Water quality and eDNA survey for Lambley Reed Pond

April 2023

Lambley Reed Pond

Approval Sheet

Customer	Lambley Reed Pond
Project title	Water quality and eDNA Assessment: April 2023
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Version	Date	Prepared by	Signature	Date
Final	13/06/2023	Scott Draper	*Scott Draper*	08/05/2023
		Approved by	Signature	Date
		Mark Jermy	*Mark Jermy*	12/06/2023

Contents

Foreword

Enitial has used its best endeavours, experience, and expertise to provide a meaningful, accurate and relevant representation of any works conducted and information procured. Any works detailed are based on a defined programme and scope of works and any data acquisition and management is in accordance with contract conditions agreed with the Client.

The findings discussed in this document relating to information acquired on behalf of the Client relates only to data and information to which we have had access. It is acknowledged that certain aspects may be superseded or rendered irrelevant by information in documentation to which we have no access.

Enitial cannot accept responsibility to any parties whatsoever, following the issue of this report, for any matters arising which may be considered outside the agreed scope of works.

This report is issued solely to the Client, Enitial does not accept any responsibility to any third parties to whom this report may be circulated, in part or in full, and any such parties rely on the contents at their own risk.

Executive Summary

Enitial have been commissioned by Lambley Reed Pond committee to carry out an eDNA test for Great Crested Newts and a sweep net survey of the pond to assess the water quality. The site is characterised by an approximately five-acre grazing pasture with a large pond with a small island. The pond is surrounded by patches of small woodland. The site has been community owned since 2021 and local volunteers have started to manage the area for wildlife and the public, by planting native wildflowers and carrying out vegetation management mainly via bramble reduction. The eDNA results were negative meaning it is likely that Great Crested Newts are not present in the reed pond, the sweep net survey returned a result of fair water quality. This is likely to be due to the number of wildfowl present at the reed pond and/or the build-up of organic matter for a number of years.

1.0 Introduction

1.1 Enitial have been commissioned by the Lambley Reed Pond committee to conduct a water quality and eDNA (for Great Crested Newts, GCN) assessment of Lambley Reed Pond.

1.2 The survey assessed the water quality of the reed pond via a handheld thermo scientific water meter, elite PCTS and from sweep netting the pond at regular intervals to check for presence/absence of indicator invertebrates. A sample of water was sent for testing for GCN eDNA to show if GCN's are present at Lambley Reed Pond.

1.3 The survey was carried out on the 25/04/23 by a suitably experienced ecologist. At the time of the survey, the weather conditions were overcast. The air temperature was approximately 9^{o}C.

1.4 The objective of this report is to show if GCN's are present at Lambley Reed Pond and to give an understanding of the quality of the water present at Lambley Reed Pond and to assess where any potential pollution may be originating from.

2.0 Site Description

2.1 Lambley Reed Pond is a small community owned nature reserve. The site is located to the east of Lambley in Nottinghamshire. It is believed that the reed pond has some extensive history, once being a medieval stew or stock pond. The site is approximately five acres in size with the reed pond being found to the western side. The reed pond measures approximately 50m by 30m with a small island. A number of mature trees are found around the pond and within the hedgerows associated to the site. Reed Pond House is a private dwelling and is found to the east with agricultural land beyond. To the north of the site is agricultural land, to the west is the village of Lambley and to the south is Cocker Beck, Park Lane, housing and Lambley Cemetery with agricultural fields beyond. There is a gated public footpath leading from Park Lane across the western edge of the site and exiting in the north-west corner.

2.2 The Reed Pond is considered to be spring fed and rarely dries up.

2.2 The centre of the site is situated at OS grid reference SK 63395 45424.

3.0 Methodology

3.1 eDNA sample. The methodology is presented in Appendix A. A detailed methodology of eDNA sample collection by Sure Screen Scientifics was followed to obtain the eDNA sample, this was immediately sent to the Sure Screen lab for testing.

