

What is eDNA?

eDNA is DNA that is collected from the environment in which an organism lives, rather than directly from the plants or animals themselves. In aquatic environments, animals including amphibians and fish, shed cellular material into the water via their saliva, urine, faeces, skin cells etc. This DNA may persist for several weeks, and can be collected through a water sample, and analysed to determine if target species of interest have been present in the waterbody.

Who's involved in the current project?

Water samples for analysis of Great Crested Newt DNA are being collected by PondNet and NARRS volunteers co-ordinated by the Freshwater Habitats Trust and Amphibian and Reptile Conservation respectively. There is an additional trial, undertaken by John Poland, to collect multiple eDNA from a small number of ponds. The cost of analysis is mainly funded by Defra with a contribution from JNCC and Natural England. The analysis is being undertaken by SPYGEN, who are one of the main groups to have researched and developed the eDNA technique in Europe.

Survey Protocol

Overview

You use the eDNA sampling kit provided, to collect water from a pond known to support Great Crested Newts in May 2013. It usually takes around 30 minutes to collect the sample. But may take double this the first time that you do it. After collection, the sample should be kept in the fridge and then sent off to Pond Conservation as soon as possible. The sample will then be couriered to France for analysis by SPYGEN.

How do I get my eDNA sampling kit?

In most cases, kits will be sent by post to your address in early May.

What do I do when my kit arrives?

- Place the kit in a plastic bag and put it in the kitchen fridge. Don't freeze it.
- Please use the kit within about two weeks of receiving it, and use one kit per pond

Why? The kit already contains a small amount of 'control' DNA – this ensures we will know if the final sample has been poorly stored since this DNA will be lost. Keeping the sample in the fridge stops the 'control' DNA from degrading. Putting the kit in a plastic bag, just stops the kit from coming into contact with DNA from food in the fridge. It's not a major problem, but a good principle to minimise all sources of 'other' DNA in the French labs where the samples will eventually be unpacked and analysed.

Sampling the pond

1. Don't go in the water - but treading on muddy edges is OK

- Collect your eDNA water sample *before* you do any other surveys at the pond.
- Take the sample whilst standing the pond bank or muddy edges. Don't tread in the pond water itself either before or during collection of the DNA water sample.

Why? 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. This is a real risk: DNA can remain on surfaces even after they have been dried, and can persist in soil for many years. There are recorded examples of eDNA cross-contaminating pond water samples from surveyor's boots.

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

- In a moment you will take 20 water samples from around the pond: so roughly plan where you will collect them.
- The aim is to spread the samples out evenly around the pond edge (e.g. one sample every 2m).
- The samples should be taken from both open water and vegetated areas if present.
- If you can't access all areas of the pond (=most ponds!), spread the samples out as best you can without entering the water.

Why: 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.

3. Collect the sample

- Open your kit. Inside you will find:
 - 1 sterile Whirl-Pak bag
 - 2 pairs of gloves
 - 1 blue sampling ladle
 - 6 conical tubes two thirds full of preserving fluid (mostly alcohol)
 - 1 sterile pipette
 - 1 protocol sheet
- Put on a pair of gloves.
- Open the sterile Whirl-Pak bag by tearing off the clear plastic strip c 1cm from the top (along the perforated line), then pulling the tabs. The bag will stand-up by itself.
- Collect 20 samples of 40 ml of pond water from around the pond (see 2 above) using the blue ladle (fill the ladle), and empty each sample into the Whirl-Pak bag. At the end the Whirl-Pak bag should be just under half full.
- 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.

Why: 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 can be absorbed in sediment particles and persist for a very long time. If you collect sediment, your sample might show a false positive indicating GCN was present recently, when in fact this was a long time in the past.

4. Preserve the sample

- When you have collected your 20 samples, close the bag securely using the top tabs and shake the Whirl-Pak bag for 10 seconds. This mixes any DNA across the whole water sample.
- Put on a new pair of gloves to keep the next stage as uncontaminated as possible.
- Using the clear plastic pipette provided take c15 ml of water from the Whirl-Pak bag, and pour into one of the six conical tubes with preserving fluid (i.e. fill tube to the 50 ml mark).
- Close the tube. Ensure the cap is tight – leaky samples could later contaminate the analysis laboratory with DNA.

