Water Sampling for Environmental DNA Analysis

Based on documents provided by M. Kinnison, University of Maine

Modified 2018, UNH to focus on preparation and sampling, assuming that location and number of sampling is specified in a sampling plan.

This sampling protocol is intended for use by members of the general public, agencies or NGOs wanting to collect water samples for environmental DNA (eDNA) analysis to detect aquatic species. The lab assays we use are able to detect extremely small concentrations of eDNA. HENCE, CARE MUST BE TAKEN TO AVOID CONTAMINATION OF SAMPLES WITH TARGET SPECIES DNA ACCIDENTALLY TRANSFERRED FROM OTHER OBJECTS OR WATERBODIES. DNA contamination during field sampling can result in false detections, so it is important that you be attentive of contamination risks and follow the procedures of this protocol.

DEFINITIONS FOR THIS PROTOCOL:

eDNA (Environmental DNA): Small fragments of DNA shed by organisms into their environment as part of their normal life and death processes

Target species: The organism you seek to detect with eDNA water samples

Waterbody: The estuary, pond, stream, or other location where you require independent eDNA detection. This can be the whole waterway, or different important locations within a given waterway

Sampling Site: One location, often among several, where field samples will be collected within a waterbody to account for movements or local habitat use of organisms.

Sample Kit: A pre-assembled and labelled set of supplies used to collect a given field water sample

Field Sample: Volume of water collected for eDNA analysis

Trip Blank: Volume of pure water that is handled much like a field sample but contains no actual field sample water. May also be referred to as a control sample.

SUPPLIES SHOPPING LIST

The follow list provides enough materials for approximately 24 samples (field or control)

1. Sample container. May be single-use container such as a whirl-pak bag or a cleaned Nalgene bottle. When sampling for a single species, bottles of commercial water may be used, and emptied at the site prior to sampling.
2. Ziplock Slider All Purpose 1 Gallon Storage Bags (sliding zipper type is important in field)
3. Disposable Exam Gloves (50 count box – Vinyl, Latex or Nitrile – no dish gloves)
4. Paper towels (1-2 rolls)
5. Unscented Kitchen Trash Bags (about a dozen.
6. Indelible Ink Marker (Sharpie or similar waterproof)
7. Clorox Clean-Up Cleaner + Bleach Spray (1.84% Sodium Hypochlorite on label) or 10% bleach solution.

NOTE: THIS PROTOCOL DOES NOT OUTLINE A SPECIFIC SAMPLING DESIGN FOR WHERE, WHEN OR HOW MUCH YOU SHOULD SAMPLE. Your sampling design is determined by the nature of your target species, the nature of the waterbody (e.g., size, flow), and your detection goals (e.g., presence-absence, relative abundance).

PART 1: ASSEMBLING AND PREPARING SAMPLING KITS

The above shopping list provides resources for collecting water samples that can be allocated to either actual field sampling or to field contamination controls. You will assemble these materials into kits that help to reduce the chances of contamination during field sampling. These kits should be assembled in a clean location before you head to the field for water sampling.

Alternative sampling containers: Sample containers must be clean to avoid contamination from previous uses. In some cases it may be simpler to purchase single-use sampling bags, such as Whirl-Pak 1 liter bags. If reusing sample bottles clean with acid wash or soak in 50% bleach solution.

1. Identify a clean and uncluttered work surface of about 2-foot-by-3-foot area to use for preparing your kits.
2. Wash your hands with soap and tap water if available. Dry them with paper towels and put on a pair of the disposable gloves (leaving the rest in their original container).
3. Spray down the work surface with the Chlorox bleach spray, let spray sit for 2-3 minutes and then wipe up with paper towels. (WARNING: follow safety precautions on bleach spray. Bleach can injure or irritate eyes, skin etc. It can also damage or discolor some fabrics and other surfaces)
4. Cover the work area with a layer of paper towels and place the empty sample containers, the box of Ziplock bags, the remaining gloves, your kitchen trash bags, and your marker on this surface, leaving some space to write on bottles and bags.
5. This is your work area for all remaining steps. If you leave or handle objects outside this space (e.g., bathroom break, phone, etc.) you should remove and replace your gloves.
6. Label a Ziplock bag with either the intended waterbody, date of sampling, site etc., or with a sample ID number/code you can later record along with these details.
7. Add one paper towel per sample bottle to the bag before sealing it shut to create a 'sample kit'. It may be convenient to fold each paper towel in half one or two times to fit them neatly. The complete kit now includes one or more bottles and the same number of clean paper towels, all sealed up inside a single labelled Ziplock bag.
8. Repeat steps 8-11 for each of the remaining samples/sites you plan for a given body of water.
9. In most cases you will also want to create and label some kits to be used as 'Contamination Controls' (see below). You might also find it useful to make an extra sample kit as a back-up in case of any mishaps or sampling opportunities that arise while in the field.
10. While still wearing gloves, you should label and fill a 'glove bag' for each waterbody (or critical site) where you wish to have an independent assessment of eDNA presence or abundance. Fill each glove bag with at least enough pairs of gloves to allow you to remove and replace your gloves whenever you move more than a short distance between sampling locations. We recommend you add a couple extra pairs per bag to accommodate torn or dropped gloves. Keep in mind, you should not reuse

these glove bags among waterbodies due to their potential field contamination. After creating the glove bags you can remove the gloves currently on your hands.