3.2 Field data from a handheld field pen was collected from spot sampling the pond at regular intervals mixing the sample for ten seconds and then taking the readings from the water sample. This gives a representative sample of the entire pond.

3.3 Sweep net sampling was carried out in accordance with pond net guidance: 'How to survey ponds for aquatic macroinvertebrate families'. Five areas of the pond were netted in total. The results are then assessed using the Trent biotic index calculation, to give a water quality score of excellent, good, fair or poor. The results are presented in Appendix C.

4.0 Survey Limitations

4.1 The information found in this document is effectively valid for one year. Should any work be conducted on site after a year the results of this document should be reviewed for continued relevance. After three years this survey will be out of date and therefore should not be relied upon, and a full re-survey would be necessary. In general, this survey only offers a single snapshot of the site, no account is made for seasonal differences or for species that subsequently decide to accept residence. A lack of signs of a particular species does not confirm absence, only that there was no indication of its presence at the time of the survey.

5.0 Results

5.1 eDNA sample. Please refer to the eDNA lab report in Appendix B. No GCN markers were found in the sample sent for testing therefore, eDNA from GCN's is below the threshold detection level and the test result should be considered as evidence of GCN absence, however, this does not exclude the potential for GCN presence below the limit of detection.

5.2 Field data, collected from handheld water monitoring device.

Water temperature	9.4^{o}C
Electrical conductivity	118
pH	6.35

5.3 Sweep net results

Picture 1 shows the position of each sample point. Each area was sampled for 30 seconds.

	Area 1	Area 2	Area 3	Area 4	Area 5	Total
Tadpoles (likely to be common frog)	2	4	7	12	5	30
Lesser Water Boatman	0	1	3	2	1	7
Midge Larvae (sp.)	14	21	19	16	18	88
Mosquito Larvae	8	13	4	5	3	33
Dragon Fly Nymph	0	3	5	4	1	13

6.0 Discussion and Recommendations

6.1 The negative eDNA result for GCN's is disappointing, however with the previous Habitat Suitability Index (HSI) score of 0.68 being of an average potential to support GCN's, there is room to improve and potentially increase the likelihood of GCN's on site. However, careful consideration should be made as to if GCN's are in the area and will occupy the reed pond if it is managed to be a suitable habitat for GCN's. Having said this the proposed management detailed in 6.7 of Baseline Assessment of Lambley Reed Pond 2022 will generally benefit further aquatic and amphibian species.

6.2 Field data collected shows that the water is of good quality. pH 6.35 is slightly acidic and slightly low, this may be a result of the spring that feeds the pond.

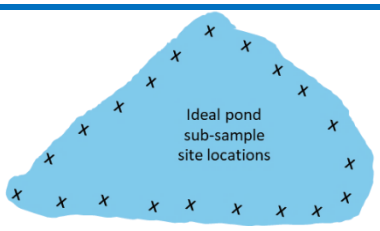
6.3 The sweep net results were compared to the Trent biotic index to give an idea of the water quality. As the water quality degrades the key organisms disappear in the following order: *Plecoptera* (stoneflies): *Ephemeroptera* (mayflies, damsel flies etc): *Trichoptera* (caddis flies): *Gammarus* (freshwater shrimp): *Asellus* (water hog louse): *Chironomidae* ('blood worms'): *Oligochaeta* (tubificid worms). The biotic index score for Lambley Reed Pond came back as 44 being of fair water quality, please see Appendix C. This along with dragon fly nymphs *SP*. being present also indicates that the water quality is at least a moderate to good water quality. As no inflows were observed from the surrounding area it is likely that the water quality is fair due to the abundance of wildfowl at the reed pond. During the survey at least eight wildfowl were observed. Foraging behaviours from wildfowl and defecation into the pond will affect the quality of the water. This along with the build up of leaf litter and further organic matter over the years will also lower the oxygen content as it decomposes. To address this dredging of the pond would be recommended.

