

The 2011 UK Chytrid Survey (aka The Big Swab 2011)

Protocol for Surveyors

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Welcome to the chytrid survey 2011!

Firstly, a huge thank you for offering your time, experience and energy to this project.

The amphibian chytrid fungus (or, to give it its proper name, *Batrachochytrium dendrobatidis*), has been much talked since it was first identified, little more than a decade ago.

It has also been subject to considerable research effort.

We now know that this remarkable organism is capable of infecting a huge range of amphibian hosts (almost 450 different species at last count), and has achieved a global distribution.

We also know that the effects of infection vary greatly from species to species. Some species appear capable of carrying infection with no evidence of clinical disease, whilst other species experience mortality rates of up to 100%.

We do not know, at the moment, where our own native species lie within this spectrum.

However, whilst parallel research projects grapple with this question, it is vital that we continue to build our understanding of the distribution of *Bd* in the UK in order to minimise further spread of infection and, if necessary, to allow effectively targeted mitigation.

Through the highly successful 2008 chytrid survey, we have already identified a number of chytrid-positive sites in the UK.

The basic design of this year's survey will be very similar: collection of skin swabs from amphibian populations around the UK, in order to detect chytrid, where present.

Repeating the survey this year is a really exciting opportunity, as it allows us to ask the question 'Where are you *now*?'. Is distribution increasing? Or decreasing? Or staying the same? And if it's not staying the same, where is it going?

In addition, by increasing coverage in previously underrepresented areas of the UK we can also gain a more accurate picture of chytrid distribution across the UK as a whole.

Furthermore, by slightly modifying the protocol (here come the sandwich bags), we can have greater confidence in species-specific data which will help us to identify those species most susceptible to infection.

I hope that being a chytrid swabber will be an enjoyable experience. I will be your lead contact throughout, so please, if you have any questions, comments or observations, do not hesitate to drop me a line.

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With regards to the all important results, I can assure you that I will get these to you as soon as possible. However, do please be aware that with a survey of this magnitude, it may take up to a year before we can get through all the samples. Keep an eye on my page on the ARG-UK website (www.arguk.org) for updates (follow projects, 2nd national chytrid survey 2011).

Survey protocol

The protocol this year has been slightly updated from that used in 2008 (based on results, feedback and lessons learnt in 2008). So, even if you have swabbed before, please take a moment to read through the instructions carefully.

The key points that I would like you to take away with you are:

- **30 amphibians** to be sampled per site (can be a mix of species)
- Single survey between **March and June** (you can start as soon as you have your swab packs).
- All samples collected on a **single day/evening** to avoid resampling.
- **No frogs!**
- No tadpoles! (**post metamorphic** animals only please)
- Capture **from the water** (i.e. not migrating toads en route to the breeding ponds)
- **Avoid contamination between animals.** Prior to swabbing this can be achieved by temporarily housing amphibians in individual sandwich bags. During swabbing, there are two options to avoid passing infection between individuals: **changing gloves** between amphibians, **or** handling amphibians **through their sandwich bags** (so that they do not come into direct contact with your gloves)

The basic run of events, for the skim readers, should be

- Get to your site on an amphibian friendly day/night
- Catch 30 amphibians (from the water) and put them in individual sandwich bags
- Swab hind legs, drink patch/lower abdomen and tail of newts (according to illustrated protocol).
- Label swabs clearly and complete recording forms
- Release animals at point of capture
- Make sure you haven't left any rubbish behind

Swabs, sandwich bags, gloves and a stamped return envelope (for return of samples) will all be provided

The text/illustrations below will explain in detail. If you have any questions after reading through all of the below, let me know and we'll sort it out.

1) Choose a site

By this stage, most sites will already be confirmed. However, previously, there has been some confusion as to the exact definition of a 'site'. When you collect your 30 samples, all animals sampled should be from the same population. Therefore if you have several adjacent ponds, as long as there are no physical barriers preventing movement between the ponds, and all ponds are within a 500 m radius (i.e. the two most distant ponds are no more than 1km apart), the complex of ponds can be considered a single 'site'. If your site does consist of more than one pond, it would be helpful if you could provide a quick sketch, numbering the ponds (there is a space for this on the Site Recording Sheet). On the Sample Recording Sheet, there is a space for noting down the origin of each sample.

2) Preparing for your survey

If you are surveying after dark, please make sure that you are familiar with the site, and any potential hazards by making a visit during daylight.