- Shake the tube vigorously for 10 seconds to mix the sample and preservative. Otherwise they will stay as separate layers and the DNA will degrade.
- Repeat for each of the six conical tubes in the kit. NOTE: Before taking each sample from the bag, stir the water in the bag to homogenize the sample – this is because the DNA will constantly sink to the bottom.
- Empty the remaining water from the whirl-Pack bag back into the pond.

5. Label the sample

- Each kit has a single unique identifier letter /number code. This number is printed on the box and on each sample tube label.
- It is essential that you record this number together with the site name, because this is the only way we will be able to tie the DNA sample results to your site.
- To reduce the potential for errors, please record this information in two places.

1. Complete the sample information box (grey box) below, and return this information with your sample.

2. Keep a separate record of your own.

PondNet surveyors: to do this please write the code number on your amphibian site recording sheet (in the box provided), and enter the data on the PondNet website with your other amphibian results. We'd also be grateful if you could use the Notes Box to provide any feedback about how you found the eDNA sampling!

Returning the kit

- On returning home, store the six preserved sample tubes in their box in a kitchen fridge (i.e. at normal 2-4° C fridge temperature). Put the box in a plastic bag so it does not touch food. Don't freeze the sample.
- **As soon as possible, mail the tubes in their original box, to Freshwater Habitats Trust using the pre-paid, addressed envelope and the packaging provided.**

If the original packaging is lost please send samples to the following address:

Freshwater Habitats Trust
c/o Faculty of Health & Life Sciences
Oxford Brookes University, Gypsy Lane, Headington,
Oxford, OX3 0BP

Thank-you very much for helping with the project!

Great Crested Newt eDNA sample record

Sample code number (from the box or tubes):

Site name:

Your name:

Sampling date:

Survey type (e.g. PondNet, NARRS, if other please state):

Please complete and return this slip with your water sample

FAQ's

Only one kit per pond – what about very big ponds?

The sampling protocol has been optimised to detect Great Crested Newt at sites with an area less than 1 hectare. It is unlikely that there will be larger sites sampled as part of PondNet or NARRS, since these large waterbodies are rarely optimal for GCN. However, if your site is larger than 1 ha, alert your regional co-coordinator who will provide an additional kit.

Does it matter if I get things like duckweed, algae or zooplankton in my sample?

No, small amounts don't matter. However try not to collect bottom sediment in the sample, because the DNA can be absorbed by sediment and may give false positive results (see above).

What happens if I spill the preservative – or the sample tube itself

If you spill some of the preservative from one of the tubes, just add proportionally less water from your pond sample. The samples from all six tubes are later combined for the lab analysis, so it's not disastrous if some sample is lost.

Won't my samples degrade in the post?

The preservative (alcohol) in your sample bottle will slow, but not eliminate degradation of any DNA. Keeping the samples in the fridge also slows this process. Sending the samples by post at ambient temperatures will mean the DNA will degrade little faster during this time, but it won't be sufficient to degrade the sample completely.

Out of interest - how much does it cost to analyse an eDNA sample?

It's still quite expensive – the lab costs are currently c£100 per sample (i.e. per pond).

When will I get the eDNA results from my pond back?

The eDNA analysis will be completed by SPYGEN by mid summer. So the *results for all ponds will be circulated to volunteers by early September at the latest.*

Why is this protocol so damn long?

Because it's good to get it right, and interesting to know why. But here's a checklist of the essentials:

1. At the pond put on waterproof gloves and use the blue ladle to take 20 samples from different places around the pond. Don't stand in the water.
2. Before taking each water sample, mix the pond water column. Don't disturb the sediment.
3. Put all 20 samples into the Whirl-pak plastic bag.
4. Then close the bag securely and shake vigorously for 10 secs.
5. Put on a new pair of gloves.
6. Use the pipette to put 15 ml of water from the Whirl-Pak bag into each of the six conical tubes with preserving fluid (fill tube to the 50 ml mark). Mix the bag water before taking each pipette sample.
7. Tighten the six tube caps securely and shake each tube for 10 secs to mix well.
8. Double label: (i) fill out the grey information box (p3 of this protocol) and return it with the sample (ii) Keep your own record. PondNet vols: fill in your Amphibian Sheet and submit the data online.
9. On returning home put the boxed samples in a plastic bag in the kitchen fridge. Don't freeze.
10. Use the SAE to return the sample in the post to Freshwater Habitats Trust ASAP. Thank-you!

Contact us

If you have questions or queries please contact Freshwater Habitats Trust:

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