Appendix A eDNA sample method

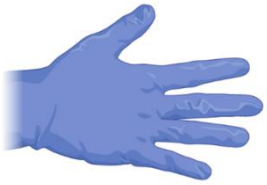
SureScreen Scientifics

Great Crested Newt eDNA Guidance

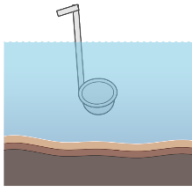
Step by Step Sample Collection Guide



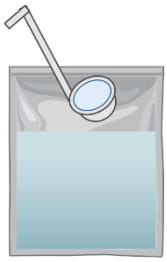
1. Identify 20 evenly spaced sites around the pond perimeter- include areas likely to contain GCN.



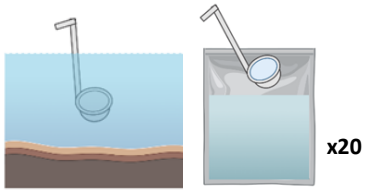
2. Put on the gloves provided. Gloves should be worn at all times during collection.



3. Collect one ladle of pond water from each pre-identified sites. Gently mix the water column and avoid disturbing sediment.

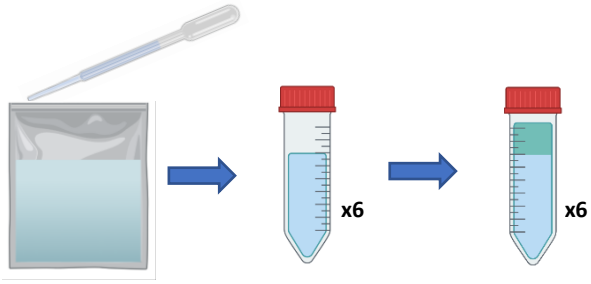


4. Transfer the ladle full of water to the bag provided. Avoid entering the water and ensure that any sediment in the pond is not disturbed.



5. Repeat steps 4-5 at each of the sites identified in step 1 until 20 ladles of water have been placed within the bag.

6. Once all sites have been sampled, tightly scrunch the bag and shake vigorously for 10 seconds (to mix any DNA within the sample equally).



7. Using the pipette, transfer 15ml of water from the bag to each of the preservative filled tubes. You will need to use the pipette multiple times to take the level of the solution from 35ml to 50ml.

Repeat this step for all 6 tubes.

8. Close and ensure the lids are tight/not cross threaded. Shake vigorously for 3 seconds. Leaky tubes = DNA loss and could cross contaminate other samples.

9. Place all the tubes in the rack inside in the box. Fill in the form, place it in the box with the sample.

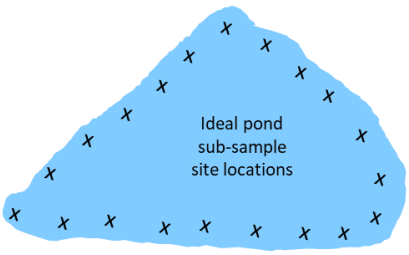
10. Return to the laboratory for analysis.

11. Results will be sent via the provided email address within your selected turnaround time.

SureScreen Scientifics, Morley Retreat, Church Lane, Morley, Derbyshire, DE7 6DE
01332 292003 | eDNA@SureScreen.com | SureScreenScientifics.com

SureScreen Scientifics

Full detail - Sample Collection



1. Identify 20 sites around the perimeter of the pond where you plan to collect your subsamples from. To ensure the sampling effort is representative of the site, space these as evenly as possible and include vegetative areas and areas likely to contain Great Crested Newts.
2. Put on the gloves. Gloves should be worn at all times during sample collection to avoid contamination of samples.
3. Using the ladle, collect a subsample from at least 5-10cm deep from each of the sites previously identified in step 1 (total 20 samples). Transfer each ladle full of water to the bag provided.

   Note: Mix the water column as you collect the sample, but avoid stirring or disturbing sediment. Avoid entering the water where possible and ensure that any sediment in the pond is not disturbed. Disturbed sediment could cause inhibition of the sample analysis, or disturb ancient DNA which has been preserved within the sediment.
4. Once all sites have been sampled, tightly scrunch the bag and shake vigorously for 10 seconds (to mix any DNA within the sample equally).
5. Using the pipette, transfer water from the bag to each of the preservative filled tubes. Repeat this step multiple times until 15ml is transferred (tube is filled to the 50ml mark).