The equipment you require will include: net(s) (and/or bottle traps for newts), pen(s) (a marker pen is useful for numbering swab tubes, biro for recording sheets), disposable vinyl gloves (one pair each per site), swabs, sandwich bags, black bin bag/several buckets for storing bagged amphibians, and disinfectant solution (see ARG Advice Note 4).

Watch the weather and go on a day when you are reasonably sure there will be good numbers. Visit with at least one other person.

3) Timing the survey

Surveys should take place, for the most part, between **April and June**. However, some of you may wish to start sampling in March, if you are hoping to catch toads or early-starting newts. If you **are planning to survey in March**, you might want to drop me an email to make sure I send out your kit in good time.

All samples should be collected on the same night. If samples are collected over a period of different sampling dates, some animals may get swabbed more than once, which muddles up the data.

4) Numbers

We are asking surveyors to collect 30 samples per site. This number is based on the need to make statistical inferences and means that, assuming the background prevalence of infection is at least 15%, we can be 99% confident of sampling at least one positive animal. So, if at all possible, do try and stick to this sample size. There is no need to collect more than 30 samples.

However, if you have completely exhausted the site, and you haven't managed to collect 30 samples, please do send in your swabs anyway. They will still be useful.

5) Species

The results of the 2008 survey suggest that all UK native species can carry infection. However, prevalence in the common frog (*Rana temporaria*) was very low, and this finding is supported by chytrid surveillance elsewhere in Europe. As a result, even at infected sites, the common frog is unlikely to test positive, meaning that we are more

likely to miss infection if we are sampling this species. We are therefore excluding the common frog from this survey.

The 30 samples can be made up of all other UK species as well as any non-native species you come across. If you are sampling great crested newts (*Triturus cristatus*) or natterjack toads (*Epidalea calamita*) you will need your licences amended to include swabbing. If you need assistance with this, please let me know. If you are sampling non-native species this will also require a licence to allow you to re-release animals after swabbing (even if they are going back to exactly the same location) so please make sure you have discussed this with me.

6) Pre sampling segregation

Prior to swabbing, we are asking people to keep individuals separately in order to avoid cross contamination between animals. **This is a new addition to the 2008 protocol.** The advised method is as follows:

Using a plastic sandwich bag to glove your hand, pick up the amphibian. Pull the bag over your hand (now gently clasping the amphibian) to turn the bag inside out (as though you were cleaning up after your dog...). Add a few damp leaves, or a small quantity of pond water and seal the bag with a tight knot, being careful to leave a pocket of air. Once you have collected the full 30 animals, you can start swabbing. Gently untie or, if necessary, break open the bag at the neck. Drain the water/remove leaves and, handling the amphibian **through** the bag (so that it doesn't come into direct contact with your gloves), swab according to the illustrated method below. If you prefer, rather than handling animals through their sandwich bags, you can simply **change gloves** between individuals. Do whatever feels easiest. The main thing is that we avoid passing infection from one animal to another. The amphibian can then be released directly at point of capture.

Pre-swabbing, you will need something solid to store the bagged animals in to prevent escape of the hamster-ball variety (toads, in particular, can make an impressive bid for freedom even within the confines of a plastic bag). Two or three large buckets should suffice. If surveying during the day, ensure that animals are kept out of direct sunlight so that they do not overheat. Please ensure that you remove all rubbish when you leave.

7) Swabbing

Swabbing methodology is best explained by illustration (see below). The method has not changed from 2008.

8) Recording

This year there are two recording sheets. One for recording site information, and one for recording swab details. Please label all swabs clearly (with pen, not pencil) with the **first three letters of your site name**, followed by the swab number. e.g. Lon 1 (for swab number 1 from London Zoo)

9) Sending in swabs

On returning from the field please post the swabs to:
UK Chytrid Survey, Wellcome Building, Institute of Zoology, Regent's Park, London NW1 4RY.

If you are unable to post the swabs straight away, please keep in the fridge for the interim period.

Swabbing by pictures

The sandwich bag technique – for keeping individuals separate prior to swabbing



When you have caught the amphibian, gently pick it up using a plastic, water tight sandwich bag. Invert the bag so that the amphibian is now enclosed within it. Add a small amount of water or some damp leaves for the amphibian to hide in, before sealing the bag with a knot. Please be sure to trap a pocket of air within the bag before you seal it. Provided the animals are kept in a cool place (i.e. out of the sun if you are surveying during the day) they should be perfectly happy for the duration of your catch period. Once you have assimilated your 30 individuals, you can start swabbing (see below). You will need to take several buckets with you to store the bagged animals in to prevent runaways (amphibians can propel themselves even inside a plastic bag!).

