Preliminary Environmental Information Report

Volume 4
Appendix 7.4

Great Crested Newt Survey Report 2023

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Introduction

Background

WSP Binnies Joint Venture (WBi) was commissioned by the Environment Agency (EA) to undertake great crested newt (GCN) surveys within 250m of the proposed River Thames Scheme (hereafter referred to as RTS or the project). These GCN surveys include an initial waterbody screening survey, Habitat Suitability Index (HSI) assessments and environmental DNA (eDNA) surveys.

Scheme description and proposed development

The project, pending Outline Business Case approval and Development Consent Order (DCO) approval from the Secretary of State, will include large-scale engineering works to construct a new flood channel measuring between 20 and 50 metres wide and 8km long. The new flood channel will be built in two sections (the Runnymede Channel and the Spelthorne Channel) and connect to a series of existing lakes between Egham Hythe and Desborough Island, and will also include capacity improvements works comprising bed lowering of the River Thames downstream of the Desborough Cut, increasing the capacity of existing weirs at Sunbury, Molesey and Teddington and priority areas for habitat creation, mitigation and enhancement works across several areas, the latter including:

- Land South of Wraysbury Reservoir
- Royal Hythe
- Laleham Reach
- Former Laleham Golf Course
- Littleton Lane
- Sheepwalk Lakes
- Sheepwalk
- Manor Farm
- Land South of Chertsey Road
- Desborough Island
- Land between Desborough Cut and Engine River
- Abbey River
- Abbey Meads
- Drinkwater Pit
- Penton Park
- Grove Farm
- Norlands Lane

The proposed Runnymede and Spelthorne channels, weirs and priority areas for habitat creation are collectively referred to as the area within the Project Boundary for EIA Preliminary Environmental Information Report (PEIR).

Previous Surveys

GCN surveys have previously been undertaken based on earlier project footprints.

GCN surveys were undertaken in 2017 (GBV 2017) for the RTS project. A summary of the 2017 survey results is provided below. Due to the age of the data, the surveys were repeated in 2021. The original results of the 2017 surveys, including the locations of surveyed waterbodies and HSI scores, were factored into the 2021 screening process.

In 2017, a total of 77 waterbodies were identified as needing HSI assessments. A summary of the results has been provided below (Table 1). A total of 39 waterbodies were subject to an HSI assessment.

Table 1: Summary of 2017 HSI Results

Suitability	Number of Waterbodies
Excellent	4
Good	10
Average	6
Below average	10
Poor	9
Not assessed	38

Of the 38 waterbodies not subject to an HSI assessment: 22 were dry, 10 had been filled in, 3 were not ponds, 2 landowners denied access and 1 was not able to be accessed safely.

Following on from the HSI assessments, 30 waterbodies which scored as below average suitability or above were scheduled for eDNA surveys. However, due to some waterbodies not being either safe to access or being too shallow at the time of survey only a total of 22 eDNA samples were taken. All eDNA results were returned as negative. Presence/absence surveys were also undertaken at one pond in 2017. No GCN were found in this waterbody.

A Phase One Habitat Validation Survey (GBV 2021) was undertaken in August 2020 to validate the ecological information reported within the previous Phase 1 Habitat Survey (P1HS) for the Scheme (GBV 2016) and the weirs (GBV 2015). In addition, P1HSs were carried out in 2020 across the fourteen sites proposed as HCAs at that time (GBV 2020a – GBV 2020n). Together, the fifteen reports identified a number of habitats that provide potential for GCN.

Surveys for GCN were subsequently carried out between March and April 2021. During these surveys 52 waterbodies within a previous iteration of the RTS project boundary plus a 500m buffer were subject to HSI assessments. eDNA samples were taken from 38 of these waterbodies (GBV, 2021). No evidence of GCN was found during any of the surveys.

Scope of the report

The brief provided to WBi for the GCN surveys in 2023 is as follows:

- To assess records of GCN provided by the local records centres;
- To undertake a screening process to determine the number of waterbodies within, and up to 250m of, the Project Boundary for EIA PEIR (hereafter referred to as the 'Survey Area') which may have suitability for GCN;
- To undertake HSI assessments to determine the suitability of the screened waterbodies for GCN;
- To undertake eDNA surveys to determine the presence or likely absence of GCN in these waterbodies;
- To provide recommendations to enable compliance with relevant nature conservation legislation and planning policy; and
- To identify the need for avoidance, mitigation, compensation and/or enhancement of the proposed options of the project.

Legislation

GCN are a European Protected Species under the Conservation of Habitats and Species Regulations 2019, listed under Schedule 2. This makes it illegal to intentionally kill or injure a GCN, as well as causing disturbance, destroying eggs, damaging breeding sites and impairing their ability to hibernate, breed or migrate.

GCN are also protected under the Wildlife and Countryside Act 1981 (as amended), making it illegal to intentionally or recklessly disturb GCN, obstruct their access to shelter, or to sell GCN.

Methodology

Desk study

A comprehensive ecological desk study was conducted by Galliford Binnies JV Ltd (GBV) in July 2022 for the Project Boundary for EIA Scoping plus a 2km buffer (referred to as the 'Wider Study Area'), including the retrieval of data from relevant biological record centres. This data informed the GCN screening survey. It is noted that the area within the Project Boundary for EIA PEIR most entirely falls within the Project boundary for EIA Scoping and therefore whilst the Project Boundary for EIA PEIR was defined after the desk study, it is anticipated that the results of this exercise will be sufficient for the purposes of this survey report.