   Note: the tube is prefilled to 35ml, adding 15ml will require several pipette movements from the bag to the tube. See markings on tube for 50ml mark.
6. Close the tubes and ensure the lids are tight and not cross threaded. Shake each vigorously for 3 seconds.

   Note: Cross threaded/leaky tubes result in loss of DNA and could cross contaminate other samples within your order. A significant loss of liquid before analysis could result in an inconclusive result.
7. Carefully place the tubes back in the cardboard rack.
8. Fill in the form, place it in the box with the sample and return to the laboratory for analysis.

   Note: you can return unwanted components back to the laboratory for recycling/disposal.
9. Results will be sent via the provided email address within your selected turnaround time.

Tips:

- Avoid entering the water as this will disturb sediment and can lead to inaccurate results.
- Kit components are single use only and must not be reused for other samples.
- If storage of samples is necessary before returning to the lab, samples should be refrigerated where possible. At a maximum, samples can be kept in the fridge for 4 weeks prior to analysis.
- Sending in a batch of samples? No need to fill out contact details multiple times, just include it on one of the forms in the box and we will work out the rest!
- Our commitment to sustainability: we are continuously working to reduce the environmental impact of eDNA testing: switching to more sustainable suppliers, reducing single use plastics and utilizing recyclable and re-usable consumables where possible.

Frequently Asked Questions

Before you start:

How long will the kit last before sampling?

Kits are guaranteed to last for the entire season which is in line with Natural England guidelines (all kits guaranteed until 30th June minimum or for 3 months – whichever is the latest date). However, if they are kept in good conditions (cool, dark) the kits should last longer than that, but outside the use by date Natural England will not accept the result.

How many kits do I need per pond?

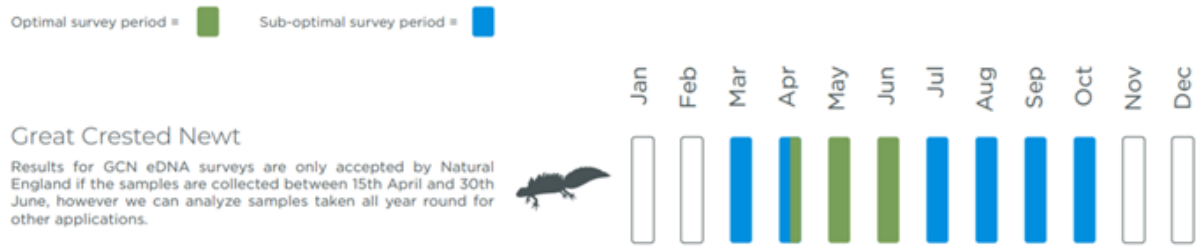
One kit is required for one pond up to approximately 1 hectare (2.5 acres). For ponds larger than this it is recommended to collect one kit per hectare. Generally, the most accepted way of collecting samples for a large pond of say 2 hectares, is that two kits will be used, one to collect 20 sub-samples around one half of the pond and the other kit to collect 20 sub-samples around the other half of the pond. These kits are analysed separately (and therefore charged as two kits and two analyses) as to not dilute the sample and give best chance to detect GCN DNA.

There are two ponds close to each other, do I need 2 kits?

Yes, you need separate kit for each pond.

When can I collect a sample? Is there any advice according to weather conditions?

Natural England has defined the GCN eDNA sample collection period to coincide with great crested newt breeding and will accept only samples collected between the 15th of April to 30th June. Samples may be taken outside of this time, provided GCN activity is noted in the area (positive result). Dates outside of this can sometimes be accepted on a case-by-case basis with prior Natural England approval.



eDNA samples can be collected at any time of day and in any reasonable weather conditions, including light rain. It may be best to avoid heavy rain as this makes sampling more difficult and might increase the risk of cross contamination (e.g. splashing of mud which could contain great crested newt DNA from wet ground). There is evidence that unpreserved amphibian eDNA decays slightly more quickly in full sun than shaded conditions, becoming undetectable after 8 and 11 days respectively, but as long as samples are preserved the impact on detection should be slight. On the other hand, we advise not to sample during or recently after heavy rainfall - the DNA is more diluted than normal.

