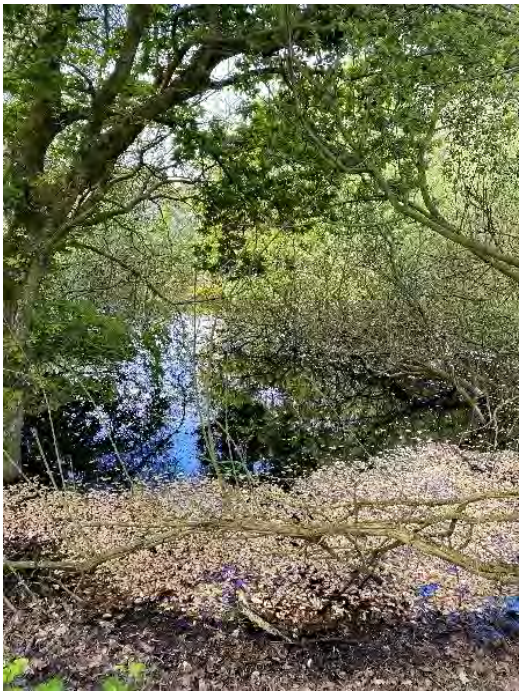
Pond Ref.	Image
1	
2	

Pond Ref.	Image
3	
4	

Pond Ref.	Image
5	
6	

Pond Ref.	Image
7	
8	

Pond Ref.	Image
9	
10	
11	Water body not present

Pond Ref.	Image
12	
13	

Pond Ref.	Image
14	
15	

Pond Ref.	Image
16	
17	

Pond Ref.	Image
18	
19	

Pond Ref.	Image
20	
21	
22	No access in 2020
23	No picture taken

Pond Ref.	Image
24	
25	
26	Water body not present

Pond Ref.	Image
27	
28	

Pond Ref.	Image
29	
30	
31	

Pond Ref.	Image
32	
33	No access in 2020
34	

Pond Ref.	Image
35	
36	
37	No access in 2020
38	No access in 2020
39	No access in 2020

Pond Ref.	Image
40	
41	No access in 2020
42	No access in 2020
43	No access in 2020
44	No access in 2020
45	

Pond Ref.	Image
46	
47	
48	Surveys not required

Pond Ref.	Image
49	

Appendix F

LABORATORY RESULTS

Client: [redacted]
WSP

ADAS
Spring Lodge
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WA6 0AR

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

www.adas.uk

Sample ID: 2020-0334	Condition on Receipt: Low Sediment	Volume: Passed
Client Identifier: pond 2, nwl	Description: pond water samples in preservative	
Date of Receipt: 26/05/2020	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	28/05/2020
Degradation Control[§]	Within Limits	Real Time PCR	28/05/2020
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	28/05/2020
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:	01/06/2020	Date of issue:	01/06/2020

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.

Client: [redacted]
WSP

ADAS
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Sample ID: 2020-0259	Condition on Receipt: Low Sediment	Volume: Passed
Client Identifier: 3, nwl	Description: pond water samples in preservative	
Date of Receipt: 26/05/2020	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	28/06/2020
Degradation Control[§]	Within Limits	Real Time PCR	28/06/2020
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	28/06/2020
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:	01/06/2020	Date of issue:	01/06/2020

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.

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

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

Client: [redacted]
WSP

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Sample ID: 2020-0283	Condition on Receipt: Low Sediment	Volume: Passed
Client Identifier: 4, nwl	Description: pond water samples in preservative	
Date of Receipt: 26/05/2020	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	28/05/2020
Degradation Control[§]	Within Limits	Real Time PCR	28/05/2020
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	28/05/2020
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:	01/06/2020	Date of issue:	01/06/2020

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.

