Legend

Habitats

- Site Boundary
- B5 - Marsh/marshy grassland
- C3.1 - Other tall herb and fern - ruderal
- G1 - Standing water
- J1.2 - Cultivated/disturbed land - amenity grassland
- J2.1.1 - Intact hedge - native species-rich
- J2.1.2 - Intact hedge - species-poor
- Bat Roost Potential Trees - T1 T2 T3 T4 T5 T6
- Target Notes

0 20 40 m

N

APEM	P9809
Figure 4 Carreghofa - PEA Habitat Map	
Canals and Rivers Trust	30/11/2022

(Overleaf)

Target Notes

1 Reptile mat

Appendix 2 – Site Photographs

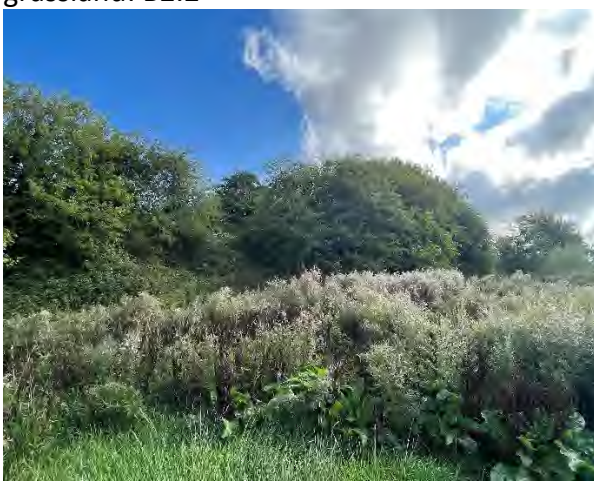
Wern

Photograph 1: Dense Scrub: A2.1

Photograph 2: Scattered trees: A3.1

Photograph 3: Semi-improved neutral grassland: B2.2

Photograph 4: Swamp: F1



Photograph 5: Tall ruderal: C3.1

Photograph 6: Species rich intact hedgerow: J2.1.1

Photograph 7: Species poor intact hedgerow: J2.1.2

N/A

Photograph 8: Species rich Defunct hedgerow: J2.2.1

Photograph 9: Standing water: G1

Carreghofa

Photograph 10: Scattered trees: A3

Photograph 11: Marshy grassland: B5

Photograph 12: Tall ruderal: C3.1

Photograph 13: Standing water: G1

Photograph 14: Species rich intact hedgerow: J2.1.1

Photograph 15: Species poor intact hedgerow: J2.1.2

Photograph 16: Arable field: J1.1

Appendix 3 – Relevant Legislation

An overview of the legislation protecting wild animals and plants relevant to the Site is provided below.

Bats

In the United Kingdom (UK) all bat (*Chiroptera* spp.) species and their roosts are legally protected, by national legislation. This protection is detailed in the Wildlife and Countryside Act 1981 (as amended) (HMSO, 1981)and the Conservation of Habitats and Species Regulations 2019 (amendment (EU Exit)) (HMSO, 2019).

Together these pieces of legislation make it a criminal offence to:

- Deliberately take, injure or kill a wild bat;
- Intentionally or recklessly disturb a bat in its roost or deliberately disturb a group of bats;
- Damage or destroy a place used by bats for breeding or resting (roosts) (even if bats are not occupying the roost at the time);
- Possess or advertise/ sell/ exchange a bat of a species found in the wild (dead or alive) or any part of a bat; and
- Intentionally or recklessly, obstruct access to a bat roost.

Badgers

Badgers are protected and so are the setts they live in. Under the Protection of Badgers Act 1992, in England and Wales it is an offence to:

- Wilfully kill, injure or take a badger (or attempt to do so).
- Cruelly ill-treat a badger.
- Dig for a badger.
- Intentionally or recklessly damage or destroy a badger sett, or obstruct access to it.
- Cause a dog to enter a badger sett.
- Disturb a badger when it is occupying a sett.

Reptiles

Reptiles (adder, grass snake, common lizard and slow worm) are protected through Section 9(1) of the Wildlife & Countryside Act 1981 (as amended) against intentional killing and injuring (note the provision in Section 9(1) of Wildlife & Countryside Act 1981 prohibiting “taking” does not apply to reptiles).

Hedgehog

Hedgehogs have some degree of legal protection in the UK:

- they are listed on schedule 6 of the Wildlife and Countryside Act (1981) which makes it illegal to kill or capture wild hedgehogs, with certain methods listed
- they are also listed under the Wild Mammals Protection Act (1996), which prohibits cruel treatment of hedgehogs
- They are a species of 'principal importance' under the NERC Act (2006) and Environment Wales Act (2016) which is meant to confer a 'duty of responsibility' to public bodies.

Wild Birds

Nesting and nest building birds are protected under the Wildlife and Countryside Act (HMSO, 1981). It is an offence to:

- Intentionally kill, injure or take any wild bird;
- Take, damage or destroy the nest of any wild bird when it is in use or is being built;
- Take or destroy an egg of any wild bird.

Some bird species are listed on Schedule 1 of this act, making it an offence to intentionally or recklessly disturb birds and their young at, on or near an 'active' nest.

Hedgehog

Hedgehogs have some degree of legal protection in the UK:

- they are listed on schedule 6 of the Wildlife and Countryside Act (1981) which makes it illegal to kill or capture wild hedgehogs, with certain methods listed
- they are also listed under the Wild Mammals Protection Act (1996), which prohibits cruel treatment of hedgehogs
- They are a species of 'principal importance' under the NERC Act (2006) and Environment Wales Act (2016) which is meant to confer a 'duty of responsibility' to public bodies.

Common amphibians

Native amphibians are protected under the Animal Welfare Act 2006. This states that is an offence to cause unnecessary suffering to an animal.

The four widespread species of amphibian, the smooth and palmate newts, the common frog and common toad, are protected only by Section 9(5) of the Wildlife and Countryside Act 1981 (as amended). This section prohibits sale, barter, exchange, transporting for sale and advertising to sell or to buy.

Otter

Otters (*Lutra lutra*) are fully protected as a European protected species under listed under Annex II of the Habitats Directive and under sections 9 and 11 of the Wildlife and Countryside Act 1981 (HMSO, 1981).

It is an offence to:

- capture, kill, disturb or injure otters (on purpose or by not taking enough care);
- damage or destroy a breeding or resting place (deliberately or by not taking enough care);
- obstruct access to their resting or sheltering places (deliberately or by not taking enough care); and.
- possess, sell, control or transport live or dead otters, or parts of otters.