11. We recommend that you transport your sampling kits, and associated glove bags, to the field inside clean trash bags that you loosely knot or twist shut. This provides an extra layer of contamination protection and makes it less likely you will drop or lose a kit. If you have many kits to transport, it may be handy to further carry the trash bags of kits inside a 5-gallon bucket or a picnic cooler, but you should spray/wipe down those containers with 10% bleach first.
12. Finally, you may want to place some extra kitchen trash bags, some extra Ziplock bags, a spare marker, paper towels, a pencil, and some note paper in a final Ziplock bag labelled with the waterbody name and "SUPPLIES". You may or may not need these materials while field sampling, but it is best to have them handy if you do. Unless you make a fresh supply bag for each waterbody, you should treat these supplies bags as a contaminated surface during later field sampling and not mix them with your sampling kits.

PART 2: PLANNING YOUR FIELD WORK

A SAMPLING TEAM: Field sampling of eDNA water samples involves two people. One person serves as the 'sampler' and the other person serves as a 'helper'. The helper can look up details in these instructions when needed, keep track of samples, handle objects that are contamination risks, serve as a second set of eyes for potential contamination, and ensure safety of the sampler in potentially hazardous field conditions.

A SAMPLING PLAN: You should plan where and in what order field samples will be collected before heading to the field so that you can decontaminate any associated field gear, collect your samples efficiently and legally, and minimize risks of sample contamination. Here are some tips:

- Reduce contamination by sampling any sites with suspected low odds of target species presence BEFORE sampling locations where the species is known or strongly suspected.
- You should always complete and store away samples from one waterbody before beginning sampling at another waterbody on the same day.

EXAMPLES OF CONTAMINATION SOURCES:

- Waders, hats, gloves, sunglasses or other fishing clothing
- Fishing gear (rods, nets, tackle boxes)
- Boat gunnels, paddles, cushions
- Vehicle upholstery, matts or carpeting
- Dogs that swim in other waterbodies
- Food preparation or serving areas
- Your lunch. Don't store it in the sample coolers!

The shared feature in all of these cases is the risk that the surfaces of objects might carry fragments of the target organism's DNA that could be accidentally transferred by direct contact or your hands.

- If you will be sampling more than one waterbody, you will need to plan for time to wash your hands or wash down and Chlorox bleach spray your field gear (buckets, coolers, boots, boats, paddles etc.) between waterbodies.

ACCESSING SAMPLING SITES: Part of your planning process involves a decision about how you will access waterbodies for sampling. There are pluses and minuses to different access methods.

Shore sampling: Shore access to water is often preferred for smaller waterbodies or detecting organisms that inhabit shoreline regions during the sampling period. Avoid sampling shore water that is very dirty from wave action. You may not want to sample shore areas close to busy boat launches or beaches where boats, swimmers or pets might introduce DNA. Docks are often convenient places to sample from shore, but boaters, swimmers, pets and birds can contaminate dock surfaces. This does not mean you cannot sample from docks, but you should take this into consideration. As with all sampling, avoid touching docks, boats or other surfaces with your gloved hands or the sample container during sampling. A clean trash bag can be laid on such surfaces to provide a non-contaminated work area if needed.

Wading: Entering the water does not necessarily provide better detection than shore sampling, but can help where water is too shallow, muddy or stagnant at the shoreline. Your boots or waders should be sprayed with bleach between sites, which may damage them in the long term, so inexpensive rubber boots may be preferred. Whenever wading, try to avoid sampling where you have just walked through the water. Instead, try to collect your sample at an arms-length in an upstream or into-the-waves direction.

Boats: Boats can introduce eDNA contamination from other areas. Consider not sampling immediately upon launching the vessel, but instead travel for a few minutes in an indirect approach to your sampling site to 'rinse' the boat's hull. Finally, try to avoid sampling in the boat's wake or on the upwind side of drifting vessel.

