Environmental DNA Stream Sampling Protocol

State of Wisconsin Department of Natural Resources

STANDARD OPERATING PROCEDURES

December 2023

EGAD #: 3200-2024-05

Table of Contents

Equipment & Materials

Field equipment:

- Boat (and associated required safety equipment)
- Waders or boots
- Box of latex or nitrile gloves (powder free). 2 pairs/site (extras are good in case one rips, or contamination is suspected)
- Bleach Solution 500 parts per million (10% freshly made)- approximately 1 gallon to remove any DNA and avoid false positives. See table on converting household bleach concentration in Best Management Practices for Boat, Gear, and Equipment Decontamination.
- 3 – 1 L (quart) clean WQ sampling bottles (since sampling will be done in 3 replicates)
- 1 – sterile 50 mL centrifuge tube
- eDNA Monitoring Form/Labslip Form 4800-026-
  (this hyperlink only available to DNR – all other collectors must generate a labslip)
- Disinfection equipment per Manual Code 9183.1

Filtering equipment

- Pump tubing (Masterflex® L/S Platinum-Cured Silicone Tubing, Cat. No. 76049-300 from VWR)
- Drill
- Optional: Means to charge drill between monitoring stations (Power Inverter)
- 12 mm socket
- Peristaltic pump
- Filter holder
- Filters 100 pk (Glass microfiber 4.7cm, Cat. No. 28333-129 from VWR)
- Tweezers (start with the blank, then disinfect with bleach and the can be used for filtering)
- 1-500 mL bottle with distilled water for a filtration blank
- 4 - 5 mL microtubes per station prefilled with preservative (100% absolute non-denatured ethanol)
- Tube tray/rack for storing completed samples
- Sampling tray for carrying bottles and filtering equipment
- Bleach batch container (Tupperware type container) with bleach solution to disinfect the desired items (tweezers, filter holders, tubing, and bottles, when needed) between sites (min 15 minutes soak)

Protocols

Preparation & collection

Environmental DNA (eDNA) only detects DNA in the water to identify areas for further surveillance. Live adults are needed to determine if there is an established population.

Check with the Regional DNR Aquatic Invasive Species Coordinator to determine whether sampling is needed: https://apps.dnr.wi.gov/lakes/invasives/Contacts.aspx?role=AIS_POC

1. Identify a station with a known or suspected population of the target species. **A station is up to 800 m on wadable streams** (Figure 1). Consult the Aquatic Invasive Species Monitoring Lead to identify appropriate stations/sites.

a. Identify 10 eDNA sampling evenly spaced locations, evenly spaced apart and targeting preferred habitat (Figure 1). **Beware of private property. If the land surrounding is private property you will have to sample from in the water (via a boat) or get permission from the landowner.**
b. Target areas of slow flow where surface scum has accumulated. These areas include protected bays or along water edges with little, low flow. If specific locations of concern are not known on large waterbodies, target the preferred habitat for the species (e.g., for *Corbicula,* target sandy beaches; for zebra mussels, target rock, gravel, wood, or other hard substrates). Ensure clean field gear (waders, boats, anchors, etc.) between stations.
c. Avoid areas with excessive vegetation as that will bind with DNA and decrease detection

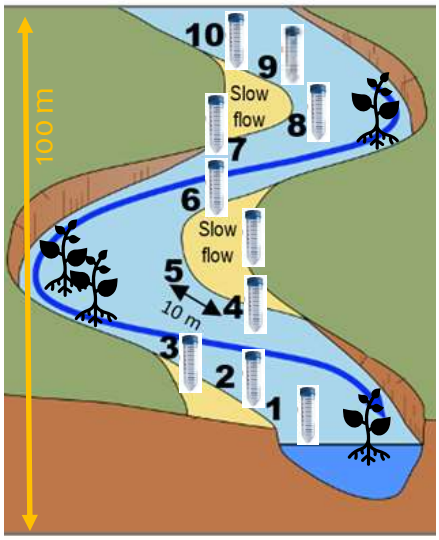


Figure 1 Diagram of 10 water collection sites for environmental DNA sampling along a 100 m stream station. Target water sample collection at areas suitable for the target species that avoid vegetation.