Data was sourced from:

- Greenspace Information for Greater London (GiGL);
- Thames Valley Environmental Records Centre (TVERC);
- Surrey Biodiversity Information Centre (SBIC); and

Additionally, an existing ecological report produced by eCountability 2020 was consulted as part of the desk study (eCountability, 2020).

As part of the desk study, GBV used Ordnance Survey (OS) mapping to identify any mapped waterbodies and watercourses within 500m of the Project Boundary for EIA Scoping.

GBV also reviewed the previous screening process undertaken as part of the GCN surveys in 2017 and 2020. The results of the original screening process were added into the updated screening process to provide the most up-to-date dataset possible.

Screening Survey

The 2021 GCN survey results, UK Habitat Classification surveys completed in 2022 and MasterMap (the latter for the 250m buffer beyond the project boundary) were used to determine the locations of waterbodies.

Waterbodies above 2000m^2 in size were discounted as highly unlikely to be favourable for GCN. Waterbodies with the presence of significant obstacles to GCN movement between waterbodies and the project ground works (such as large rivers, roads or urban areas) were also discounted.

A total of 69 waterbodies within the Project Boundary for EIA PEIR, plus a 250m buffer were identified as potentially suitable for HSI surveys. These are shown on Figure ENVIMSE500260-CBI-ZZ-3ZZ-DR-EN-00004, Appendix A.

Habitat Suitability Index (HSI) Assessments

The HSI assessment (Oldham et al. 2000) considers 10 indices that are known to affect likelihood of GCN including geographical location, pond area, pond drying, water quality, shade, waterfowl, fish, pond count, terrestrial habitat and macrophyte coverage. The assessment allows an accurate and quick appraisal of a ponds suitability to support GCN.

Each of the ten factors is given a Suitability Index (SI) value between 0.1 and 1 and the geometric mean is the overall HSI score for the waterbody. The overall HSI score can then be interpreted as one of five suitability categories as shown in Table 2.

Table 2: Habitat Suitability Categories and Scoring

Suitability	HSI Score
Poor	<0.5
Below Average	0.5 – 0.59
Average	0.6 – 0.69
Good	0.7 – 0.79
Excellent	>0.8

Where a pond is categorised as having greater than 'poor' suitability for GCN, it was subject to an eDNA survey to determine whether any GCN eDNA is present, as per the approach for the 2017 and 2021 surveys. Any ponds which were identified for survey through desk study but were dry or found to be unsuitable for survey in the field were not subject to eDNA survey.

Any ponds categorised as 'poor' were not subject to eDNA surveys as the presence of GCN in a pond assessed as 'poor' is deemed to be unlikely. A study by ARG UK found that roughly 3% of ponds categorised as 'poor' were found to be occupied by GCN (ARG UK, 2010). All ponds scoring 'below average' suitability or above were subject to further eDNA survey.

HSI assessments were undertaken by GCN licensed ecologists Alexander Bell (2019-44114-CLS-CLS) or Amy Roberts (2016-20189-CLS-CLS) between 17th April and 5th May 2023.

Environmental DNA (eDNA) Surveys

eDNA surveys were conducted in ponds 2a, 4, 6, 7, 11, 12, 19, 226, 228, 231, 233 and 236. They were undertaken in accordance with the latest guidance (Biggs et al 2014) between 17th April and 5th May 2023 by GCN licenced surveyors Alexander Bell (2019-44114-CLS-CLS) and Amy Roberts (2016-20189-CLS-CLS).

eDNA surveys involved one visit to each pond by a licenced surveyor to collect a water sample. Water samples were collected under a strict protocol to prevent cross-contamination. The samples were sent for analysis by an accredited laboratory (ADAS) and it was recorded whether DNA from GCN (such as skin cells, urine, faeces etc) was present in the sample, indicating the recent or current presence of GCN in the pond where the sample was taken from.

Limitations

Every effort was made to gain as much survey information as possible within the Survey Area. However, in some circumstances, surveys could not be completed as a result of being unable to agree access with landowners or due to health and safety concerns. Access issues encountered during the HSI assessments and/or eDNA surveys are as follows:

- Ponds 10, 22, 23, 24, 32, 33, 35, 38, 215, 215a, 216, 220, 225, 229, 232, 235 and 237 - No response from landowners. Access not granted.
- Pond 8, 15, 26, 30 and 227 - HSI assessment undertaken but eDNA samples could not be safely taken or waterbody was on land where access was not available

A number of waterbodies were dry over the course of the survey season. Due to this, it was not always possible to conduct HSI assessments or eDNA surveys. Suitability issues encountered during the HSI assessments and/or eDNA surveys are as follows:

- Ponds 5, 13, 16, 25, 29, 31, 34, 36, 37, 41, 219, 223, 251, 252 and 254 - No HSI assessment conducted due to unsuitability such as dry waterbodies or running water. Pond 27 was the subject of an HSI assessment but due to flow was deemed unsuitable for eDNA survey.
- Ponds 21, 39 and 40 - No waterbody present upon surveying.

In relation to the desk study, it should be noted that data from biological record centres may be incomplete, inaccurate or missing. Therefore, records cannot be relied on and serve only as an indication of which species may or may not be present in an area. The absence of records does not necessarily denote the absence of a species.

However, despite the above limitations it is still considered that in combination with previous surveys undertaken in 2017 and 2021 this survey gives an accurate representation of the current status of GCN within the Survey Area.

Results

Desk study

There were no records of GCN returned by the desk study within the Project Boundary for EIA Scoping. However, the desk study reported 30 records of amphibians from four species - GCN, smooth newt, common frog and common toad - in the Wider Study Area (within 2km of the Project Boundary for EIA Scoping).