TRANSPORTING SAMPLES IN THE FIELD:

Part of planning involves preparing for how you will manage and transport your samples in the field. Sampling kits do not require any special care prior to sampling. However, once samples are actually collected in the field they do require protection from contamination, heat and UV light. Collected samples should not be mixed in the same bag or container with unused kits. The best and most common option is to isolate samples by waterbody or site (usually in different trash bags) inside a picnic cooler filled with ice. That cooler should be decontaminated (inside and out) with Chlorox bleach spray before heading into the field.

PART 3: COLLECTING WATER SAMPLES

With good planning, actual sample collection is a fairly straightforward process. The essential points to remember are:

1) NEVER OPEN A SAMPLE CONTAINER UNTIL THE INSTANT YOU WILL TAKE THE SAMPLE

2) ONLY OPEN A BOTTLE WHILE WEARING CLEAN GLOVES

3) DON'T LET YOUR GLOVES, SAMPLE CONTAINERS OR BOTTLE CAPS TOUCH POTENTIALLY CONTAMINATED SURFACES (SEE EXAMPLES ABOVE)

Collecting Surface Water Samples:

1. Once you have assigned a sampler and a helper, the sampler should be the only person to ever place hands inside of a field sampling kit, and only while wearing exam gloves.
2. Do not open any field sampling kits until you reach the actual location where you plan to take a water sample.
3. Once at the sampling location, the helper holds open the glove bag so the sampler can grab one glove at a time and put them on. If you tear a glove while putting it on, the helper can provide you with a replacement from the spare field supplies.
4. **Once gloves are on, the sampler should try to avoid touching any surfaces other than the bottle, bottle cap and field water** (including clothing, backpacks, coolers etc.). For example, the helper should be the one to reclose the glove bag.
5. The helper now unzips and holds open the sampling kit (without placing their hands inside).
6. The sampler is then able to reach into the kit bag with gloved hands and remove a sample bottle The sampler should NOT open the container until they are in place to collect the sample.
7. Once the sampler is in position, if using commercial water bottles, open the bottle and pour the drinking water out a few feet from where the actual water sample will be collected. Do not set down the bottle cap or put in a pocket. Keep it in a gloved hand. (Do not drink from the bottle).
8. To collect the sample, simply immerse the container in the surface water of the lake, pond, stream or river. Tip the container so that it fills slowly and move it about gently to collect water from a larger area. Some airspace (1/2 inch or so) reduces risk of container damage during later freezing.
9. The sampler then seals or recaps the container tightly. Tip it upside down briefly to make sure there's no leakage.
10. If you have more than one sample at a site, you can set your completed sample aside or hand it to your helper, and proceed with the remaining samples (following steps 6-9).
11. Once all samples have been collected at that location, use the paper towels from the field sample kit to dry the container(s) and your gloves and place the completed samples back into their original labelled Ziplock sample kit bag.
12. Remove your gloves by grabbing them at the wrist cuffs and pulling toward your fingertips (turns the glove inside out). Dispose of the glove and paper towels in a kitchen trash bag or spare Ziplock bag designated for the purpose. Do not use that same trash bag at another waterbody.

13. You should store your completed samples away from unused kits, out of direct sunlight (e.g., in a cooler or trash bag).

Collecting Trip Blanks:
Blanks are not true field water samples, but are instead mock or mimic samples meant to help detect contamination in your field sampling supplies or field procedures. When target species DNA is detected in a contamination control that is strong indicator that other samples with positive detections could also be contaminated.

For a control to be meaningful it must be prepared and handled in all the same ways as your actual field samples, with the only exception being you won't collect actual field water.

1. Follow all of the above initial steps for field samples through step #6 (removing the sample bottle from the sampling kit bag).
2. OPEN THE SAMPLE CONTAINER BUT DO NOT POUR OUT THE CONTENTS OR IMMERSE IN THE WATERBODY.
3. Leave container open for about 10 seconds and then close it.
4. Proceed with sealing the bottle, testing the seal, drying it, and placing it back into the sample bag (steps 9, 10, 11).
5. After sampling, be sure to transport, freeze or ship your contamination control samples in the same bags, coolers etc. you use for their associated field samples.

PART 4: STORING AND SHIPPING SAMPLES

Environmental DNA in water samples can degrade due to physical, chemical and biological processes. Samples should be filtered or frozen within 24 hours. The goal is to stabilize samples until they can be processed in the lab.

Freezing: If you plan to freeze your eDNA samples this should occur as soon as possible following field collection. They can be stored frozen for several weeks, but it is preferable that they be shipped within a day or two of collection to avoid complications with modern frost-free freezers that can subject samples to repeated freeze-thaw cycles that damage eDNA. As noted above, samples from different waterbodies and dates should not be mixed, but should instead be kept grouped by waterbody and date within separate kitchen trash bags before, during and after freezing. Do not reopen the Ziplock sample bags if you do not have to.