Sites of Special Scientific Interest (SSSI)

SSSIs are the most important sites for Wales' natural heritage. They are highly protected to safeguard the range, quality and variety of habitats, species and geological features in all parts of Wales. They are the cornerstones of conservation work, protecting the core of natural heritage.

Each SSSI has a list of activities that NRW think are likely to damage the site's special interest.

Before you carry out, or allow someone else to carry out, activities on that list, you must notify NRW in writing and obtain our consent. You should include what you propose to do, and give details about where, when and how it will be carried out.

European sites - Natura 2000

The European Union have identified the most important sites for wildlife in Europe as the Natura 2000 sites. There are two types of Natura 2000 sites:

- Special Protection Areas - designated because of rare or migratory birds and their habitats
- Special Areas of Conservation - for a wide range of habitats and species other than birds

The Special Protection Areas (SPAs) in Wales are areas that have been designated specifically to conserve wild birds that are listed as rare and vulnerable in the Birds Directive. They also include the sites in Wales that migratory birds use as stop-off points on their journeys across the planet.

The Special Areas of Conservation (SACs) have been chosen to make a significant contribution to conserving habitats and wildlife species that live there, named in the EC Habitats Directive.

Marine SACs are also being developed to protect marine habitats and species.

Appendix E: GCN and WCC Report

Memo

SUBJECT
Great Crested Newts and White Clawed Crayfish – Montgomery Canal

TO
Evie Challinor, Sara James

DATE
15/09/2023

DEPARTMENT
Ecology (Environmental Planning)

PROJECT NUMBER
10048826

COPIES TO
Donna Ryan

FROM
Brandon Murray
07809230662

R.E. Great Crested Newt and White Clawed Crayfish Survey 2022 / 2023

This letter reports the findings of the great crested newt and white clawed crayfish surveys conducted in relation to the proposed works of bridge construction and creation of nature reserves along the Montgomery Canal. Specifically, this report relates to an assessment of potential impacts to great crested newts which may result from the creation of Nature reserves and from the construction of Walls Bridge and Carreghofa Lane Bridge by The Canal and River Trust. Mitigation measures are outlined where appropriate.

Introduction

Arcadis was commissioned in 2022 by the Canal and River Trust to conduct ecological assessments necessary to inform a proposed scheme of works to build two new bridges to permit boat traffic along the Montgomery Canal and build three reserves (at the time of writing reduced to two reserves) to provide compensatory habitat to permit the restoration of boat traffic along the Montgomery Canal. As a component of this, it was necessary to conduct surveys for great crested newt (GCN) and white clawed crayfish (WCC) in order to assess the potential impacts from the proposed works.

Assessments were conducted in relation to ponds and water bodies identified within the potential zone of influence (ZOI) of the works.

This report presents results from the 2022 surveys for GCN, update surveys for GCN conducted in 2023 and White Clawed Crayfish surveys conducted on the canal in 2023. The 2023 surveys were modified to account for a reduction in scope (as the proposed Carreghofa reserve was removed form the proposals).

Methodology

Great Crested Newts

In line with current guidelines, ponds within 500 metres of the proposed works were identified from aerial mapping. Ponds were identified using Ordnance Survey mapping. Initially, 25 ponds were identified from mapping as potentially requiring assessment. Ponds identified are presented in Figure 1, Appendix F (numbered 1 – 23 (with 21a and 22a)). Other ponds present on the ordinance survey mapping that were not assessed were either over 500m from the proposed works, hydrologically connected to ponds that were assessed / sampled or separated from the proposed works by significant barriers to great crested newt dispersal.

Where possible, ponds were initially assessed on site through HSI (habitat suitability index) assessment methodology which allocates each pond a condition and therefore likelihood of supporting great crested newt. Following this, where it was safe to do so, water samples were collected from the ponds and eDNA assessments were conducted. eDNA assessments identify the presence of great crested newt DNA within the pond water (a description of the eDNA survey technique is presented in Appendix A). The same process was used and eDNA assessments were conducted to identify the presence of white clawed crayfish, signal crayfish and crayfish plague in two sites, Williams Bridge and Carreghofa Lane Bridge. The HSI assessments and the eDNA samples for great crested newts were collected by suitably qualified ecologists on the following days:

- 16/06/2022; and 17/06/2022.
- 12/06/2023; and 13/06/2023.

White Clawed Crayfish

Two sites were identified from mapping as potentially requiring assessment for white clawed crayfish presence. The sites identified are presented in Appendix F. eDNA assessments were conducted to identify the presence of white clawed crayfish, signal crayfish and crayfish plague in two sites, Williams Bridge and Carreghofa Lane Bridge. The eDNA samples for white clawed crayfish were collected by suitably qualified ecologists on the following day:

- 12/06/2023

Samples were taken from canal water at the locations of Williams and Carreghofa Lane Bridge, at OSGR SJ 2535919849 and SJ 26270 20814 respectively. Both locations are connected by the Montgomery Canal.

Limitations

It was not possible to access all 25 ponds initially identified for survey. In 2022, of the 25 ponds initially identified, nine could not be accessed, and of the remaining 16, only 14 ponds were suitable for HSI and eDNA assessment. In 2023 11 ponds were not accessed, for various reasons (including two ponds that were removed from the scope as Carreghofa reserve was removed from the proposals). The table below outlines the reason that the water body could not / was not surveyed. The potential impact upon the validity of the overall result due to the omission of the pond from the assessment is also presented.

In 2023, both sites identified for eDNA assessments for the presence of white clawed crayfish were accessed and surveys were conducted. No constraints were identified.