Screening Survey

The waterbody screening process was undertaken in March 2023. Following the screening process, a total of 69 waterbodies were identified within the Survey Area as requiring HSI assessment surveys.

Habitat Suitability Index (HSI) Assessments

A survey map is included in Appendix A (Figure ENVIMSE500260-CBI-ZZ-3ZZ-DR-EN-00004). An additional map which identifies ponds surveyed for HSI assessments and eDNA surveys, is also included in Appendix A (Figure ENVIMSE500260-CBI-ZZ-3ZZ-DR-EN-00067). All survey results are reported in Appendix B.

During the HSI surveys, five additional waterbodies were found, creating a new total of 74 waterbodies requiring HSI assessment surveys. Of these, a total of 39 were subject to an HSI assessment. The remaining 35 waterbodies were either dry (12), had running water (2), access was denied (17), or were found not to be waterbodies (4).

Photographs are provided in Appendix C showing why certain waterbodies did not undergo HSI assessments.

A summary of the HSI results is shown in Table 3.

Waterbodies categorised as ‘poor’ are considered to be low risk for GCN occupation and these ponds were therefore screened out from further survey. Furthermore, all waterbodies categorised as ‘poor’ are in close connectivity to other waterbodies which are either dry or returned negative eDNA results. This further supports the likelihood of GCN absence in the ‘poor’ waterbodies. A total of 18 waterbodies were identified as requiring eDNA surveys.

Table 3: Number of waterbodies per HSI category

HSI Category	Number of Waterbodies
Excellent	1
Good	1
Average	8
Below Average	8
Poor	21
Scoring not possible	35

Environmental DNA (eDNA) Surveys

Of the 18 waterbodies requiring an eDNA survey, a total of 12 were successfully surveyed. The remaining six waterbodies were not sampled for eDNA due to the following reasons:

- Ponds 8, 15, and 227 were not safe and had limited access to conduct eDNA surveys.
- Pond 26 had limited access for eDNA sampling, thus it was not suitable to conduct these surveys as it was not possible to obtain sufficient water samples.
- Pond 27 had flowing water and therefore was deemed as having reduced suitability as a breeding site for GCN and unsuitable for eDNA survey.
- Pond 30 was on private property where HSI assessment was conducted from a public right of way. There was no access to carry out eDNA sampling.

The above ponds which could not be eDNA surveyed are considered in more detailed in the following section. Photographs are provided in Appendix C, showing why certain waterbodies did not undergo eDNA surveys.

All 12 waterbodies which were surveyed for eDNA returned negative results. Full survey results are recorded in Appendix B. A copy of the eDNA laboratory results is provided in Appendix D.

Ponds which could not be surveyed

In total, 26 waterbodies were not surveyed in 2023 due to land access issues, inaccessibility/safety concerns or unsuitability for HSI/eDNA.

Previous survey records from 2021 showed a lack of GCN evidence for a number of the ponds which were not surveyed in 2023, consolidating the likelihood of GCN absence from these waterbodies. Of the 26 waterbodies that could not be surveyed, 10 (26, 27, 30, 215, 215a, 220, 225, 229, 235 and 237) were previously eDNA sampled in 2021 and returned with negative results. Three waterbodies (22, 23, 24) were previously surveyed and no pond was found to be present at that time, and it is therefore considered likely that these ponds no longer exist. Pond 38 was found to be filled in and pond 232 was found to be dry in 2021. Pond 216 scored 'poor' during the 2021 HSI assessment and is within close proximity to ponds 215, 215a and 220, which returned negative when sampled for eDNA in 2021.

Six waterbodies (5, 10, 29, 32, 33 and 35) were not surveyed in 2023 or 2021. Pond 5 is a small waterbody connected to a large lake with known fish stocks. The waterbody has flow and was considered unsuitable for HSI or eDNA survey. This pond is also located 50m from pond 6 which tested negative for eDNA in 2023. Pond 10 is surrounded by urban areas, a main road and large connecting lakes. The closest pond (9) is across a main road and scored 'poor' for HSI. Pond 29 is a ditch with running water which connects into the River Thames. This pond was deemed unsuitable for HSI or eDNA surveys. Nearby ponds (28 and 34) were either dry or scored 'poor' for HSI. Pond 32 is in close proximity to ponds 31 and 34 which were found to be dry. It is also close to ponds 27 and 28 which scored 'poor' and 'below average' (had litter and water flow) for HSI assessments. The surrounding habitat and nearby ponds with poor connectivity are likely unsuitable for a GCN population. Pond 33 has a 3m wall surrounding it, making it highly unlikely for any potential GCN to leave this pond and be affected by the proposed works. Pond 35 is close to pond 232 (dry) and 237a ('poor' HSI), with a busy road on one side and the River Thames on the other side. These habitats are unlikely to be suitable for a GCN population surrounding the waterbodies due to poor connectivity and surrounding habitats.

Three waterbodies (8, 15 and 227) were surveyed for HSI assessments in 2023 but were not subject to eDNA sampling due to limited safe access. Pond 8 scored 'below average' for the HSI assessment but due to unsafe access eDNA surveys were not conducted. However, it is in close proximity to ponds 2a, 4 and 7, which returned negative results for eDNA samples in 2023, making it unlikely there would be GCN presence in this waterbody. Pond 15 scored 'below average' for HSI but could not be safely accessed for eDNA sampling. It was also surveyed in 2021 and recorded as 'poor' for HSI assessment. The habitats surrounding this waterbody consist of large waterbodies over 2000m^2 and the River Thames, which are highly likely to be unfavourable for GCN. Pond 227 scored 'average' for HSI but was inaccessible for eDNA. This waterbody is in close proximity to ponds 256 and 230 which scored 'poor' and to pond 228 which returned a negative result for eDNA. With the potential for GCN

to travel between these four waterbodies, the ‘poor’ HSI and negative eDNA results would indicate that it is unlikely that GCN would be present in pond 227.