2. Ensure clean field gear (waders, boats, anchors, etc.) between stations.
   a. Follow the Boat, Gear, and Equipment Decontamination and Disinfection Manual Code for all equipment prior to sampling. Use 2% Virkon if previously in or proximal to waters known to harbor New Zealand mudsnail, *Corbicula*, or faucet snails prior to sample collection.
   b. Spray and soak genetic sampling gear (tweezers, filter holders, tubing, etc., when needed) with 1 part bleach to 9 parts water mixed fresh that day) to remove any DNA. Ensure 15 min contact time by either soaking, using a continuous spray, or spraying and bagging.

c. Rinse the bleached gear a minimum of 10 times in the site (surface) water prior to sample collection at a location that is downstream of sampling reach. *Residual bleach on gear could degrade or inhibit DNA in samples.* Gear may also be rinsed with municipal water.

d. Put on a new pair of gloves, **only handle clean or disinfected equipment**. One pair of gloves may be used for the entire station unless they become ripped or otherwise dirty.

e. Rather than disinfecting all tubing between stations, it may be preferable to use a new segment of tubing that goes from the sample to the filter to decrease the possibility of

3. Always begin at the downstream end of a station, and always move/wade upstream to not further disturb sediments/DNA in upstream/wind locations (Figure 1).
4. From each location, dip the 50 mL centrifuge tube just below the surface, attempting to collect from the top 1 cm of water *a*s DNA is increased in concentration near the surface. Collect 50 mL of water in the tube and pour in a 1 L (quart) bottle.
5. Repeat this 2 more times per each of the 10 sites to yield a total of 3 composite samples, each consisting of 500 mL in 1 L (quart) bottles (Figure 2).
6. Samples may now be either filtered in the field or transported back to a lab for filtering. If transporting to a lab, place the 1-liter (quart) bottles on ice.

Filtering samples

1. For assembly and operation of field filtering equipment please see this video https://widnr.widen.net/s/sjtfbdn8lt/wy_ednasamplingprotocol.

   This can be done in the field or at a "lab" and is usually done with 2 people

2. Prior to sampling a site, tubing, tweezers, and filter holder with the rubber O-ring should be disinfected by placing in a container with 10% bleach for 15 minutes, then rinsed 10 times in clean (municipal or other "clean") water. The drill is not normally disinfected but can be wiped with DNA wipes if necessary.