Table 1: Ponds not assessed in one or both of the surveys, rationale and assessment of impact upon the assessment

Pond Number	Reason for no survey in 2022	Reason for no survey in 2023	Assessment of impact upon results and assessment
3	No access obtained. Over 350m from the proposed works	No access obtained. Over 350m from the proposed works	Due to the distance from the proposed works and the nature of the works, the omission of this pond is not considered to impact upon the veracity of the assessments or change the proposed approach to managing risk to GCN.
10	No access obtained. Over 370m from the proposed works	Access Denied	Due to the distance from the proposed works and the nature of the works, the omission of this pond is not considered to impact upon the veracity of the assessments or change the proposed approach to managing risk to GCN.
11	No access obtained. Over 370m from the proposed works	Access Denied	Due to the distance from the proposed works and the nature of the works, the omission of this pond is not considered to impact upon the veracity of the assessments or change the proposed approach to managing risk to GCN.
12	No access obtained. Over 370m from the proposed works	Access Denied	Due to the distance from the proposed works and the nature of the works, the omission of this pond is not considered to impact upon the veracity of the assessments or change the proposed approach to

Pond Number	Reason for no survey in 2022	Reason for no survey in 2023	Assessment of impact upon results and assessment
			managing risk to GCN.
14	No access obtained.	It was confirmed by the landowner that there was no pond in this location	No impact no pond in this location.
15	Accessed	Not surveyed as modified works are not within 500m of the pond	No impact
16	No access obtained. Over 458m from the proposed works	No access obtained. over 500m from the updated proposed works	Due to the distance from the proposed works and the nature of the works, the omission of this pond is not considered to impact upon the veracity of the assessments or change the proposed approach to managing risk to GCN.
18	No access obtained.	Access Denied	This pond is located relatively close to the proposed works. A precautionary assessment of presence will need to be made.
19	No access obtained. Over 500m from the proposed works	Landowner confirmed there is not a pond at this location.	No impact no pond in this location.
22a	Accessed	Not surveyed as presence of GCN confirmed in 2022	Presence confirmed – no further survey needed.
23	No access obtained.	Access obtained	No impact – surveyed in 2023

Where it was assessed that the lack of a survey of a water body could affect the value of the assessment or result in potential impacts to great crested newts, a precautionary assessment of presence was assumed. For each pond where precautionary assessment of presence is made the approach to safeguarding great crested newt in this area will be the same as ponds where presence was confirmed (unless confirmed otherwise at a later date). Through this approach it will be possible to ensure that the conservation status of great crested newt is maintained and therefore does not impact upon the validity of the assessment made in this memo.

Results

Great Crested Newts

Of the 25 ponds initially identified for survey, nine were not surveyed in 2022 or 2023 and of the remaining 16, only 15 ponds were suitable for eDNA assessment. A table presenting the results of the HSI assessment and eDNA surveys is presented below in Table 2. The certificates for the 2022 eDNA water sample testing are provided in Appendix B, and the full HSI assessment results are presented in Appendix C, and the 2023 eDNA surveys are presented in Appendix E.

Table 2: Results of the HSI assessments and eDNA surveys conducted for great crested newts

Pond	HSI Assessment	eDNA Result 2022	eDNA Result 2023
1	Good	Negative	Negative
2	Good	Negative	Negative
4	Excellent	Negative	Negative
5	Good	Negative	Negative
6	Excellent	Negative	Negative
7	Below Average	Negative	Negative
8	N/A Pond Dry		
9	N/A Pond Dry		Negative
13	Below Average	Negative	Negative
15	Good	Negative	N/A not needed (out of impact area)
17	Average	Negative	Negative
20	Good	Negative	Negative
21a	Excellent	Negative	Negative
21	Good	Negative	Negative
22	Good	Negative	Negative
22a	Poor	Positive	N/A not needed - presence confirmed
23	N/A	N/A	Negative

White Clawed Crayfish

Neither of the sampled water bodies had traces of white clawed crayfish eDNA. The results of the assessments are presented below in Table 3. One of the sampled water bodies contained signal crayfish eDNA. White clawed crayfish are out competed by signal crayfish and signal crayfish transfer crayfish plague to white clawed crayfish (although crayfish plague was not detected), they do not tend to be present in the same locations. These results strongly suggest that white clawed crayfish are absent from the two locations sampled.

Table 3: Results of the eDNA surveys conducted for white clawed crayfish

Site name	HSI	White clawed crayfish eDNA Result 2023	Signal crayfish eDNA Result 2023	Crayfish plague eDNA Result 2023
Williams Bridge	N/A	Negative	Positive	Negative
Carreghofa Lane Bridge	N/A	Negative	Negative	Negative

Conclusions

Overall, of the 25 ponds identified from mapping it is assessed that only one pond (22a) has confirmed great crested newt presence, and one pond (18) is assessed as having presence on a precautionary basis until access can be obtained. For works within the vicinity of these ponds, it will be necessary to employ measures to ensure that impacts to great crested newt are managed in such a way that the favourable conservation status of the great crested newt populations can be maintained. The subsequent sections of this report outline an assessment of the potential impact of the proposed works upon great crested newt populations (utilising the rapid risk assessment provided by Natural England within the great crested newt method statement template) and a subsequent section includes recommendations for completing the works whilst safeguarding great crested newt.

Of the two bridge sites identified, both were accessed and surveyed for white clawed crayfish presence. No evidence of WCC was found at either site, and at Williams Bridge, eDNA for signal crayfish was found, reinforcing the evidence that no white clawed crayfish are present.

Rapid risk assessment

The tables below present a rapid risk assessment in relation to the potential impact upon great crested newt. This assumes removal of the areas shown in in pink in Figure 4, Appendix E. The rapid risk assessment tool from Natural England is presented as Image 1[1]. The results of the assessment are presented in Table . An explanation of what the colour coded risk assessment result means is presented in the subsequent section.

Image 1: Example Rapid Risk Assessment from the Natural England method Statement

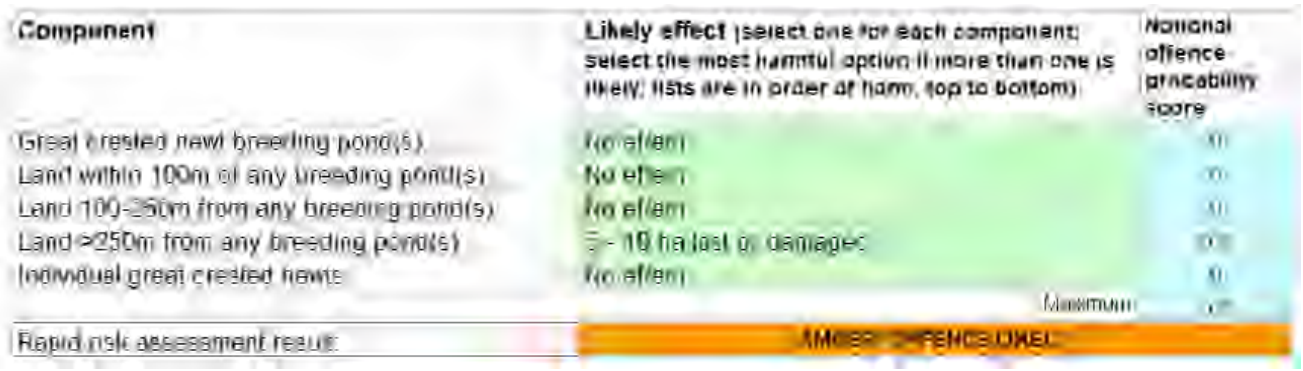


Table 4: Rapid risk assessment output for works

Area	Risk assessment	Advice
In the vicinity of 18	**AMBER: OFFENCE LIKELY**	Reasonable avoidance measures
In the vicinity of 22a	**AMBER: OFFENCE LIKELY**	Reasonable avoidance measures