Why are sampling points spread around the pond?
Existing data shows that eDNA can be very patchy depending on where the animals have been. By sampling in many areas, you considerably increase your chance of collecting their DNA successfully.

Why is the water column mixed before sampling?
DNA 'sinks' and so will often be present in larger amounts close to the pond bottom. However, it is important not to collect sediment because DNA may persist in the sediment for substantially longer than in the water column. If you collect sediment, there is a risk your sample might show a false positive indicating great crested newts were present recently, when in fact this was a long time in the past.

Limited access to entire pond perimeter?
Try using an extendable pole or attaching the ladle to a long stick to get better access to the water. It is important to collect as best a representative sample as possible and avoid collecting the entire sample from a very small proportion of the site. Make sure you write down how much perimeter was accessible.

Pond is murky/brown/bright orange/bright white (calcification) – should I still take a sample?
This can cause issues with analysis (such as inhibition of the sample). Whilst in most cases our laboratory procedures mean that we can account for this, we cannot guarantee reliable analysis if the sample is highly inhibited. Where possible, try to avoid sediment and highly untypical areas, make a note on the sample collection form of any abnormalities so we can use it in the interpretation of results. If these conditions occur after rain or a heavy storm, try to collect sample on different day, 3-5 days later, when the sediment has settled and the risk of picking up in the sample is reduced.

What if the site has mostly dried up?
Avoid collecting sediment. Where possible, samples should be collected where the pond/river is at least a minimum depth of 10cm to avoid sediment. If unavoidable, please contact us for further advice.

Returning your samples:

How long can I store the kit after sample collection, before sending to the lab?
For best results, return samples back to the lab as soon as possible. The longer the sample is stored before analysis, the more opportunity and time has passed which could lead to increased degradation of the GCN DNA by temperature, light, enzymes etc. Post sample collection, samples can be stored for a maximum of 2 weeks at room temperature and 4 weeks in the fridge. If you are working on a large project, feel free to store samples briefly until you have a larger batch to return to the lab.

What is the best way to send samples to you?
Ideally next day delivery, using a courier of your choice, try not to send on weekend as we are unable to control the conditions in which the couriers store the kits, especially during hot weather.
Please send completed kits back to our laboratory by courier at: SureScreen Scientifics Ltd, Morley Retreat, Church Lane, Morley, Derbyshire, DE7 6DE. We also accept hand-delivered kits between 08.00 and 16.30 Monday to Friday (16:00 on Friday), and by appointment 24/7. For a collection charge of £40 per batch, we can arrange sample collection from you with our preferred courier, DPD. However, it may be logistically or financially beneficial to arrange the return courier service yourself.

Will samples degrade in the post?

The ethanol-based preservative in the sample bottle will slow, but not eliminate, degradation of DNA. Keeping the samples refrigerated also slows this process. As long as your kits arrive at our labs within three weeks of collection the analysis/results are unlikely to be affected.

Analysis & Results:

What methods are used for analysis?

At SureScreen Scientifics GCN eDNA samples are 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 have to be correct before a result is reported. Stages of the DNA analysis are also conducted in different buildings at our premises for added security.

How accurate is the test?

Lab analysis detecting GCN DNA is highly sensitive. A study from 2014 focused on GCN shows that eDNA analysis detected GCN in 99.3 % (139 times out of 140 samples from ponds where we knew newts were present). There were no false positive results.

Where can I find my results?

Once your results are available, they will be sent to the email address provided on the sample collection form before the deadline. If you have not received your results before this date, please get in touch and we will assist you (please make sure that you check your spam or junk email folder).

How do I interpret the results?