Client: [redacted]
WSP

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Sample ID: 2020-0238	Condition on Receipt: Low Sediment	Volume: Passed
Client Identifier: 5, nwl	Description: pond water samples in preservative	
Date of Receipt: 26/05/2020	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	28/06/2020
Degradation Control[§]	Within Limits	Real Time PCR	28/06/2020
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	28/06/2020
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:	01/06/2020	Date of issue:	01/06/2020

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.*

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

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

Client: [redacted]
WSP

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Sample ID: 2020-4532	Condition on Receipt: Low Sediment	Volume: Passed
Client Identifier: NWL 6	Description: pond water samples in preservative	
Date of Receipt: 03/07/2020	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	20/07/2020
Degradation Control[§]	Within Limits	Real Time PCR	20/07/2020
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	20/07/2020
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:	21/07/2020	Date of issue:	21/07/2020

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.

Client: [redacted]
WSP

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Sample ID: 2020-0431	Condition on Receipt: Good	Volume: Passed
Client Identifier: 7, nwl	Description: pond water samples in preservative	
Date of Receipt: 26/05/2020	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	28/05/2020
Degradation Control[§]	Within Limits	Real Time PCR	28/05/2020
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	28/05/2020
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:	01/06/2020	Date of issue:	01/06/2020

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.

Client: [redacted]
WSP

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Sample ID: 2020-0324	Condition on Receipt: Good	Volume: Passed
Client Identifier: 9, nwl	Description: pond water samples in preservative	
Date of Receipt: 26/05/2020	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	28/05/2020
Degradation Control[§]	Within Limits	Real Time PCR	28/05/2020
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	28/05/2020
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:	01/06/2020	Date of issue:	01/06/2020

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.

Client: [redacted]
WSP

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Sample ID: 2020-0244	Condition on Receipt: Good	Volume: Passed
Client Identifier: 10, nwl	Description: pond water samples in preservative	
Date of Receipt: 26/05/2020	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	28/06/2020
Degradation Control[§]	Within Limits	Real Time PCR	28/06/2020
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	28/06/2020
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:	01/06/2020	Date of issue:	01/06/2020

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.

Client: [redacted]
WSP

ADAS
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Sample ID: 2020-0322	Condition on Receipt: Good	Volume: Passed
Client Identifier: 12, nwl	Description: pond water samples in preservative	
Date of Receipt: 26/05/2020	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	29/05/2020
Degradation Control[§]	Within Limits	Real Time PCR	29/05/2020
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	29/05/2020
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:	01/06/2020	Date of issue:	01/06/2020

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: 2020-0285	Condition on Receipt: Low Sediment	Volume: Passed
Client Identifier: 13, nwl	Description: pond water samples in preservative	
Date of Receipt: 26/05/2020	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	29/05/2020
Degradation Control[§]	Within Limits	Real Time PCR	29/05/2020
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	29/05/2020
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:	01/06/2020	Date of issue:	01/06/2020

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: 2020-0226	Condition on Receipt: Low Sediment	Volume: Passed
Client Identifier: 14, nwl	Description: pond water samples in preservative	
Date of Receipt: 26/05/2020	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	28/06/2020
Degradation Control[§]	Within Limits	Real Time PCR	28/06/2020
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	28/06/2020
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:	01/06/2020	Date of issue:	01/06/2020

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: 2020-0221	Condition on Receipt: Algae Present	Volume: Passed
Client Identifier: 15, nwl	Description: pond water samples in preservative	
Date of Receipt: 26/05/2020	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control†	2 of 2	Real Time PCR	28/05/2020
Degradation Control§	Within Limits	Real Time PCR	28/05/2020
Great Crested Newt*	10 of 12 (GCN positive)	Real Time PCR	28/05/2020
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:	01/06/2020	Date of issue:	01/06/2020

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: 2020-0325	Condition on Receipt: Good	Volume: Passed
Client Identifier: 16, nwl	Description: pond water samples in preservative	
Date of Receipt: 26/05/2020	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	28/05/2020
Degradation Control[§]	Within Limits	Real Time PCR	28/05/2020
Great Crested Newt*	1 of 12 (GCN positive)	Real Time PCR	28/05/2020
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:	01/06/2020	Date of issue:	01/06/2020

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: 2020-0430	Condition on Receipt: Good	Volume: Passed
Client Identifier: 17, nwl	Description: pond water samples in preservative	
Date of Receipt: 26/05/2020	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	28/05/2020
Degradation Control[§]	Within Limits	Real Time PCR	28/05/2020
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	28/05/2020
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:	01/06/2020	Date of issue:	01/06/2020

