

# Monitoring otters using water samples from water bodies: A practical guide for eDNA sample collection

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## Introduction

Understanding the geographic distribution of a species is critical for species management. Effective management involves identification of the species, which can often be very low in density. The numbers of these species are typically estimated employing traditional methods, especially in the case of wildlife. In freshwater ecosystem, the presence of target species of invertebrates and vertebrates is increasingly being detected through environmental DNA (eDNA), which is based on the detection of DNA fragments released into the ecosystem. eDNA allows aquatic species to be monitored effectively due to the potential for greater sensitivity over traditional survey methods, which can be time-consuming and costly. The ability to detect eDNA makes it possible to detect the species at low densities in a robust manner, but also makes samples highly susceptible to contamination. Sample contamination in field sampling is a major concern. As a result, it is imperative that eDNA-based methods follow protocols that allow for efficient sampling while taking proper precautions. Here we present a protocol that has been developed for *Lutra lutra* detection. It minimizes contamination issues while maximizing species detection and sampling efficiency. Our step-by-step guide describes how reliable eDNA samples can be collected from lotic and lentic ecosystems. This protocol has not been extensively tested in ponds, lakes or large rivers but with proper developments, it can be applied in these environments.

## Aims

This research is intended to provide scientists and managers with an overview of the process for collecting and preserving samples of environmental DNA (eDNA) for molecular testing.



eDNA SAMPLING KIT

1. Bag containing eDNA sampling equipment
2. Clean gloves
3. Vacuum flask
4. Rubber stopper
5. Silicone tubing
6. Manual vacuum pump
7. MicroFunnel™ Filter Funnel with Supor® Membrane, 100 mL, 0.2 µm
8. Sterile bottles for water sampling
9. Notepad
10. Permanent marker
11. GPS
12. Sterile sampling bags
13. Silica gel pouches
14. Black polyethene's bags
15. Bleach
16. Forceps set
17. Bucket

## Step 1. Sample site selection

- Choose a flat area where you can lay out all of the sampling equipment without risk of it being blown away or falling into the stream.
- Always stay downstream of the sampling location including you and the equipment
- During sampling, the water should be collected from the well-mixed portion of the flow
- Avoid eddies or splash pools where water could wash off contaminated materials and contaminate the sample.

## Step 2. Filter set

- Put the rubber stopper on the vacuum flask.
- Attach the disposable MicroFunnel™ Filter Funnel with Supor® Membrane on the vacuum flask.
- Put the vacuum pump on the flask using silicone tubing

## Step 3. Water and filter

If filtering on-site:

- Use a new filter with Supor® Membrane to filter water. When using MicroFunnel with Supor, wear new gloves.
- Pour slowly through a filter funnel. If you swirl the MicroFunnel™ Filter Funnel around, you will reduce sediment formation.
- Begin filtration using a vacuum pump. Inspect vacuum pressure to maintain it between the gauge or watch the water level to make sure water is flowing into the funnel.
- If there is more than one filter, remove vacuum from funnel when you add more volume. Don't use the pump's pressure release button, this may contaminate the sample. The pressure release is located in the bottom of the pump.
- The filter may not be completely in the water. It takes one drop every several seconds. Consider a drip-rate or cut-off time. The drip rate is set to 3 drips every 10 seconds.
- Note any unusual events, conditions, or problems. Make a note of the contamination if there was any.

If you are taking samples off-site:

- Collect water in sterile bottles. Wear new gloves for handling plastic bags.
- Rinse sample 3 times in sample site water. Shake in each rinse. Keep rinsed water away from the collection spot.
- Pouring water into the container while stirring itself reduces contamination. When sedimenting, gently stir.
- Label the sample number, and take GPS location
- Keep the samples at cold temperatures until they can be filtered.

## Step 4. Membrane removal

- Remove the vacuum to remove the tubing from the flask.
- Tilt the funnel to detach it from the base.
- Open the bottle to prepare the filter.
- Wear a new glove. Do not touch the membrane with your skin.
- Remove the filter with gloved fingers. In Nalgene bottles, the membrane sits on top of a paper disc. Save the thin membrane and discard the disc. Open the filter and fold it in half.
- Roll the tube into a cylinder. Use a gloved finger to hold the filter steady. Insert the filter and then add ethanol.
- Secure the vial to the label with sample ID and date. Attach label. Remove glove.
- Remove the stopper from the funnel.
- Look at the vacuum filter before each and every sample. Use sterile gloves when handling the forceps tips.
- Store vials at room temperature or colder.
- Note: Alcohol is not allowed in some shipments. Contact your carrier.