Recommendations

As shown in the table above (Table), for the ponds that were surveyed (or given a precautionary assessment of presence), which fall within a great crested newt impact zone, the assessed risk of conducting works is amber. An amber assessment as is stated in the section below can be avoided through non-licensed avoidance

1 https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/879595/gcn-method-statement.xlsm

measures.

"Amber: offence likely" indicates that the development activities are of such a type, scale and location that an offence is likely. In this case, the best option is to redesign the development (location, layout, methods, duration or timing; see non-licensed avoidance measures tool) so that the effects are minimised. You can do this and then re-run the risk assessment to test whether the result changes, or preferably run your own detailed site-specific assessment. Bear in mind that this generic risk assessment will over- or under-estimate some risks because it cannot take into account site-specific details, as mentioned in caveats above. In particular, the exact location of the development in relation to resting places, dispersal areas and barriers should be critically examined. Once you have amended the scheme you will need to decide if a licence is required; this should be done if on balance you believe an offence is reasonably likely." (Source: the instructions for the rapid risk assessment tool).

Considering the detail of the proposed works (in both locations this will be the creation of a reserve offering significantly enhanced habitat for GCN) it is considered that a reasonable avoidance approach will be the correct methodology for avoiding impacts in relation to these works. The section below outlines the likely prescriptions for the reasonable avoidance measures. Any reasonable avoidance measures which are required must be secured within a method statement and followed by the appointed contractors at all times. Whenever works are being conducted within the great crested newt impact zones, it will be necessary for an ecological clerk of works to attend the site. An example toolbox talk which the ecological clerk of works would provide to the appointed contractors prior to work commencing is provided in Appendix D.

As WCC are considered absent, no further input in relation to WCC is considered necessary.

Likely Methodology for Reasonable Avoidance Measures

The section below presents an example of the likely reasonable avoidance measures likely to be recommended to be implemented to safeguard great crested newt. The approach will need to be updated and expanded once the timings for the proposed works and the exact details of the construction location and methods are known.

In addition to these measures to be employed in areas where potential presence has been identified, contractors will also need to be aware of the potential presence of great crested newts elsewhere along the route, potentially associated with water bodies that were not identified from the aerial imagery. Details of safeguarding measures to be employed elsewhere along the route will need to be finalised once the details of the proposed works are known.

Example Reasonable Avoidance Measures

Works should be undertaken in the great crested newt (GCN) active season.

Prior to commencement of the works, an Ecological Clerk of Works (ECoW) will liaise with the contractor to clearly demarcate the required working areas, including those required for vehicular access. Where possible, excavations will be located within areas of suboptimal GCN habitat and avoid areas of optimal GCN habitat. Where it is necessary to undertake works within areas of suitable GCN habitat the following precautionary measures will be put in place to avoid encountering and accidentally injuring GCN:

- *Where possible, the ECoW will work with the contractor to microsite the location of the works into habitat less suitable to support sheltering GCN.*
- *Amphibian sheltering features (i.e. log and vegetation piles) will be avoided. If this is not possible, these will be checked by the ECoW before their removal (should this be required).*
- *Where excavation or ground-disturbing works are necessary, an excavator will be used to slowly and gradually strip the upper layer of vegetation and top soil. Deeper excavations will then be made where required. All of these works shall be overseen by the ECoW.*
- *All excavtions left open over night shall be checked in the morning for amphibians. If any are discovered, the ecologist must be contacted.*

Summary

Of the ponds assessed for the potential presence of great crested newt, one pond (22a) has confirmed presence of Great Crested Newts and one pond (18) was given a precautionary assessment of presence. As such, within the vicinity of these ponds, methodologies to safeguard great crested newt will need to be employed during the construction. Of the sites assessed for the potential presence of white clawed crayfish, no sites had confirmed presence and this species is considered absent. No further inputs in relation to WCC is needed.

Appendix A – eDNA information and Protocol

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

eDNA SURVEY PROTOCOL

Kits should be kept at room temperature in an appropriate solvent store, consistent with Home Office regulations.

Kit contents: 1 sterile Whirl-Pak bag; 2 pairs of sterile gloves; 1 sterile sampling ladle; a sample box containing 6 x 50 mL sample tubes two thirds full of preserving fluid (contains alcohol); 1 sterile pipette; 1 protocol sheet.

Please **keep all packaging** as you will require this for couriered return of samples (see instructions emailed upon ordering and overleaf).

Don't go in the water.

- Collect your eDNA water sample before you do any other surveys at the pond.
- Take the sample whilst standing on the pond bank.
- Don't tread in the pond water itself either before or during collection of the DNA water sample as there is a considerable risk of contaminating your pond sample by bringing in Great Crested Newt DNA in mud and water from other areas on your boots and equipment.

Walk around the pond, to identify areas where you can take your eDNA samples

Roughly plan where you will collect the 20 water samples from. The aim is to spread the samples out evenly around the pond edge. The samples should be taken from both open water and vegetated areas if present and if possible should avoid water that is less than 10 cm deep. If you cannot access all areas of the pond, spread the samples out as best you can without entering the water. Existing data shows that eDNA can be patchy depending on where the animals have been. Sampling in many areas considerably increases the chance of collecting their eDNA successfully.

NOTE: Before you take each ladle sample, be sure to mix the pond the water column by gently using the ladle to stir the water from the surface to close to the pond bottom WITHOUT disturbing the mud in the bottom. DNA 'sinks' and so will often be present in larger amounts close to the pond bottom. **It is important not to collect sediment as this may cause inhibition of the PCR analysis which could lead to an inconclusive result** (please see examples of different sediment levels within sampling tubes at http://www.adas.uk/Service/edna-analysis-for-great-crested-newt).