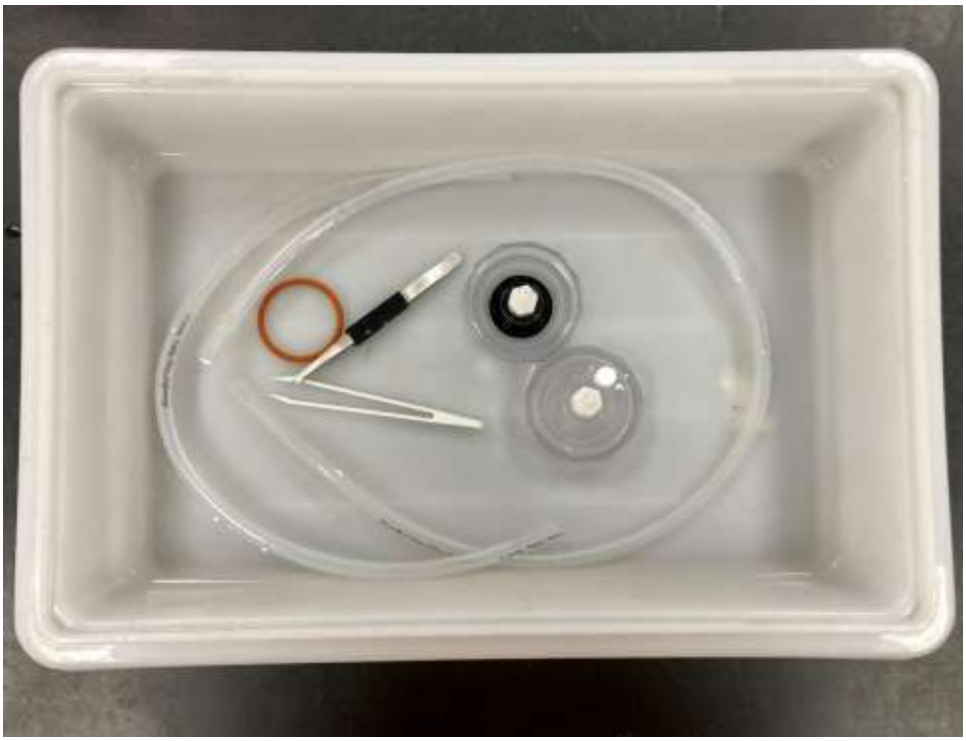


Figure 2 Soak tubing, pump and tweezers soaking in 10% sodium chlorine (aka bleach) solution for 15 minutes prior to filtration.

3. Put on a new pair of gloves.
4. ***Before running any actual (field) samples through the filter****, a field blank should be run. This is to ensure there is no residual eDNA contamination prior to filtering an actual sample.* To do this, pour 500 ml of distilled, deionized, or other source of clean water into a clean 1 L bottle.
    a. Filter should be placed in the filter holder with tweezers (tweezers that handle clean filters only) on top of black screen, then red O-ring should be placed on top of filter and filter holder should be closed making sure the O-ring does not get caught in the threads.
    b. Place clean (new or disinfected) tubing in the 500 ml of blank sample and hook up to the top of the filter holder (water will hit the filter before going through the black screen on which it is placed) and the other piece of tubing to the peristaltic pump.
    c. Use drill with 12 mm socket to operate the pump ensuring right direction of water flow: from bottle through filter, then black screen, and finally the pump (filtrate can be wasted onto the ground).

d. After ensuring the water is pumping in the proper direction, pump the rest of the sample.
e. Once the full 500 ml of sample has passed through the filter, remove the top of the filter holder and use the “wet tweezers” to carefully fold the filter in half 2-3 times, making sure it will fit in the microtube and will be immersed in the preservative.
f. Place the folded filter in the labeled 5 mL microtube with the prefilled preservative.

Figure 3 Sample bottles, peristaltic pump, tubing, filter tubes, tweezers, and gloves used for eDNA filtration.

5. Repeat this procedure for the actual samples (triplicates). Be sure to agitate (shake) the sample bottle before filtering. It is not necessary to disinfect between replicates, nor to run a blank between replicates. However, disinfection and blank procedures should be done between stations.