Please also ensure that you have accounted for all bags used at the end of the survey and have left no litter behind you.

How to swab

The purpose of swabbing is to dislodge loose skin, chytrid sporangia or zoospores. This means that you will need to be quite firm with your swabbing.

When handling animals during swabbing, it is important to avoid cross contamination between animals (or swabs). This can be achieved either by handling animals through the sandwich bag (which means that the amphibian does not come in direct contact with your gloves), or, if you prefer, by changing gloves between individuals. A sufficient supply of gloves will be provided. Soil and other organic matter can make it more difficult to detect chytrid. If you have a particularly muddy specimen please give it a quick watery dunk (pond water is fine) to clean off the worst of the mud.

The pictures below show the technique that should be used for swabbing.

- 1 Take a firm but careful hold of the amphibian, on its back, holding its throat/head region with your thumb/index finger. This allows you to manipulate the lower limbs with your other fingers.

Amphibians do not have a rib cage so care should be taken not to damage internal organs by pressing the abdomen.



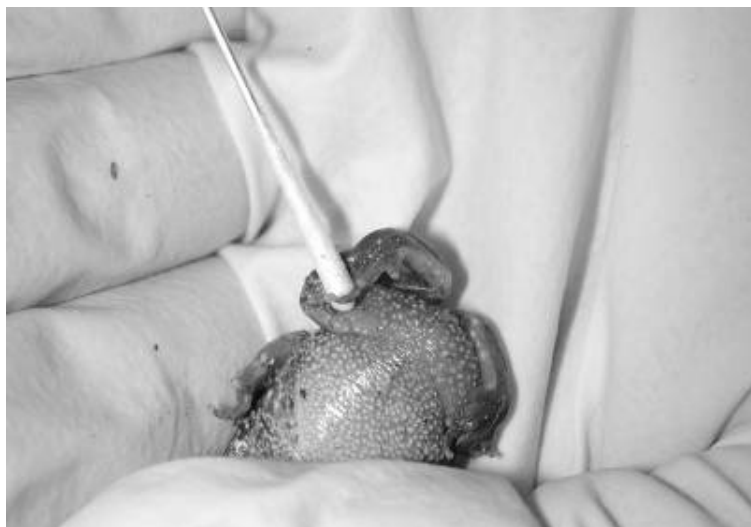
- 2 Start by swabbing the inner thigh of the hind leg with a firm continuous action. Repeat three times on each leg.



- 3 Repeat the swabbing procedure in 2 above but this time concentrating on the lower hind leg. Repeat three times.



- 4 Repeat the swabbing procedure in 3 above but this time concentrating on the underside of the foot/toes. Repeat three times.



- 5 Repeat the swabbing procedure in 3 above but this time concentrating on the 'drink patch', this found just above the pelvis.



6. For newts a similar approach is taken. Swab the rear limb in one action, ensuring that all of the inner leg and toes are covered. Repeat three times.



7. For newts the base of the tail and lower abdomen should also be swabbed. Repeat three times.



- 8 After swabbing, the amphibian can either be released directly, or stored in a secure container until release. However, prior to returning it to its capture location, please observe the animal for a few moments to ensure that it is unharmed.

Dead amphibians: Please do keep your eyes peeled for any dead amphibians you might encounter during your survey. If you find a carcass, please freeze immediately and contact me (freya.smith@ioz.ac.uk, 02074496621) to discuss whether submission for post mortem examination is appropriate. To store the carcass safely, seal within two plastic bags (one inside the other) and put in a rigid, crush proof container (e.g margarine tub) before placing in the freezer.

Extras

Although the primary aim of this study is to investigate the distribution of chytrid fungus in the UK, we are also very interested to find out more about another amphibian pathogen, amphibiocystidium, which affects newts.

Unlike chytrid, amphibiocystidium infection results in very obvious skin lesions as shown below. Should you encounter any newts showing these lesions during your survey, please do jot down your findings on the swab record sheet (under 'reporting of abnormal individuals'). It would be helpful if you could mark your comments as 'possible amphibiocystidium'.