SAMPLE COLLECTION

- Open your kit and put on a pair of gloves.
- Open the sterile Whirl-Pak bag by tearing off the clear plastic strip along the perforated line, then pull the tabs.

Collect 20 samples of 30 mL of pond water from around the pond (in the areas you have already identified) using the sampling ladle (fill the ladle), and empty each sample into the Whirl-Pak bag.

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

SAMPLE PRESERVATION

1. When you have collected your 20 samples, close the bag securely using the top tabs (fold over several times and bend tabs over) and shake the Whirl-Pak bag for 10 seconds. This mixes any DNA across the whole water sample.
2. Put on a fresh pair of gloves to keep the next stage as uncontaminated as possible.
3. Using the clear plastic pipette provided take 15 mL of water from the Whirl-Pak bag, and transfer into one of the six conical tubes containing preserving fluid (i.e. fill tube to the 50 mL mark).
4. **Label the box** containing the six tubes with the date, your name (sampler), the pond name, and grid reference/co-ordinates.

NOTE: Please do not overfill or under fill the tubes.

5. Close the tube and ensure the cap is tight - leaky samples could later contaminate the laboratory with DNA.
6. Shake the tube vigorously for 10 seconds to mix the sample and preservative.
7. Repeat for each of the 6 conical tubes in the kit.
8. Double check that the lids are on tightly if they have leaked during shaking please also wipe the tubes.
9. Empty the remaining water from the whirl-Pack bag back into the pond.
10. Place all used gloves, pipettes, rubbish into the sampling bag and dispose.

If storage of samples is necessary prior to their return please store refrigerated (2-4°C). Samples can be stored in this way for up to 1 month prior to analysis.

RETURNING THE KIT - DROP OFF OPTION

Should you wish to return your items directly to us, they can be dropped off at **Vet School Stores. SVMS, Nottingham University, Sutton Bonington Campus, Loughborough, LE12 5RD**. (please note opening times: 8.30am - 4.00pm Monday-Friday) or outside of these times at **Main Reception** on **College Road**. Please clearly mark your box "FAO Helen Rees: ADAS".

BOOKING YOUR DHL COLLECTION

Please email us at eDNAcouriering@adas.co.uk so we can arrange your collection.

We require the address of where the parcel will be, the number of parcels/number of kits, your contact details and the date of collection. Wherever possible we will try to book the requested date between 9am-5pm. Once we have booked your return we will email you the DHL collection documents, these will need to be printed off and attached to your parcel before your driver arrives. Please use original packaging wherever possible, if alternative packaging is used you **MUST** attach an **LQ label** ( , we send along with your DHL collection documents just in case) and write **UN1170** onto the box or DHL will not transport your parcel. Should you have any problems please call the office on 01159 516747.

Memo

Appendix B – 2022 eDNA results certificates

ARCADIS

Client: Brandon Murray,
Arcadis

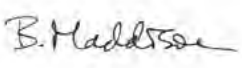
ADAS
Spring Lodge
172 Chester Road
Helsby
WA6 0AR

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

www.adas.uk

Sample ID: ADAS-5420	Condition on Receipt: Low Sediment	Volume: Passed
Client Identifier: 21 Montgomery Canal	Description: pond water samples in preservative	
Date of Receipt: 24/06/2022	Material Tested: eDNA from pond water samples	

Determinant	Result	Method	Date of Analysis
Inhibition Control[†]	2 of 2	Real Time PCR	28/06/2022
Degradation Control[§]	Within Limits	Real Time PCR	28/06/2022
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	28/06/2022
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:	30/06/2022	Date of issue:	30/06/2022

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.

ARCADIS

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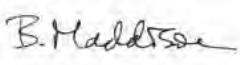
Sample ID: ADAS-5421	Condition on Receipt: Good	Volume: All tubes low volume
Client Identifier: 31A Montgomery Canal	Description: pond water samples in preservative	
Date of Receipt: 24/06/2022	Material Tested: eDNA from pond water samples	

Determinant	Result	Method	Date of Analysis
Inhibition Control[†]	2 of 2	Real Time PCR	28/06/2022
Degradation Control[§]	Within Limits	Real Time PCR	28/06/2022
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	28/06/2022
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:	30/06/2022	Date of issue:	30/06/2022

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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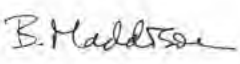
Sample ID: ADAS-5422	Condition on Receipt: Low Sediment	Volume: Passed
Client Identifier: 20 Montgomery Canal	Description: pond water samples in preservative	
Date of Receipt: 24/06/2022	Material Tested: eDNA from pond water samples	

Determinant	Result	Method	Date of Analysis
Inhibition Control[†]	2 of 2	Real Time PCR	28/06/2022
Degradation Control[§]	Within Limits	Real Time PCR	28/06/2022
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	28/06/2022
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:	30/06/2022	Date of issue:	30/06/2022

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-5423	Condition on Receipt: Low Sediment	Volume: Passed
Client Identifier: 17 Montgomery Canal	Description: pond water samples in preservative	
Date of Receipt: 24/06/2022	Material Tested: eDNA from pond water samples	

Determinant	Result	Method	Date of Analysis
Inhibition Control[†]	2 of 2	Real Time PCR	28/06/2022
Degradation Control[§]	Within Limits	Real Time PCR	28/06/2022
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	28/06/2022
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:	30/06/2022	Date of issue:	30/06/2022

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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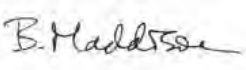
Sample ID: ADAS-5424	Condition on Receipt: Low Sediment	Volume: Passed
Client Identifier: 13 Montgomery Canal	Description: pond water samples in preservative	
Date of Receipt: 24/06/2022	Material Tested: eDNA from pond water samples	

Determinant	Result	Method	Date of Analysis
Inhibition Control[†]	2 of 2	Real Time PCR	28/06/2022
Degradation Control[§]	Within Limits	Real Time PCR	28/06/2022
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	28/06/2022
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:	30/06/2022	Date of issue:	30/06/2022

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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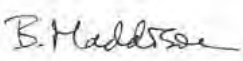
Sample ID: ADAS-5425	Condition on Receipt: Low Sediment	Volume: Passed
Client Identifier: 15 Montgomery Canal	Description: pond water samples in preservative	
Date of Receipt: 24/06/2022	Material Tested: eDNA from pond water samples	

