eDNA Sampling Manual

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Minnesota Aquatic Invasive Species Research Center

maisrc.umn.edu

Thank you for volunteering to help us collect eDNA samples!

Our Objective: We are working to develop efficient strategies for monitoring aquatic invasive species (AIS) in Minnesota lakes using environmental DNA. Environmental DNA, or eDNA, is simply DNA left in an environment (in this case, lake water) by organisms that live there. We are studying the effectiveness of eDNA sampling conducted by volunteers in hopes that we can increase our sampling capacity and improve early detection of AIS. Early detection allows us to have a better chance at effective management, keeping the lakes we all love healthy.

General Overview: We are providing training and sampling equipment with the goal of volunteers independently collecting water samples and filtering for eDNA. Our process consists of 4 steps.

1. Review training materials and resources to gain an understanding of the sampling procedure.
    a. Resources include this packet, demonstration videos, and the recorded pre-sampling Q&A webinars (maisrc.umn.edu/surveill-eDNA).
2. Conduct the sampling on your own using the information & equipment provided.
    a. Collect a water sample during the sampling window (June 30th - July 9th) at the lake specified in your kit.
    b. If you have access to the lake via private property or boat, you are welcome to sample at that location or use a public access instead. Some public locations have been identified on the map provided.
    c. Filter the water using the equipment provided.
3. Return the filter, datasheet, and equipment with the included prepaid shipping.
    a. Return the sample and datasheet in the pre-labeled envelope as soon as possible upon completion.
    b. Return the equipment in its original box using the prepaid shipping label.
4. Fill out a post-sampling survey about your experience, and attend an optional webinar to share thoughts and offer feedback or ideas for improvement.

Final Notes: Throughout the sampling process, please take note of any directions that are unclear, steps that are difficult, or issues that arise. Our goal is to refine this protocol so that it can be used as an effective form of AIS monitoring in the future, and your feedback is crucial for that to happen.

Results: Preliminary results will be shared with volunteers upon completion of the project. Processing eDNA samples takes time, so it may be a while before there is information to share. Options for receiving results are provided in the post-sampling survey and will likely be via email or webinar.

Included Materials

			
Gloves (x3 pairs)	500ml Bottle	Siphon	eDNA Filter Pack
			
Plastic Tube	Filter Housing	Pencil	Lake Map
			
Data Sheet	Survey	Return Envelope	Box

*return label and box seal are also included

Pre-Sampling Protocol

1. Review the sampling protocol and how-to videos (maisrc.umn.edu/surveill-eDNA) to make sure you have all the necessary information to successfully perform the eDNA sampling.
2. Make sure to check the weather before sampling. Avoid days with rain or high winds. Wait at least one day to sample after rain.
3. Verify that all sampling materials are present in your kit.
4. Safety is important! Make sure to collect your sample from a safe, accessible location. Sample with a partner if possible.
5. Throughout the sampling and filtering process, avoid touching equipment without gloves or allowing it to contact any surfaces as this could introduce DNA contamination.

Sampling Protocol

1. **Collect a water sample.**
    a. Open the testing kit (save the box!). Find the data sheet, pencil, nitrile gloves, and the 500mL Nalgene bottle, this will be used to collect the sample.
    b. Proceed to a sampling location and find an accessible spot along the shoreline. See the included map for site options, or choose your own if you have private access.
        i. This could be a dock, boat launch, boat, beach, or anywhere you can safely and easily reach the water.
        ii. If you have access to a private dock, shoreline location, or boat, we encourage you to take a sample at that location. If on private property or a boat, please record the coordinates, an address, or description of the location on the data sheet.
        iii. Try to find an area with clear water and minimal floating debris.
    c. Fill out the applicable portions of the data sheet (1-8, 11, 12).
        i. For box #7 (Site): record either the recommended site or “private” followed by the type of location (i.e., dock, boat, shoreline). If the site was on private property or a boat, add details in box #11.
    d. Put on a pair of gloves.
    e. Rinse the bottle three times by completely filling it with lake water and emptying.
    f. Move slightly away from where you rinsed the bottle. If there is floating debris, clean the area out first by moving the bottle side-to-side across the surface of the water.

g. Fill the bottle by completely submerging it under the lake's surface. Once full, tightly screw the cap on immediately.