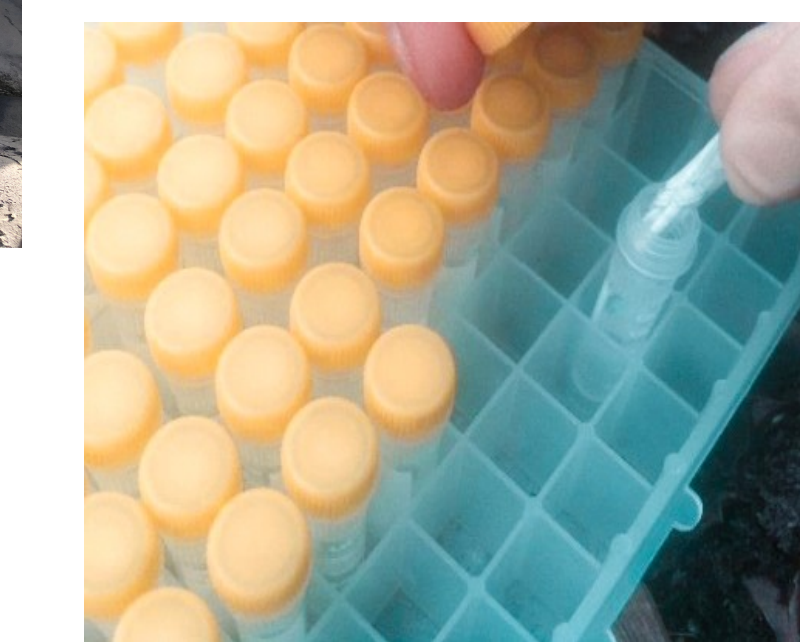
Water sample collection



Remove funnel cup

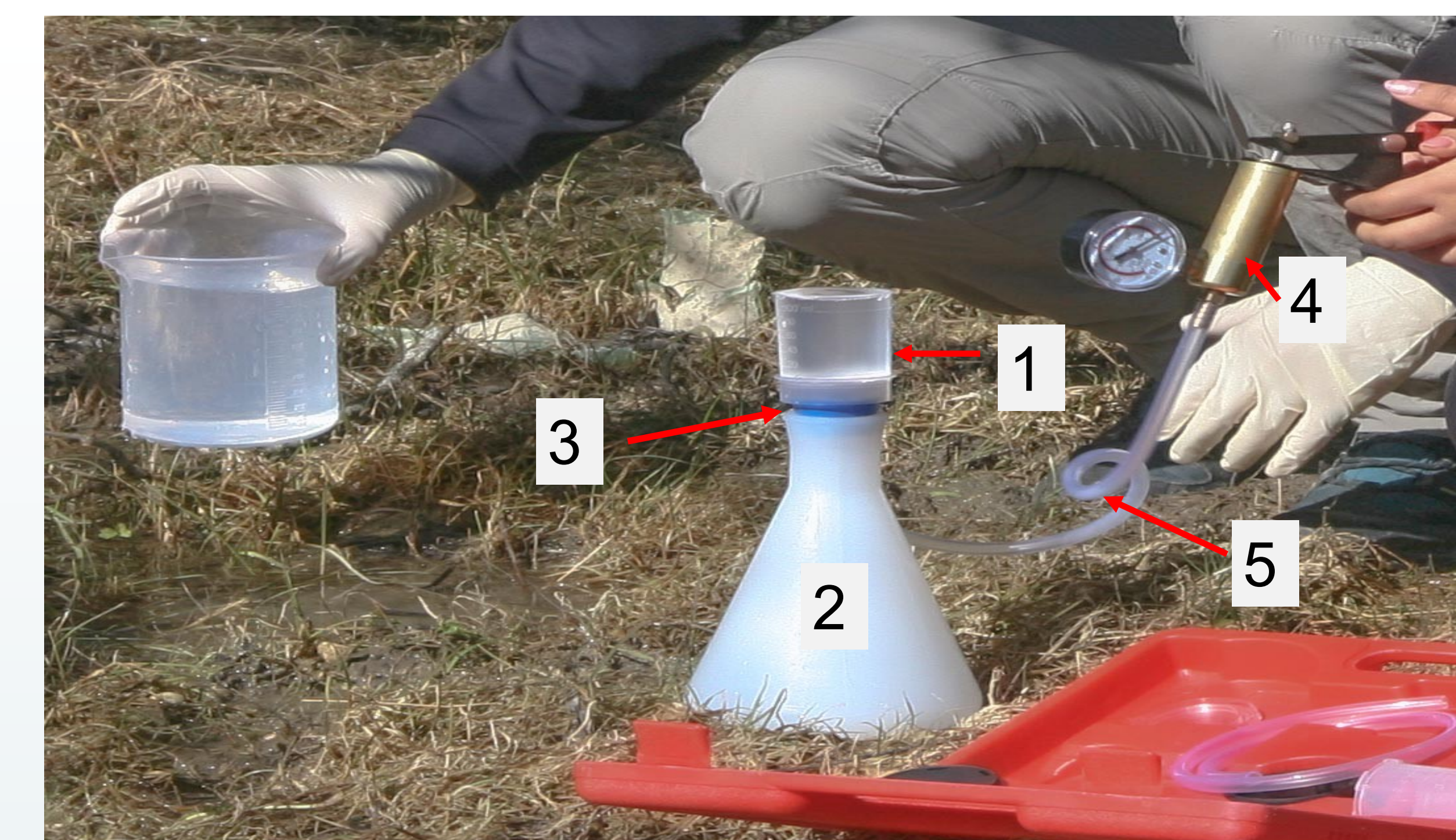


Fold filter



Place filter in 2 mL tube

Filtering water sample using vacuum pump



1. MicroFunnel™ Filter Funnel with Supor® Membrane, 100 mL, 0.2 µm
2. Vacuum flask
3. Rubber stopper
4. Manual vacuum pump
5. Silicone tubing

## Techniques to avoid contamination

- Used equipment goes into the black bag. Sources of contamination include anything that has touched the target species. Hands, clothes, waders, pumps, used supplies, and even the field vehicle.
- If you suspect that forceps, filter holders, or anything else have become contaminated, start over with a new kit.
- Carry spare site kits for these circumstances.
- The pump, tubing, and container are sources of contamination because they are subjected to the environment.
- Avoid handling these items once you have donned clean gloves.
- We use a white bag for clean things, and a black bag for used or contaminated items.
- Generally, remove the clean site kits from the white plastic bag only when needed.
- However, a kit that leaves the white bag should never be returned to the white bag.