Pond 25 was not surveyed separately as it is considered to be part of pond 231 which was subject to an eDNA survey.

Conclusion and recommendations

A total of 74 waterbodies were scoped into the GCN assessment. Of these, 39 were subject to HSI assessments, identifying a total of 18 waterbodies that had potential to support GCN.

eDNA samples were taken from 12 waterbodies, of which all were found negative for GCN. No evidence of GCN was found during any of the surveys. The results of the 2023 surveys are consistent with the results of the 2017 and 2021 GCN surveys, and the records provided by the local record centres.

A total of 12 waterbodies found to be dry in 2023 could theoretically support GCN populations in wetter years, as GCN are long lived and can endure several dry years. However, given the lack of GCN evidence in all of the surveyed waterbodies and lack of historic records within 250m of any of the waterbodies, it is believed that GCN are likely absent.

The same result (of likely absence) is also postulated for the 23 waterbodies to which access could not be gained for HSI or eDNA surveys, given the distribution of these ponds, poor HSI assessments and the negative eDNA results for nearby ponds.

Given the lack of GCN evidence in previous survey results (2017 and 2021), the desk study records and 2023 survey results, it is concluded that GCN are likely absent from the Survey Area and are therefore unlikely to be present within the Project Boundary for EIA PEIR. As such, no further mitigation for GCN is required at this time.

Despite the considered likely absence of GCN as outlined above, further pre-commencement surveys are recommended for the ponds which could not be accessed in 2023. These surveys are required to allow a full assessment of the potential impacts of the Scheme on GCN.

Additionally, GCN surveys are valid for two years, after which resurveys of all ponds are recommended to ensure data are up to date. When resurveys are undertaken the 12 ponds which were previously found to be dry should also be re-assessed, and

surveys completed if they are found to be holding water and have suitability as breeding sites for amphibians.

The Survey Area was based on the RTS project boundary at the time of survey (April to June 2023). If the project boundary were to change, any new waterbodies which come within the 250m zone of the new boundary will require survey. If GCN are subsequently found, then a protected species licence may be required. This would likely require sufficient survey effort to establish population estimates, not just presence or absence.

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GBV (2020b). *River Thames Scheme Capacity Improvements and Flood Channel Project. Phase One Habitat Survey for Chertsey Meads.*

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GBV (2020d). *River Thames Scheme Capacity Improvements and Flood Channel Project. Phase One Habitat Survey for Desborough Island.*

GBV (2020e). *River Thames Scheme Capacity Improvements and Flood Channel Project. Phase One Habitat Survey for Funky Footprints.*

GBV (2020f). *River Thames Scheme Capacity Improvements and Flood Channel Project. Phase One Habitat Survey for Ham Lands.*

GBV (2020g). *River Thames Scheme Capacity Improvements and Flood Channel Project. Phase One Habitat Survey for Hurst Park.*

GBV (2020h). *River Thames Scheme Capacity Improvements and Flood Channel Project. Phase One Habitat Survey for Laleham Reach.*

GBV (2020i). *River Thames Scheme Capacity Improvements and Flood Channel Project. Phase One Habitat Survey for Land between Desborough Cut and Engine River.*

GBV (2020j). *River Thames Scheme Capacity Improvements and Flood Channel Project. Phase One Habitat Survey for Land South of Chertsey Road.*

GBV (2020k). *River Thames Scheme Capacity Improvements and Flood Channel Project. Phase One Habitat Survey for Land South of Wraysbury Reservoir.*

GBV (2020l). *River Thames Scheme Capacity Improvements and Flood Channel Project. Phase One Habitat Survey for Ockwells Park.*

GBV (2020m). *River Thames Scheme Capacity Improvements and Flood Channel Project. Phase One Habitat Survey for Penton Hook Marina.*

GBV (2020n). *River Thames Scheme Capacity Improvements and Flood Channel Project. Phase One Habitat Survey for Sheepwalk East.*

GBV (2021). *River Thames Scheme Capacity Improvements and Flood Channel Project. Phase One Habitat Validation Survey for the Flood Channel and Weirs.*

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Datasets

- Surrey Biodiversity Information Centre (SBIC). Datasets for statutory and non-statutory designated sites, protected species and rare records.
- Thames Valley Environmental Records Centre (TVERC). Datasets for statutory and non-statutory designated sites, protected species and rare records
- Greenspace Information for Greater London (GiGL). Datasets for statutory and non-statutory designated sites, protected species and rare records.