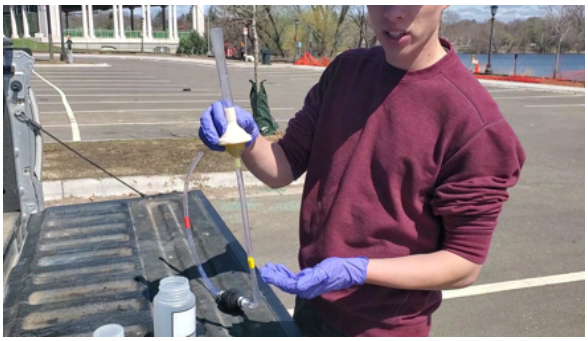
2. Filter the water sample (refer to pictures that follow!).

a. Take the water sample to a comfortable location, preferably somewhere with a flat surface to complete the filtering process. Make sure the siphon outflow is draining to an appropriate location for disposing of lake water (sink, ground, etc.), and not into another body of water.
b. Locate the nitrile gloves, siphon pump, and filter pack in the kit. Lay out materials so they are accessible and ready to use.
    i. Record the sample ID found on the filter pack in box #9 on your datasheet.
c. Put on a new pair of gloves.
d. Open filter pack by tearing off the top, remove the filter unit and short piece of plastic tubing.
    i. Attach the plastic tubing to the beige end of the filter unit.
    ii. Attach the siphon tube labeled with yellow tape to the yellow end of the filter unit.
    iii. **Make sure that the arrow on the squeeze bulb is pointing away from the filter unit.** It should already be correct but verify.
e. Once everything is prepared, uncap the water sample and insert the plastic tubing of the filter unit into the water contained in the bottle.
    i. Support the sample with one hand, use the siphon with the other.
f. Squeeze the bulb on the siphon to move water through the filter.
    i. Do this by completely compressing the bulb and allowing it to fully return before squeezing again.
    ii. If the filter clogs, stop and note/record the amount of water filtered.
g. Once the bottle is empty, lift the filter unit above the water level and allow excess water to drain out of the filter unit back into the bottle. Once water has drained, flip the filter unit upside down and remove the siphon tube to drain water stuck in the filter unit.
    i. Note how much water remains in the bottle.
h. Separate the plastic tube from the filter unit, place the filter unit back inside the original package, and seal tightly.
i. Fill out the remainder of the data sheet (13, 14) and collect materials.
    i. The volume of water filtered can be estimated by subtracting the volume remaining in the bottle from 500. If empty, record 500ml.
    ii. Any remaining water can be discarded, pick up any trash.

Post-Sampling Protocol

1. Complete the post-sampling survey included in the kit or found on the MAISRC website (maisrc.umn.edu/surveil-eDNA).
2. Return materials.
    a. Place data sheet and filter in the pre-labelled return envelope and seal.
    b. Place the sampling equipment and post-sampling survey back in the box. Seal the box with the included round, clear sticker. Place the included return label over the original label.
1. Drop off both items at your local post office shortly after sampling.

Sampling How-To Pictures

	
1 (f) After rinsing, collect the water sample by submerging the bottle.	**2 (c)** The siphon hose with yellow tape attaches to the yellow end of the filter housing.
	
2 (c) Verify that the arrow on the squeeze bulb points away from the filter housing.	**2 (d-e)** Filter the sample by squeezing the bulb until the bottle is empty.
	
2 (f) Remove the filter from the sample and turn upside down to allow water to drain.	**2 (g)** Place filter housing back in filter pack and seal.

Troubleshooting

- Contamination: occurs when the sample is exposed to outside sources of DNA.
    - Avoid contamination by keeping equipment in packaging until ready to use. Don't open the bottle until you are ready to collect the sample.
    - Wear the gloves provided whenever using sampling equipment or performing any parts of the sampling process.
    - If the collection bottle is exposed to contamination while open, don't collect a sample. Wash the bottle in water with bleach, let air dry upside down, and cap. Proceed with sampling as normal.
    - If the filter housing is exposed to contamination after it's removed from its sealed package, it cannot be cleaned. Notify the research team at MAISRC (eDNAproject@umn.edu), and we will send a new filter pack.
    - Extra gloves are included in the kit. If you drop a glove, touch a dirty surface, or feel the glove is dirty, put on a new pair before continuing.
- Siphon not working: this is most likely due to a bad seal at one of the connection points allowing air into the siphon.
    - Check each connection between the siphon hose, filter housing, and squeeze bulb. Make sure the hose is attached completely to the object.
    - Ensure the funnel is attached tightly to the filter housing.
    - Check for cracks in the hose. If the hose is cracked: remove it, carefully cut off the affected area using a scissors or knife and reattach.
    - If you have checked the equipment over completely and the siphon still does not work, it may be faulty. Contact the research team at MAISRC (eDNAproject@umn.edu) to get a new siphon sent to you.
- For any other questions or problems, please contact the research team at MAISRC (eDNAproject@umn.edu). We will work with you to resolve any issues that occur.

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