Determinant	Result	Method	Date of Analysis
Inhibition Control[†]	2 of 2	Real Time PCR	28/06/2022
Degradation Control[§]	Within Limits	Real Time PCR	28/06/2022
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	28/06/2022
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:	30/06/2022	Date of issue:	30/06/2022

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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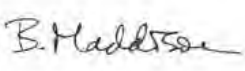
Sample ID: ADAS-5426	Condition on Receipt: Low Sediment	Volume: Passed
Client Identifier: 22 Montgomery Canal	Description: pond water samples in preservative	
Date of Receipt: 24/06/2022	Material Tested: eDNA from pond water samples	

Determinant	Result	Method	Date of Analysis
Inhibition Control[†]	2 of 2	Real Time PCR	28/06/2022
Degradation Control[§]	Within Limits	Real Time PCR	28/06/2022
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	28/06/2022
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:	30/06/2022	Date of issue:	30/06/2022

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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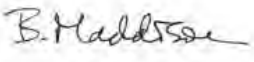
Sample ID: ADAS-5427	Condition on Receipt: Low Sediment	Volume: Passed
Client Identifier: 22A Montgomery Canal	Description: pond water samples in preservative	
Date of Receipt: 24/06/2022	Material Tested: eDNA from pond water samples	

Determinant	Result	Method	Date of Analysis
Inhibition Control[†]	2 of 2	Real Time PCR	27/06/2022
Degradation Control[§]	Within Limits	Real Time PCR	27/06/2022
Great Crested Newt*	2 of 12 (GCN positive)	Real Time PCR	27/06/2022
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:	30/06/2022	Date of issue:	30/06/2022

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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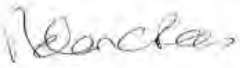
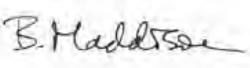
Sample ID: ADAS-5428	Condition on Receipt: Low Sediment	Volume: Passed
Client Identifier: 1 Montgomery Canal	Description: pond water samples in preservative	
Date of Receipt: 24/06/2022	Material Tested: eDNA from pond water samples	

Determinant	Result	Method	Date of Analysis
Inhibition Control[†]	2 of 2	Real Time PCR	27/06/2022
Degradation Control[§]	Within Limits	Real Time PCR	27/06/2022
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	27/06/2022
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:	30/06/2022	Date of issue:	30/06/2022

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** 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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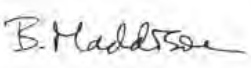
Sample ID: ADAS-5431	Condition on Receipt: Good	Volume: Passed
Client Identifier: 7 Montgomery Canal	Description: pond water samples in preservative	
Date of Receipt: 24/06/2022	Material Tested: eDNA from pond water samples	

Determinant	Result	Method	Date of Analysis
Inhibition Control[†]	2 of 2	Real Time PCR	28/06/2022
Degradation Control[§]	Within Limits	Real Time PCR	28/06/2022
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	28/06/2022
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:	30/06/2022	Date of issue:	30/06/2022

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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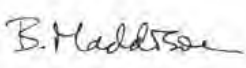
Sample ID: ADAS-5432	Condition on Receipt: Good	Volume: Passed
Client Identifier: 6 Montgomery Canal	Description: pond water samples in preservative	
Date of Receipt: 24/06/2022	Material Tested: eDNA from pond water samples	

Determinant	Result	Method	Date of Analysis
Inhibition Control[1]	2 of 2	Real Time PCR	28/06/2022
Degradation Control[§]	Within Limits	Real Time PCR	28/06/2022
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	28/06/2022
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:	30/06/2022	Date of issue:	30/06/2022

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.*

[1] 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.

Memo

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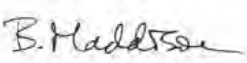
Sample ID: ADAS-5433	Condition on Receipt: Low Sediment	Volume: All tubes low volume
Client Identifier: 4 Montgomery Canal	Description: pond water samples in preservative	
Date of Receipt: 24/06/2022	Material Tested: eDNA from pond water samples	

Determinant	Result	Method	Date of Analysis
Inhibition Control[†]	2 of 2	Real Time PCR	28/06/2022
Degradation Control[§]	Within Limits	Real Time PCR	28/06/2022
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	28/06/2022
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:	30/06/2022	Date of issue:	30/06/2022

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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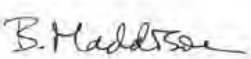
Sample ID: ADAS-5434	Condition on Receipt: Low Sediment	Volume: Passed
Client Identifier: 5 Montgomery Canal	Description: pond water samples in preservative	
Date of Receipt: 24/06/2022	Material Tested: eDNA from pond water samples	

Determinant	Result	Method	Date of Analysis
Inhibition Control[†]	2 of 2	Real Time PCR	28/06/2022
Degradation Control[§]	Within Limits	Real Time PCR	28/06/2022
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	28/06/2022
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:	30/06/2022	Date of issue:	30/06/2022

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.

Memo

ARCADIS

Client: Brandon Murray,
Arcadis

ADAS
Spring Lodge
172 Chester Road
Helsby
WA6 0AR

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

www.adas.uk

Sample ID: ADAS-5435	Condition on Receipt: Low Sediment	Volume: All tubes low volume
Client Identifier: 2 Montgomery Canal	Description: pond water samples in preservative	
Date of Receipt: 24/06/2022	Material Tested: eDNA from pond water samples	

Determinant	Result	Method	Date of Analysis
Inhibition Control[†]	2 of 2	Real Time PCR	28/06/2022
Degradation Control[§]	Within Limits	Real Time PCR	28/06/2022
Great Crested Newt*	0 of 12 (GCN negative)	Real Time PCR	28/06/2022
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:	30/06/2022	Date of issue:	30/06/2022

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

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

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

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

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

Appendix 1: Interpretation of results

Sample Condition

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

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

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

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

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

What do my results mean?