APPENDICES

Appendix A - Survey Map

Figure Number	Description of Figure
ENVIMSE500260-CBI-ZZ-3ZZ-DR-EN-00004	GCN Survey Scope Map
ENVIMSE500260-CBI-ZZ-3ZZ-DR-EN-00067	GCN Ponds Surveyed Map

Appendix B - Survey Details

Pond No.	Location	HSI Score	HSI Category	eDNA Result	Comments	Present (Y/N)
1	Royal Hythe	0.3	Poor	N/A	No further surveys required.	N
2	Royal Hythe	0.45	Poor	N/A	No further surveys required.	N
2a	Norlands Lane	0.5	Below average	Negative	N/A	N
3	Norlands Lane	0.48	Poor	N/A	Conducted HSI assessment from public right of way path. No further surveys required.	N
4	Norlands Lane	0.55	Below average	Negative	N/A	N
5	Norlands Lane	N/A	N/A	N/A	Connected to a large lake with confirmed fish stock and running waterbody. Not suitable for HSI.	N
6	Thorpe Park Lakes and adjacent land	0.66	Average	Negative	N/A	N
7	Norlands Lane	0.69	Average	Negative	N/A	N
8	Norlands Lane	0.57	Below average	N/A	Site manager informed surveyors it would not be safe to eDNA.	N
9	Thorpe Park Lakes and adjacent land	0.19	Poor	N/A	Waterbody is connected to running water and a lake with confirmed fish stock. No further surveys required.	N
10	Near Thorpe Park Lakes and adjacent land	N/A	N/A	N/A	Not part of Thorpe Park. No land access.	N
11	Near Thorpe Park Lakes and adjacent land	0.5633	Below average	Negative	N/A	N
12	Former Laleham Golf Course	0.74	Good	Negative	N/A	N
13	Littleton North Lake and surrounding area	N/A	N/A	N/A	Dry ditch. Not suitable for HSI.	N
14	Littleton North Lake and surrounding area	0.21	Poor	N/A	Ditch that is partially dry. Section is partially wet with common reed. No further surveys required.	N
15	Abbey Mead	0.5639	Below Average	N/A	Pond almost divided into two by vegetation strip. Could not access to view smaller section. Very limited safe access to water due to steep banks, not suitable for eDNA survey.	N
16	Abbey Mead	N/A	N/A	N/A	Dry upon survey. Not suitable for HSI.	N
17	Abbey Mead	0.36	Poor	N/A	Waterbody is potentially larger in wet season, as majority was dry at time of	N

Pond No.	Location	HSI Score	HSI Category	eDNA Result	Comments	Present (Y/N)
					survey. No further surveys required.	
18	Abbey River confluence with River Thames	0.23	Poor	N/A	Pond is completely sealed with fine netting and ornamental fish. No vegetation coverage except one ornamental plant. No further surveys required.	N
19	Abbey River confluence with River Thames	0.68	Average	Negative	N/A	N
21	Littleton North Lake and surrounding area	N/A	N/A	N/A	No pond present.	N
22	Abbey River confluence with River Thames	N/A	N/A	N/A	No land access.	N
23	Abbey River confluence with River Thames	N/A	N/A	N/A	No land access.	N
24	Abbey River confluence with River Thames	N/A	N/A	N/A	No land access.	N
25	Funky Footprints	N/A	N/A	N/A	Not surveyed separately because it is considered to be part of pond 231.	N
26	Funky Footprints / Manor Farm	0.84	Excellent	N/A	Long body of water near the school. Large portion was inaccessible so not suitable for eDNA sampling.	N
27	Manor Farm	0.53	Below average	N/A	Ditch with water flow adjacent to park and road, with litter. Not suitable for eDNA survey.	N
28	Manor Farm	0.4953	Poor	N/A	Not much water left, highly likely to dry out. No further surveys required.	N
29	Manor Farm	N/A	N/A	N/A	Running water ditch connecting into the River Thames. Not suitable for HSI.	N
30	Manor Farm	0.7862	Average	N/A	Within private land but viewable from public pavement. Wildlife pond within school grounds, unable to eDNA survey due to private property.	N
31	Manor Farm	N/A	N/A	N/A	Dry ditch, not suitable for HSI.	N
32	Manor Farm	N/A	N/A	N/A	No land access.	N
33	Manor Farm	N/A	N/A	N/A	Unable to survey pond as in residential area. Newts likely unable to leave area due to high parameters 3m wall. Could not conduct HSI.	N
34	Desborough island	N/A	N/A	N/A	Dry ditch other than single 6m stagnant puddle, very shallow. No further surveys required.	N

Pond No.	Location	HSI Score	HSI Category	eDNA Result	Comments	Present (Y/N)
35	Area west of Sheepwalk	N/A	N/A	N/A	No land access.	N
36	Land south of Chertsey road	N/A	N/A	N/A	Dry upon survey, not suitable for HSI.	N
37	Ferry Lane Lake (Ferris Meadow)	N/A	N/A	N/A	Dry ditch with no water, not suitable for HSI.	N
38	Ferry Lane Lake (Ferris Meadow)	N/A	N/A	N/A	No land access.	N
39	Desborough Island	N/A	N/A	N/A	No pond present. Dry and hard standing area by building.	N
40	Desborough Island	N/A	N/A	N/A	No pond present. Grassy area.	N
41	Desborough Island	N/A	N/A	N/A	Dry upon survey. Not suitable for HSI.	N
46	Littleton North Lake and surrounding area	0.28	Poor	N/A	No further surveys required.	N
47	Littleton East Lake	0.31	Poor	N/A	No further surveys required.	N
215	Land south of Wraysbury Reservoir	N/A	N/A	N/A	No land access.	N
215a	Land south of Wraysbury Reservoir	N/A	N/A	N/A	No land access.	N
216	Land south of Wraysbury Reservoir	N/A	N/A	N/A	No land access.	N
218	Land south of Wraysbury Reservoir	0.44	Poor	N/A	No further surveys required.	N
219	Land south of Wraysbury Reservoir	N/A	N/A	N/A	Dry ditch. Not suitable for HSI.	N
220	Land south of Wraysbury Reservoir	N/A	N/A	N/A	No land access.	N
221	Laleham Reach	0.42	Poor	N/A	No further surveys required.	N
222	Former Laleham Golf Course	0.25	Poor	N/A	Ditch and pond connected via pipe and trench. Full size not properly mapped. No further surveys required.	N
223	Former Laleham Golf Course	N/A	N/A	N/A	Dry upon survey. Not suitable for HSI.	N
225	Penton Hook	N/A	N/A	N/A	Council owned, not responding to access. No land access.	N
226	Manor Farm	0.51	Below average	Negative	N/A	N
227	Sheepwalk	0.674	Average	N/A	Large waterbody with HSI potential. However, over 80% was not accessible for eDNA survey.	N

