PondNet

HOW TO . . . . . .
COLLECT AN eDNA SAMPLE

Environmental DNA (eDNA) is a new and exciting way to find out if Great Crested Newts are using a pond by collecting a water sample to see if their DNA is present.

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, shed cellular material into the water via reproduction, saliva, urine, faeces, skin cells, etc. This DNA will persist for several weeks, and can be collected through a water sample which is then analysed to determine if the target species of interest have been present in the waterbody.

PondNet volunteers are collecting eDNA samples from approximately 300 ponds in 100 randomly selected 1km grid squares in England in 2015, to gather information on the presence/ absence of Great Crested Newts. Samples are being collected using kits provided by Freshwater Habitats Trust; the analysis is being undertaken by SPYGEN: one of the main contributors to the research and development of the eDNA technique in Europe.

How will I get my eDNA kit?

- **Freshwater Habitats Trust is distributing eDNA kits to our volunteers.** We will email you in advance to arrange when and where. This may be your home address, work place or we can arrange a suitable time and place to meet with a group of volunteers to drop off kits.

How should I store my eDNA kit?

- **Before you collect your sample keep your kit at room temperature** – out of direct sunlight and away from any heat source or naked flame. Store the samples out of reach of children and animals. There is no need to keep the sample kits in the fridge at this stage.
- **Keep your kits upright with the sample lids screwed on firmly, but don't over-tighten the lid.** If the lid is closed too tightly it can damage the thread and the samples will leak. Keeping the kit upright at all times will help to prevent leaks.
- **Once you've collected the sample put the sample kits in the fridge.** Place the kits in a plastic bag and put them upright in the fridge. Putting the kits in a plastic bag just stops the kits from coming into contact with DNA from food in the fridge. If you don't have room in the fridge it's not a major problem, just keep the sample kits somewhere relatively cool in the house, out of direct sunlight. The sample tubes contain ethanol and a small amount of 'control' DNA. If the kit is damaged because it was kept too warm or for too long, this control DNA will degrade and we'd know that the sample results were less reliable.

What do I sample and how do I record what I've done?

- **Use one kit per pond.** You should have received a PondNet 'Site Survey Pack' with a map showing the location of your sample ponds in your 1km grid square. If you have not received your pack please contact Freshwater Habitats Trust.
- **Allow at least 30 minutes to sample each pond using your eDNA kit.** You will need an additional 30 minutes at each pond to fill in an environmental survey pond (included with your sample kit). Big ponds may take longer and you may need a bit more time on your first pond whilst you get used to the technique.
- **Fill in the eDNA survey form and a Pond Habitat Survey form**, one for each pond. Write the pond name, pond grid reference and the eDNA kit number (which you'll find on the top of the eDNA box), in the space provided on the eDNA survey form. Also record the pond grid reference and pond name on the outside of the eDNA kit box.

PondNet is one of three projects within Freshwater Habitats Trust's People, Ponds and Water Project, funded by the Heritage Lottery Fund, with additional funding for eDNA from Natural England

Contact: peoplepondswater@freshwaterhabitats.org.uk

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How to sample a pond using your eDNA kit

You can watch a video illustrating the eDNA sampling method on You Tube: www.youtube.com/watch?v=rJYLzd5gngs

The ADAS kits in the video look slightly different and you don't need to label each tube in our kits (just the kit box), but the principle is the same

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

- **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 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. 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

- **Plan your sample.** You are going to take 20 water samples from all around the pond: so roughly plan where you will collect them.
- **Spread the samples out evenly around the pond edge.** 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 around all areas of the pond you can get to.
- **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

- **Inside your kit 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
- **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 to open out the bag. 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 to the top), 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 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. If you have collected sediment in your ladle, discard the sample and collect a new one.

4. Preserve the sample

- **Shake the water in the Whirl-Pak bag.** 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.
- **Pipette a water sample into the preserving tubes.** Using the clear plastic pipette provided, take c.15 mL of water from the Whirl-Pak bag, and pipette into one of the six conical tubes with preserving fluid **(i.e. fill the tube to the 50 mL mark)**.
- **Close the tube.** Ensure the cap is closed firmly but not too tightly - leaky tubes could result in a lost sample and may 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, shake the water in the Whirl-Pak bag again to mix 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.
- **Write the eDNA kit number, pond name and pond grid reference on the eDNA survey form.** It is essential that you record this number together with the pond name and pond grid reference on your survey form; because this is the only way we will be able to tie the DNA sample results to your site.
- **Write the pond name and pond grid reference on your eDNA kit.** You will have this information in your 'Site Survey Pack'. Double check that the eDNA kit number, pond name and pond grid reference are recorded on the box and on your eDNA survey form.

6. Complete a Pond Habitat Survey

- **Fill in a Pond Habitat Survey form.** For each pond you sample for eDNA we also need you to complete a Pond Habitat Survey form (included with you kit).
- **For more information visit the PondNet website.** Instructions on how to complete a pond habitat survey are included on the Pond Habitat Survey form, but if you need more information about how to complete the form visit the PondNet website. www.freshwaterhabitats.org.uk/projects/pondnet/survey-options/habitats
- **Enter the Habitat survey results online www.freshwaterhabitats.org.uk/projects/waternet**, or return the form to us when we pick up your kits. If you have any problems completing your form, or entering data, contact Freshwater Habitats Trust.

7. Return you eDNA sample kits and survey forms

- **We will collect your eDNA kits and survey forms at the end of the survey.** Once you have collected your eDNA sample please email Freshwater Habitats Trust to say that you are ready for us to collect your sample kits and survey forms.
- **Unused kits**. Please let us know, as soon as possible, if you don't use all of your eDNA kits. We only have a limited number of kits to use in 2015, so if a kit is not needed we can re-allocate it to another volunteer.

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. 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?

The preservative (alcohol) in your sample bottle will slow, but not eliminate degradation of any DNA. Keeping samples in the fridge slows the process. At ambient temperatures the DNA will degrade a little faster, but it won't be sufficient to degrade the sample completely. We will aim to collect the kit from you as soon as possible and deliver them to the SPYGEN laboratory for analysis.

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. We will also make the results available through our new web portal WaterNet which we are developing through People, Ponds and Water **www.freshwaterhabitats.org.uk/projects/waternet.**

This is a long protocol! What's the summary?

We've given you the detail and the reasons behind the protocol. 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 seconds.
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. Close the six tube caps firmly but not too tightly and shake each tube for 10 seconds to mix well.
8. Double label: (i) fill out the eDNA survey form, and (ii) write the details of the pond on the outside of your eDNA kit box.
9. Complete a Pond Habitat Survey form at each pond where you collect an eDNA sample.
10. On returning home keep the sample box in the fridge, or somewhere cool in the house, away from direct sunlight and heat.
11. Contact your regional coordinator or Freshwater Habitats Trust main office to arrange pick-up.

Good luck and thank you!