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

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

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

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

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

Memo

Appendix C – Full HSI Results (2022)

Table 4: Pond 1 HSI

SI_1		SI_2		SI_3		SI_4		SI_5		SI_6		SI_7		SI_8		SI_9		SI_{10}		HSI	Suitability	
Location		Pond Area		Pond Drying		Water Quality		Shade		Fowl		Fish		Ponds		Terrestrial Habitat		Macrophytes				
Zone A	1	>2000m2	0.8	Never Dries	0.9	Moderate	0.67	0-60%	1	Minor	0.67	Possible	0.67	6	0.84	Moderate	0.67	26-30%	0.6	0.073124388	0.769850195	**Good**

Table 5: Phis 2 HSI

SI_1		SI_2		SI_3		SI_4		SI_5		SI_6		SI_7		SI_8		SI_9		SI_{10}		hisoduct	HSI	Suitability	
Location		Pond Area		Pond Drying		Water Quality		Shade		Fowl		Fish		Ponds		Terrestrial Habitat		Macrophytes					
Zone A	1	>2000m2	0.8	Never Dries	0.9	Good	1	0-60%	1	Minor	0.67	Minor	0.33	6	0.84	Moderate	0.67	16-20%	0.5	0.044796629	0.733034699	**Good**

Table 6: Pond 4 HIS

SI_1 Location		SI_2 Pond Area		SI_3 Pond Drying		SI_4 Water Quality		SI_5 Shade		SI_6 Fowl		SI_7 Fish		SI_8 Ponds		SI_9 Terrestrial Habitat		SI_{10} Macrophytes		Product	HSI	Suitability
Zone A	1	800m2	0.985	Sometimes Dries	0.5	Good	1	0-60%	1	Absent	1	Absent	1	>12	1	Good	1	16-20%	0.5	0.24625	0.869235838	**Excellent**

Table 7: Pond 5 HSI

SI_1 Location		SI_2 Pond Area		SI_3 Pond Drying		SI_4 Water Quality		SI_5 Shade		SI_6 Fowl		SI_7 Fish		SI_8 Ponds		SI_9 Terrestrial Habitat		SI_{10} Macrophytes		Product	HSI	Suitability
Zone A	1	800m2	0.985	Sometimes Dries	0.5	Poor	0.33	0-60%	1	Absent	1	Minor	0.33	>12	1	Good	1	81-85%	0.95	0.050951588	0.742533021	**Good**

Table 8: Pond 6 HSI

SI_1 Location		SI_2 Pond Area		SI_3 Pond Drying		SI_4 Water Quality		SI_5 Shade		SI_6 Fowl		SI_7 Fish		SI_8 Ponds		SI_9 Terrestrial Habitat		SI_{10} Macrophytes		Product	HSI	Suitability
Zone A	1	>2000m2	0.8	Never Dries	0.9	Moderate	0.67	0-60%	1	Minor	0.67	Possible	0.67	>12	1	Good	1	51-55%	0.85	0.184066956	0.844301543	**Excellent**

Table 9: Pond 7 HSI

SI_1 Location		SI_2 Pond Area		SI_3 Pond Drying		SI_4 Water Quality		SI_5 Shade		SI_6 Fowl		SI_7 Fish		SI_8 Ponds		SI_9 Terrestrial Habitat		SI_{10} Macrophytes		Product	HSI	Suitability
Zone A	1	>2000m2	0.8	Never Dries	0.9	Moderate	0.67	0-60%	1	Minor	0.67	Major	0.01	>12	1	Good	1	61-65%	0.95	0.003070476	0.560687108	**Below Average**

Table 11: pond 13 HSI

SI_1 Location		SI_2 Pond Area		SI_3 Pond Drying		SI_4 Water Quality		SI_5 Shade		SI_6 Fowl		SI_7 Fish		SI_8 Ponds		SI_9 Terrestrial Habitat		SI_{10} Macrophytes		Product	HSI	Suitability
Zone A	1	200m2	0.4	Never Dries	0.9	Good	1	71-75%	0.7	Absent	1	Major	0.01	>12	1	Good	1	6-10%	0.4	0.001008	0.501586747	**Below Average**

Table 12: Pond 15 HSI

SI_1 Location		SI_2 Pond Area		SI_3 Pond Drying		SI_4 Water Quality		SI_5 Shade		SI_6 Fowl		SI_7 Fish		SI_8 Ponds		SI_9 Terrestrial Habitat		SI_{10} Macrophytes		Product	HSI	Suitability
Zone A	1	>2000m2	0.8	Never Dries	0.9	Moderate	0.67	0-60%	1	Minor	0.67	Minor	0.33	>12	1	Good	1	6-10%	0.4	0.042663456	0.729466922	**Good**

Table 13: Pond 17 HSI

SI_1 Location		SI_2 Pond Area		SI_3 Pond Drying		SI_4 Water Quality		SI_5 Shade		SI_6 Fowl		SI_7 Fish		SI_8 Ponds		SI_9 Terrestrial Habitat		SI_{10} Macrophytes		Product	HSI	Suitability
Zone A	1	>2000m2	0.8	Never Dries	0.9	Moderate	0.67	76-80%	0.6	Minor	0.67	Minor	0.33	6	0.84	Good	1	6-10%	0.4	0.021502382	0.681159247	Average

Table 14: Pond 20 HSI

SI_1 Location		SI_2 Pond Area		SI_3 Pond Drying		SI_4 Water Quality		SI_5 Shade		SI_6 Fowl		SI_7 Fish		SI_8 Ponds		SI_9 Terrestrial Habitat		SI_{10} Macrophytes		Product	HSI	Suitability
Zone A	1	>2000m2	0.8	Never Dries	0.9	Moderate	0.67	0-60%	1	Minor	0.67	Minor	0.33	6	0.84	Good	1	96-100%	0.8	0.071674606	0.768310081	Good

Table 15: Pond 21 HSI

SI_1 Location		SI_2 Pond Area		SI_3 Pond Drying		SI_4 Water Quality		SI_5 Shade		SI_6 Fowl		SI_7 Fish		SI_8 Ponds		SI_9 Terrestrial Habitat		SI_{10} Macrophytes		Product	HSI	Suitability

Zone A	1	>2000m2	0.8	Never Dries	0.9	Moderate	0.67	0-60%	1	Minor	0.67	Minor	0.33	6	0.84	Good	1	96-100%	0.8	0.071674606	0.7683 10081	Good

Table 16: Pond 21A HSI

SI_1 Location		SI_2 Pond Area		SI_3 Pond Drying		SI_4 Water Quality		SI_5 Shade		SI_6 Fowl		SI_7 Fish		SI_8 Ponds		SI_9 Terrestrial Habitat		SI_{10} Macrophytes		Product	HSI	Suitability
Zone A	1	>2000m2	0.8	Never Dries	0.9	Moderate	0.67	76-80%	0.6	Minor	0.67	Possible	0.67	6	0.84	Good	1	66-80%	1	0.109140877	0.8013 06645	Excellent