A positive result is indicative of GCN presence at the time of sampling or within the 3-4 weeks (GCN eDNA degrades to below detectable levels between 7-21 days). A negative result suggests that there are no great crested newts within the sample area. Inconclusive results are rare, however, indicates that although GCN DNA has not been detected there may be some underlying degradation or inhibition which could be affecting the interpretation of results. This could be due to sediment, algae or plant matter within the sample, or an unusually high concentration of inhibitory molecules within the pond water. For inconclusive results, it is recommended that analysis is repeated with a fresh sample from the site in question.

What quality checks do you perform during the analysis?

We have a number of checks on the sample to ensure quality results are provided, these are: quality check, degradation check and inhibition check.

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.

Can you give an accurate picture of population numbers?

Our eDNA assays are reported as either positive, negative or inconclusive. Positive results are given a score out of 12, for how many of the 12 qPCR replicates were found to be positive. Whilst a high score such as 12/12 could appear to suggest a higher amount of DNA within the sample when compared to one with a score of 1/12, it does not always mean a higher population at the survey site. This is due to an extremely high number of variable factors which can affect eDNA sampling and the distribution of eDNA across sites. Such factors which can affect detection include differences in seasonal species-specific activity levels; how a species sheds DNA; size of site; population size; population locality to sample collection area; weather (including temperature and flow rate); and the presence of bacteria and inhibitory molecules etc. Without having an accurate picture of all of these factors it can be difficult to link DNA concentrations within the sample to population sizes within the sample site.

My result was reported negative but we saw newts in the pond…

Like any survey method, eDNA is not fool proof and does have its limitations. The result you obtain is only as good as the sample collection, eDNA distribution in a pond is not homogeneous, so if subsamples were not taken effectively, we may not be able to detect eDNA. High levels of inhibition or degradation of sample between sampling and analysis can also result in false negative results.

My result was reported positive but we did not find newts in the pond…

False positive detections can occur from time to time with eDNA sampling. The important thing here is to have as much information about the site as possible to identify if it could be a false positive and interpret the meaning of this result. Often false positives can occur via contamination (at any stage during the collection and analysis process) or cross-contamination of the site (eDNA spread between ponds via birds, dogs, water inflow, recreational activities etc.), prior to sample collection. Due to the sensitivity of eDNA testing, it is also possible that GCN are present within the pond at extremely low levels, below detection of traditional methods. If your sample is reported as positive and you expect this to be a false positive then we would recommend re-testing, with additional samples for security.

Why has my sample failed on inhibition check and received an inconclusive result?

Inhibition can be caused by a number of factors. The presence of chemical run off from farms/industry/fertilisers etc is one such cause which is widely reported to affect the analytical process (namely the extraction and qPCR steps). Inhibition happens when a substance or molecule is present in a sample which prevents the PCR process from occurring efficiently. We are able to detect for this by including a known quantity of non-target DNA (we call this DNA spike) within each kit. When we receive kits back into the laboratory, we screen them for both GCN and this DNA spike. If we fail to detect this DNA spike, it can be hypothesised that the sample is subjected to some form of inhibition, which affects the amplification process of the qPCR. In some cases, the inhibition can be overcome by diluting the sample and re-analysing it. Usually this resolves about 60% of inhibited samples and results in a reliable result. However, if your sample was reported as inconclusive, inhibition was still present as the spike DNA was not found to be present even after diluting. Further dilutions and tests reported the same conclusion. For inconclusive results, it is recommended that analysis is repeated with a fresh sample from the site in question.

What about ancient DNA?

In ponds, when collecting subsamples make sure not to disturb sediment which may contain historical DNA from the target species (in some cases can be present for up to 2 years post species removal form the pond). If needed use an extendable pole to prevent such disturbances.

Additional FAQ's:

Are you part of the GCN eDNA proficiency testing scheme?

SureScreen Scientifics participates in the proficiency testing, have done since its birth in 2017 and were actively involved in setting the scheme up. Our results will be accepted by Natural England this season. We are proud to say that we scored 100% in the latest proficiency test.