Pond No.	Location	HSI Score	HSI Category	eDNA Result	Comments	Present (Y/N)
228	Area west of Sheepwalk	0.79	Average	Negative	N/A	N
229	Area west of Sheepwalk	N/A	N/A	N/A	No land access.	N
230	Area west of Sheepwalk	0.44	Poor	N/A	No further surveys required.	N
231	Funky Footprints	0.6044	Average	Negative	Knotweed in the area – spraying in progress. Used to be a historical fishing lake.	N
232	Land south of Chertsey road	N/A	N/A	N/A	No land access.	N
233	Land south of Chertsey road	0.71	Average	Negative	N/A	N
234	Land south of Chertsey road	0.44	Poor	N/A	No further surveys required.	N
235	Land south of Chertsey road	N/A	N/A	N/A	No land access, residential garden.	N
236	Land south of Chertsey road	0.5957	Below average	Negative	N/A	N
237	Land south of Chertsey road	N/A	N/A	N/A	No land access, residential garden.	N
237a	Land south of Chertsey road	0.4	Poor	N/A	Reed bed, done from fence side. No further surveys required.	N
248	Desborough Island	0	Poor	N/A	Settlement lagoon on Affinity water site so has pumped inflow and outflow. Pond drained every 3 years. No further surveys required.	N
249	Desborough Island	0	Poor	N/A	Concrete open pipe used as emergency drain if pumps fail and for one filter tank. No further surveys required.	N
251	Land between Desborough cut and Engine River	N/A	N/A	N/A	Completely dry. Shallow ditch depression. Not suitable for HSI.	N
252	Abbey 1 & 2 Lakes	N/A	N/A	N/A	Dry ditch, Not suitable for HSI. Unrecorded ditch added.	N
253	Littleton Lane	0.2	Poor	N/A	Unrecorded ditch added. No further surveys required.	N
254	Littleton Lane	N/A	N/A	N/A	Dry swamp recorded during PEA. Not suitable for HSI. Unrecorded swamp added.	N
255	Littleton Lane	0.23	Poor	N/A	Heavily obscured water, signs of pollution. Unrecorded ditch added. No further surveys required.	N
256	Sheepwalk	0.25	Poor	N/A	Unrecorded pond added. No further surveys required.	N

Appendix C - Survey Photographs

Plate 1. Pond 26 is a long body of water and scored excellent in HSI assessment but unsuitable for eDNA due to large portion of area inaccessible.

Plate 2. Pond 26 is a long body of water and scored excellent in HSI assessment but unsuitable for eDNA due to large portion of area inaccessible.

Plate 3. Pond 27 is a ditch with water flow, unsuitable for eDNA survey.

Plate 4. Pond 27 is a ditch with water flow, unsuitable for eDNA survey.

Plate 5. Pond 28 did not have much water left upon survey and highly likely to dry out.

Plate 6. Pond 29 is a ditch with running water connecting to the River Thames. Not suitable for HSI assessment.

Plate 7. Pond 31 is a dry ditch, unable to conduct HSI assesment.

Plate 8. Pond 31 is a dry ditch, unable to conduct HSI assesment.

Plate 9. Pond 31 is a dry ditch, unable to conduct HSI assesment.

Plate 10. Pond 33 has a 3m high wall around pond. Unable to conduct HSI assessment.

Plate 11. Pond 33 has a 3m high wall around pond. Unable to conduct HSI assessment.

Plate 12. Pond 34 is a dry ditch with the exception of a single 6m stagnant puddle, which is very shallow. Unsuitable for HSI assessment.

Plate 13. Pond 37 is a dry ditch. Unsuitable for HSI assessment.

Plate 14. Pond 41 was dry upon survey. Unsuitable for HSI assessment.

Appendix D - eDNA Test Laboratory Results

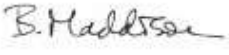
Client: Lucy Robertson,
Binnies

ADAS
Spring Lodge
172 Chester Road
Helsby
WA6 0AR

Tel: 01159 229249
Email: Helen.Rees@adas.co.uk

www.adas.uk

Sample ID: ADAS-218	Condition on Receipt: Good	Volume: Passed
Client Identifier: Pond 2a	Description: pond water samples in preservative	
Date of Receipt: 26/04/2023	Material Tested: eDNA from pond water samples	

Determinant	Result	Method	Date of Analysis
Inhibition Control[†]	2 of 2	Real Time PCR	28/04/2023
Degradation Control[§]	Within Limits	Real Time PCR	28/04/2023
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	28/04/2023
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:	02/05/2023	Date of issue:	02/05/2023

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: Lucy Robertson,
Binnies

ADAS
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Tel: 01159 229249
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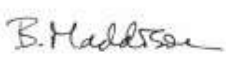
Sample ID: ADAS-226	Condition on Receipt: White Precipitate	Volume: Passed
Client Identifier: RTS 4	Description: pond water samples in preservative	
Date of Receipt: 26/04/2023	Material Tested: eDNA from pond water samples	