Table 17: Pond 22 HSI

SI_1 Location		SI_2 Pond Area		SI_3 Pond Drying		SI_4 Water Quality		SI_5 Shade		SI_6 Fowl		SI_7 Fish		SI_8 Ponds		SI_9 Terrestrial Habitat		SI_{10} Macrophytes		Product	HSI	Suitability
Zone A	1	>2000m2	0.8	Never Dries	0.9	Moderate	0.67	76-80%	0.6	Minor	0.67	Possible	0.67	6	0.84	Good	1	6-10%	0.4	0.043656351	0.7311 47069	Good

Table 18: Pond 22A HSI

SI_1		SI_2		SI_3		SI_4		SI_5		SI_6		SI_7		SI_8		SI_9		SI_{10}			

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Location		Pond Area		Pond Drying		Water Quality		Shade		Fowl		Fish		Ponds		Terrestrial Habitat		Macrophytes		Product	HSI	Suitability
Zone A	1	>2000 m2	0.8	Never Dries	0.9	Poor	0.33	76-80%	0.6	Minor	0.67	Possible	0.67	6	0.84	Good	1	<1%	0	0	0	**Poor**

Appendix D – Example Toolbox talk

Great crested Newts

Legal Protection
Great crested newts, their breeding habitat and their eggs are protected under the Habitats Directive 2010 (as amended).

Appendix E – 2023 eDNA Certificates

SureScreen Scientifics

Folio No: E18074
Report No: 1
Purchase Order: 12185
Client: APEM Ltd
Contact: Michael Underwood

TECHNICAL REPORT

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

SUMMARY

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

RESULTS

Date sample received at Laboratory: 16/06/2023
Date Reported: 23/06/2023
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
4727	Pond 21A	SJ 22283 06072	Pass	Pass	Pass	Negative	0
4728	Pond 9	SJ 25425 19904	Pass	Pass	Pass	Negative	0
4729	Pond 20	SJ 22194 05994	Pass	Pass	Pass	Negative	0
4730	Pond 22	SJ 22345 00245	Pass	Pass	Pass	Negative	0
4731	Pond	SJ 22271 06042	Pass	Pass	Pass	Negative	0
4732	Pond 13	SJ 25366 20242	Pass	Pass	Pass	Negative	0
4733	Pond 17	SJ 26008 13057	Pass	Pass	Pass	Negative	0

Forensic Scientists and Consultant Engineers
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Company Registration No. 08950940

SureScreen Scientifics

4734	Pond 2	SJ 26310 20047	Pass	Pass	Pass	Negative	0
4735	Pond 6	SJ 25366 19787	Pass	Pass	Pass	Negative	0
4736	Pond 7	SJ 25346 19865	Pass	Pass	Pass	Negative	0
4737	Pond 23	SJ 22234 00105	Pass	Pass	Pass	Negative	0
4738	Pond 1	SJ 26223 20788	Pass	Pass	Pass	Negative	0
4739	Pond 3	SJ 23301 19879	Pass	Pass	Pass	Negative	0
4740	Pond 4	SJ 25307 19773	Pass	Pass	Pass	Negative	0

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

Reported by: Chris Troth **Approved by:** Jackson Young

METHODOLOGY

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

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

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

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

INTERPRETATION OF RESULTS

SIC: **Sample Integrity Check** [Pass/Fail]

SureScreen Scientifics

When samples are received in the laboratory, they are inspected for any tube leakage, suitability of sample (not too much mud or weed etc.) and absence of any factors that could potentially lead to inconclusive results.

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

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

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

SureScreen Scientifics

Folio No: E18087
Report No: 1
Purchase Order: 12185
Client: APEM Ltd
Contact: Michael Underwood

TECHNICAL REPORT

ANALYSIS OF ENVIRONMENTAL DNA SAMPLES FOR THE DETECTION OF CRAYFISH SPECIES AND CRAYFISH PLAGUE

SUMMARY

All organisms continuously release small amounts of environmental DNA (eDNA) into their habitat. By collecting and analysing this eDNA from water samples from lakes, ponds or rivers we can detect the presence or absence of crayfish species including: the white-clawed crayfish (Austropotamobius pallipes), signal crayfish (*Pacifastacus leniusculus*), the marbled crayfish (*Procambarus virginalis*) and the crayfish plague (*Aphanomyces astaci*).

RESULTS

Date sample received at Laboratory: 16/06/2023
Date Reported: 22/06/2023
Matters Affecting Results: None

Lab Sample ID.	Site Name	O/S Reference	Species	Result	SIC	DC	IC	Positive Replicates
FK1273	Williams Bridge	SJ 25359 19849	*White-Clawed Crayfish*	Negative	Pass	Pass	Pass	0
			Signal Crayfish	Positive	Pass	Pass	Pass	5
			Crayfish Plague	Negative	Pass	Pass	Pass	0
FK1274	Walls Bridge	SJ 26270 20814	*White-Clawed Crayfish*	Negative	Pass	Pass	Pass	0
			Signal Crayfish	Negative	Pass	Pass	Pass	0
			Crayfish Plague	Negative	Pass	Pass	Pass	0

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

Reported by: Chris Troth **Approved by:** Jennifer Higginbottom

SureScreen Scientifics

METHODOLOGY

The analysis is conducted in two phases. The sample first goes through an extraction process where the filter is incubated in order to obtain any DNA within the sample. The extracted sample is then tested via real time PCR (also called q-PCR) for each of the selected target species. This process uses species-specific molecular markers (known as primers) to amplify a select part of the DNA, allowing it to be detected and measured in 'real time' as the analytical process develops. qPCR combines amplification and detection of target DNA into a single step. With qPCR, fluorescent dyes specific to the target sequence are used to label targeted PCR products during thermal cycling. The accumulation of fluorescent signals during this reaction is measured for fast and objective data analysis. The primers used in this process are specific to a part of mitochondrial DNA only found in each individual species. Separate primers are used for each of the species: white-clawed crayfish, signal crayfish and crayfish plague, ensuring no DNA from any other species present in the water is amplified.

Analysis of eDNA requires scrupulous attention to detail to prevent risk of contamination. True positive controls, negative controls and spiked synthetic DNA are included in every analysis and these have to be correct before any result is declared and reported. Stages of the DNA analysis are also conducted in different buildings at our premises for added security. These methods have been extensively tested since 2015 in a number of different environments, habitats, conditions and ecological situations in order to successfully enable the full application of eDNA for the detection of crayfish species and the crayfish plague.

RESULTS INTERPRETATION

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

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

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

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

Memo

Appendix F: Figures