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: 2020-0236	Condition on Receipt: High Sediment	Volume: Passed
Client Identifier: 18, nwl	Description: pond water samples in preservative	
Date of Receipt: 26/05/2020	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	28/06/2020
Degradation Control[§]	Evidence of degradation	Real Time PCR	28/06/2020
Great Crested Newt*	Indeterminate	Real Time PCR	28/06/2020
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:	01/06/2020	Date of issue:	01/06/2020

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: 2020-0220	Condition on Receipt: Low Sediment	Volume: Passed
Client Identifier: 19, nwl	Description: pond water samples in preservative	
Date of Receipt: 26/05/2020	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	28/05/2020
Degradation Control[§]	Within Limits	Real Time PCR	28/05/2020
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	28/05/2020
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:	01/06/2020	Date of issue:	01/06/2020

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: 2020-2388	Condition on Receipt: Good	Volume: Passed
Client Identifier: Pond 23 NWL	Description: pond water samples in preservative	
Date of Receipt: 23/07/2020	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	29/07/2020
Degradation Control[§]	Within Limits	Real Time PCR	29/07/2020
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	29/07/2020
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:	29/07/2020	Date of issue:	29/07/2020

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: 2020-4309	Condition on Receipt: Low Sediment	Volume: Passed
Client Identifier: NWL 25	Description: pond water samples in preservative	
Date of Receipt: 03/07/2020	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	20/07/2020
Degradation Control[§]	Within Limits	Real Time PCR	20/07/2020
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	20/07/2020
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:	21/07/2020	Date of issue:	21/07/2020

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: 2020-0292	Condition on Receipt: Low Sediment	Volume: Passed
Client Identifier: 28, nwl	Description: pond water samples in preservative	
Date of Receipt: 26/05/2020	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	28/05/2020
Degradation Control[§]	Within Limits	Real Time PCR	28/05/2020
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	28/05/2020
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:	01/06/2020	Date of issue:	01/06/2020

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: 2020-0239	Condition on Receipt: Good	Volume: Passed
Client Identifier: 29, nwl	Description: pond water samples in preservative	
Date of Receipt: 26/05/2020	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	28/06/2020
Degradation Control[§]	Within Limits	Real Time PCR	28/06/2020
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	28/06/2020
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:	01/06/2020	Date of issue:	01/06/2020

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: 2020-0242	Condition on Receipt: Low Sediment	Volume: Passed
Client Identifier: 30, nwl	Description: pond water samples in preservative	
Date of Receipt: 26/05/2020	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	28/06/2020
Degradation Control[§]	Within Limits	Real Time PCR	28/06/2020
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	28/06/2020
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:	01/06/2020	Date of issue:	01/06/2020

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: 2020-0269	Condition on Receipt: Good	Volume: Passed
Client Identifier: 32, nwl	Description: pond water samples in preservative	
Date of Receipt: 26/05/2020	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	29/05/2020
Degradation Control[§]	Within Limits	Real Time PCR	29/05/2020
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	29/05/2020
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:	01/06/2020	Date of issue:	01/06/2020

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: 2020-4307	Condition on Receipt: Good	Volume: Passed
Client Identifier: NWL 34	Description: pond water samples in preservative	
Date of Receipt: 03/07/2020	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	20/07/2020
Degradation Control[§]	Within Limits	Real Time PCR	20/07/2020
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	20/07/2020
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:	21/07/2020	Date of issue:	21/07/2020

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.

Client: [redacted]
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Sample ID: 2020-0295	Condition on Receipt: Low Sediment	Volume: Passed
Client Identifier: 35, nwl	Description: pond water samples in preservative	
Date of Receipt: 26/05/2020	Material Tested: eDNA from pond water samples	

Determinant	**Result**	**Method**	**Date of Analysis**
Inhibition Control[†]	2 of 2	Real Time PCR	28/05/2020
Degradation Control[§]	Within Limits	Real Time PCR	28/05/2020
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	28/05/2020
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:	01/06/2020	Date of issue:	01/06/2020

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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)

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