Determinant	Result	Method	Date of Analysis
Inhibition Control[†]	1 of 2	Real Time PCR	02/05/2023
Degradation Control[§]	Within Limits	Real Time PCR	02/05/2023
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	02/05/2023
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:	02/05/2023	Date of issue:	02/05/2023

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: Lucy Robertson,
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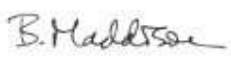
Sample ID: ADAS-225	Condition on Receipt: Good	Volume: Passed
Client Identifier: RTS 6	Description: pond water samples in preservative	
Date of Receipt: 26/04/2023	Material Tested: eDNA from pond water samples	

Determinant	Result	Method	Date of Analysis
Inhibition Control†	2 of 2	Real Time PCR	02/05/2023
Degradation Control§	Within Limits	Real Time PCR	02/05/2023
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	02/05/2023
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:	02/05/2023	Date of issue:	02/05/2023

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: ADAS-223	Condition on Receipt: Good	Volume: Passed
Client Identifier: Pond 7	Description: pond water samples in preservative	
Date of Receipt: 26/04/2023	Material Tested: eDNA from pond water samples	

Determinant	Result	Method	Date of Analysis
Inhibition Control[†]	2 of 2	Real Time PCR	28/04/2023
Degradation Control[§]	Within Limits	Real Time PCR	28/04/2023
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	28/04/2023
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:	02/05/2023	Date of issue:	02/05/2023

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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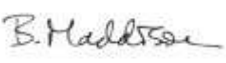
Sample ID: ADAS-217	Condition on Receipt: Low Sediment	Volume: Passed
Client Identifier: RTS Pond 11	Description: pond water samples in preservative	
Date of Receipt: 02/05/2023	Material Tested: eDNA from pond water samples	

Determinant	Result	Method	Date of Analysis
Inhibition Control[†]	2 of 2	Real Time PCR	04/05/2023
Degradation Control[§]	Within Limits	Real Time PCR	04/05/2023
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	04/05/2023
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:	05/05/2023	Date of issue:	05/05/2023

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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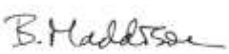
Sample ID: ADAS-214	Condition on Receipt: Good	Volume: Passed
Client Identifier: Pond 12	Description: pond water samples in preservative	
Date of Receipt: 26/04/2023	Material Tested: eDNA from pond water samples	

Determinant	Result	Method	Date of Analysis
Inhibition Control[†]	2 of 2	Real Time PCR	02/05/2023
Degradation Control[§]	Within Limits	Real Time PCR	02/05/2023
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	02/05/2023
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:	02/05/2023	Date of issue:	02/05/2023

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: ADAS-224	Condition on Receipt: Good	Volume: Passed
Client Identifier: Pond 19	Description: pond water samples in preservative	
Date of Receipt: 26/04/2023	Material Tested: eDNA from pond water samples	

Determinant	Result	Method	Date of Analysis
Inhibition Control†	2 of 2	Real Time PCR	02/05/2023
Degradation Control§	Within Limits	Real Time PCR	02/05/2023
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	02/05/2023
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:	02/05/2023	Date of issue:	02/05/2023

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: Lucy Robertson,
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172 Chester Road
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Tel: 01159 229249
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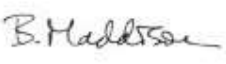
Sample ID: ADAS-234	Condition on Receipt: Low Sediment	Volume: Passed
Client Identifier: Pond 226	Description: pond water samples in preservative	
Date of Receipt: 26/04/2023	Material Tested: eDNA from pond water samples	

Determinant	Result	Method	Date of Analysis
Inhibition Control[†]	2 of 2	Real Time PCR	28/04/2023
Degradation Control[§]	Within Limits	Real Time PCR	28/04/2023
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	28/04/2023
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:	02/05/2023	Date of issue:	02/05/2023

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.

. Sample ID: ADAS-218231

Client: Lucy Robertson,
Binnies

ADAS
Spring Lodge
172 Chester Road
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WA6 0AR

Tel: 01159 229249
Email: Helen.Rees@adas.co.uk

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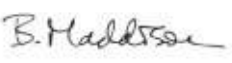
Sample ID: ADAS-231	Condition on Receipt: Good	Volume: Passed
Client Identifier: 228	Description: pond water samples in preservative	
Date of Receipt: 09/05/2023	Material Tested: eDNA from pond water samples	

Determinant	Result	Method	Date of Analysis
Inhibition Control[†]	2 of 2	Real Time PCR	10/05/2023
Degradation Control[§]	Within Limits	Real Time PCR	10/05/2023
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	10/05/2023
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:	12/05/2023	Date of issue:	12/05/2023

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: Lucy Robertson,
Binnies

ADAS
Spring Lodge
172 Chester Road
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WA6 0AR

Tel: 01159 229249
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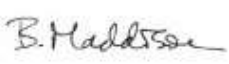
www.adas.uk

Sample ID: ADAS-232	Condition on Receipt: Good	Volume: Passed
Client Identifier: RTS 231	Description: pond water samples in preservative	
Date of Receipt: 02/05/2023	Material Tested: eDNA from pond water samples	

Determinant	Result	Method	Date of Analysis
Inhibition Control[†]	2 of 2	Real Time PCR	04/05/2023
Degradation Control[§]	Within Limits	Real Time PCR	04/05/2023
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	04/05/2023
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:	05/05/2023	Date of issue:	05/05/2023

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: Lucy Robertson,
Binnies

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Tel: 01159 229249
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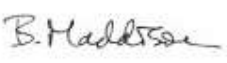
Sample ID: ADAS-233	Condition on Receipt: Good	Volume: Passed
Client Identifier: Pond 233	Description: pond water samples in preservative	
Date of Receipt: 26/04/2023	Material Tested: eDNA from pond water samples	