What is a PO number?

The purchase order /reference number is required to be on the form to help smooth out the payment process. It is mainly serving the purpose of being the reference on the invoice that you are sent regarding the charges for analysis, so it is easy to identify what project the charges relate to. This should be generated by yourselves – if you don't use purchase orders, just put a reference in there such as your name or project name. Feel free to use the same purchase order for all forms. If you have paid upfront for the analysis, please state your payment reference number. We are unable to begin processing a sample without a purchase order provided on the sample collection form. This means that there might be a delay to sample processing.

Can I send you another company's kit to analyse?

We are unable to analyse kits manufactured by other eDNA service providers as we would not be able to detect their DNA spike to check for degradation.

Can I use GCN kit from previous year?

For Natural England to accept results, these should be used within 3 months of manufacture. This applies to most cases. If you have expired kits – do not dispose of them, make use of old/out of date kits by using our GCN eDNA Refill Kit which allows you to replace the critical parts in an old kit and use it as brand new – reducing excess, unnecessary waste. If it is not critical for approval by Natural

England (I.e. for school project/conservation) and only when not planning and building related, then older kits can be used. Due to the nature of the DNA spike, which is contained within them, the kits typically have a use by date of 3 to 6 months from manufacture for which the stability of the DNA spike is guaranteed. The DNA spike is important in enabling the detection of any inhibition of the sample or degradation of sample post collection in the case that a sample turns out to be negative. With the older kits we are unable to guarantee that DNA spike will still be present as it has most likely degraded. Therefore, should the sample be negative for both GCN DNA and DNA spike, we would have to report the sample as inconclusive.

How do I dispose of my unused kits?

Instead of disposing of your unused kit, you can buy our GCN eDNA Refill Kit and use it as brand new. Otherwise, kit components can be disposed of or recycled according to your locally available recycling facilities. Sample preservative solution can be disposed of by pouring down the sink with copious amounts of water. If you would prefer, you can return kits to us for correct disposal.

GCN refill kit

Do you have some information on the preservative for our COSHH forms?

The preservative is an ethanol/sodium acetate buffer solution containing 95% absolute ethanol and 5% sodium acetate solution at pH 5.2. We can't provide a COSHH sheet as we have one just for preparing DNA preservative, not for using in field. Get in touch for more information.

Why do you separate the cost of the kit and the analysis?

We have found over the years that on average 88% of kits come back to us. The rest don't come back to us for several reasons such as ponds being dried up. However, if this happens to you, then it will have only cost you the price of the kit – in this case, the analysis is not charged.

Is it possible to order kit to Northern Ireland/Ireland and is there any additional price?

Yes, we can ship to these locations for an additional delivery fee. Contact us for more details.

How long is the report valid for?

For queries of this nature we would advise you to contact Natural England or the relevant local authority for further advice.

For further advice or queries:

01332 292003

or

eDNA@surescreen.com

Appendix B eDNA results

SureScreen Scientifics

Folio No: E16995
Report No: 1
Purchase Order: Lambley
Client: Enitial
Contact: Scott Draper

TECHNICAL REPORT

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

SUMMARY

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

RESULTS

Date sample received at Laboratory: 28/04/2023
Date Reported: 11/05/2023
Matters Affecting Results: None

Lab Sample No.	Site Name	O/S Reference	SIC	DC	IC	Result	Positive Replicates
0234	Lambley Pond	-	Pass	Pass	Pass	Negative	0

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

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METHODOLOGY

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

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

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

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

INTERPRETATION OF RESULTS

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

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

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

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

Appendix C, Trent Biotic index

Results (R)		Biotic Value	(R x Biotic value=)
0	Mayflies	10	
0	Caddisflies	10	
0	Stoneflies	10	
0	Scuds	6	
88	Midge Larva	5	440
			Total = 440

Divide the total by 10 = Biotic value 440/10 = 44

Water quality	**Biotic index**
Excellent	>80
Good	60-79
Fair	40-59
Poor	<40