Determinant	Result	Method	Date of Analysis
Inhibition Control[†]	2 of 2	Real Time PCR	02/05/2023
Degradation Control[§]	Within Limits	Real Time PCR	02/05/2023
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	02/05/2023
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:	02/05/2023	Date of issue:	02/05/2023

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: Lucy Robertson,
Binnies

ADAS
Spring Lodge
172 Chester Road
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Email: Helen.Rees@adas.co.uk

www.adas.uk

Sample ID: ADAS-228	Condition on Receipt: Low Sediment	Volume: Passed
Client Identifier: RTS Pond 236	Description: pond water samples in preservative	
Date of Receipt: 02/05/2023	Material Tested: eDNA from pond water samples	

Determinant	Result	Method	Date of Analysis
Inhibition Control[†]	2 of 2	Real Time PCR	04/05/2023
Degradation Control[§]	Within Limits	Real Time PCR	04/05/2023
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	04/05/2023
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:	05/05/2023	Date of issue:	05/05/2023

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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.

ADAS Guidance notes: 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:

- Evidence of decay - meaning that the degradation control was outside of accepted limits 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 no

Appendix E - Legislation

Note that the details provided in this appendix are for general guidance only and should not be relied upon as a definitive statement of the law. The legislation is applicable in Britain only (i.e. not the Isle of Man, the Republic of Ireland or the Channel Islands.). Only legislation applicable to this scheme is provided here.

Great crested newt

Great crested newt *Triturus cristatus* are fully protected under the Conservation of Habitats and Species Regulations 2019 as they are listed on Schedule 2 which makes it an offence to:

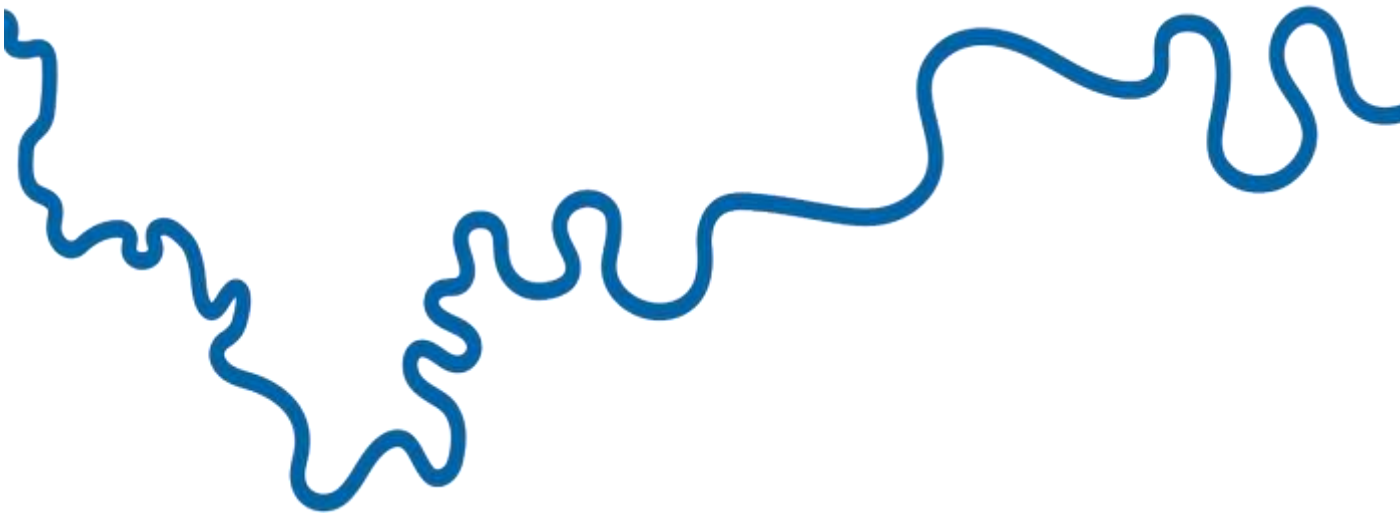
- Intentionally capture, kill or injure a great crested newt;
- Deliberate disturbance of any species as:
    - to impair their ability:
        - to survive, breed, or reproduce, or to rear or nurture young; or
        - in the case of animals of a hibernating or migratory species, to hibernate or migrate.
    - to affect significantly the local distribution or abundance of the species;
- Damage or destruction of a breeding site or resting place; or
- Deliberate taking or destroying of the eggs of great crested newts;
- Keeping, transporting, selling, exchanging or offering for sale whether live or dead or of any part thereof.

Great crested newts are afforded additional legal protection under Schedule 5 of the Wildlife and Countryside Act 1981 (as amended) which makes it an offence to:

- Intentional or reckless disturbance (at any level);
- Intentional or reckless obstruction of access to any place of shelter or protection; or
- Selling, offering or exposing for sale, possession or transporting for purpose of sale.

Impacts of legislation on development works

An EPSM Licence issued by the relevant countryside agency (e.g. Natural England) will be required for works liable to affect great crested newts, their breeding site or resting places, or for operations likely to result in a level of disturbance which might impair their ability to undertake those activities mentioned above (e.g. survive, breed, and rear young). The licence is to allow derogation from the relevant legislation but also to enable appropriate mitigation measures to be put in place and their efficacy to be monitored.



SURREY COUNTY COUNCIL

Environment Agency

The River Thames Scheme, delivered in a partnership led by the Environment Agency and Surrey County Council, will reduce flood risk for residents and businesses and improve the surrounding area.