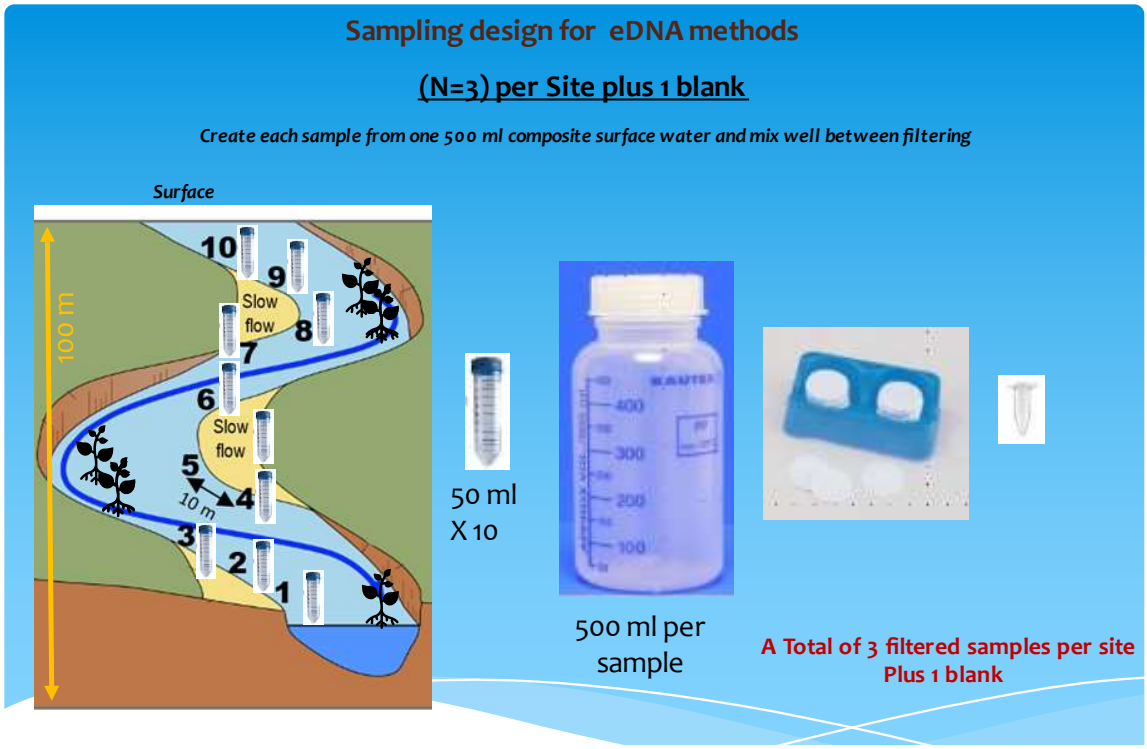


Figure 2 Diagram of 10 water collection sites for environmental DNA sampling along a 100 m stream station. At each site, 50 ml of surface water is collected and added to a composite for a total of 500 ml of surface water which is then shaken and then the entire 500 ml sample is passed through a filter which is preserved on ice.

Sample handling and preservation

- Each sample should include external labels with:
    - Field number (i.e., Fox-1; Fox-2; Fox-3)
    - Collector initials and date
- Since sample tubes are too small for large labels with all information, label them with consecutive field numbers and keep a spreadsheet with all the information cross-referenced with those numbers (i.e., WBIC; SWIMS station ID; latitude/longitude).
- Tubes should be kept on ice (or with ice packs) during transport and stored frozen (-20°C or less is best) for up to 2 months (Figure 3). Samples should be stored upright in a rack to avoid spilling and contamination. Samples in racks should be stored in a 1-gallon Ziploc bag to keep dry, if transported on ice.

- Complete the eDNA monitoring form (4800-026) that was created from the SWIMS database. Include a copy of the form with the samples. Samples from all sites will be hand-delivered or shipped on ice to WI State Lab of Hygiene (see address below). Since there are only 2 ml of ethanol per tube, theses samples will not meet Hazardous shipping requirements until 30 ml (15 samples) are shipped in one box. ***Prior to delivering samples, please notify the lab by email and/or phone when you will be sending samples and when they can expect to receive them. Thursday and Friday are general not good days to ship to the lab.***

After completing a station, soak tubing, filter holder, tweezers, and sample bottles and tubes (if reused) in 10% bleach disinfection solution in a tub for ≥15 minutes to disinfect to remove any DNA residues). Remember, spray or soak waders with bleach (or 2% Virkon solution for 20 minutes if in/near New Zealand mudsnails, *Corbicula* or faucet snail waters). Follow the Boat, Gear and Equipment Decontamination and Disinfection Manual Code for all other equipment.

Figure 6 Example of plankton tows with bagged datasheet and packaging material.

Data Management

A completed Test Request - eDNA Environmental Toxicology (4800-026) must be submitted with each sample. This labslip form is generated in SWIMS prior to sample collection (Appendix A). Some fields on this form will be pre-populated (yellow highlights) when the labslip form is generated and others will be filled out during sample collection (blue highlights) (Figure 3 and 4). The following are some definitions for fields to complete on the form.

Account Number: This field is tied to the funding source. If you are unsure what the proper account number is refer to http://intranet/int/es/science/ls/Account.htm or contact the DNR Laboratory Coordinator.

DNR User ID: Oracle ID that can be found in SWIMS. This can be the same as your SWIMS ID, but check.

Report To Name: This field will auto-populate when "DNR User ID" is entered.

Report To Email: This field is for use by non-DNR collectors.

Sample Type: Select SU Surface Water for eDNA samples.

Station ID (STORET #): use the SWIMS station ID for the sample location.

Sample Address or Location Description: This field is the SWIMS Station Name and will auto populate when Station ID (STORET #) is entered.

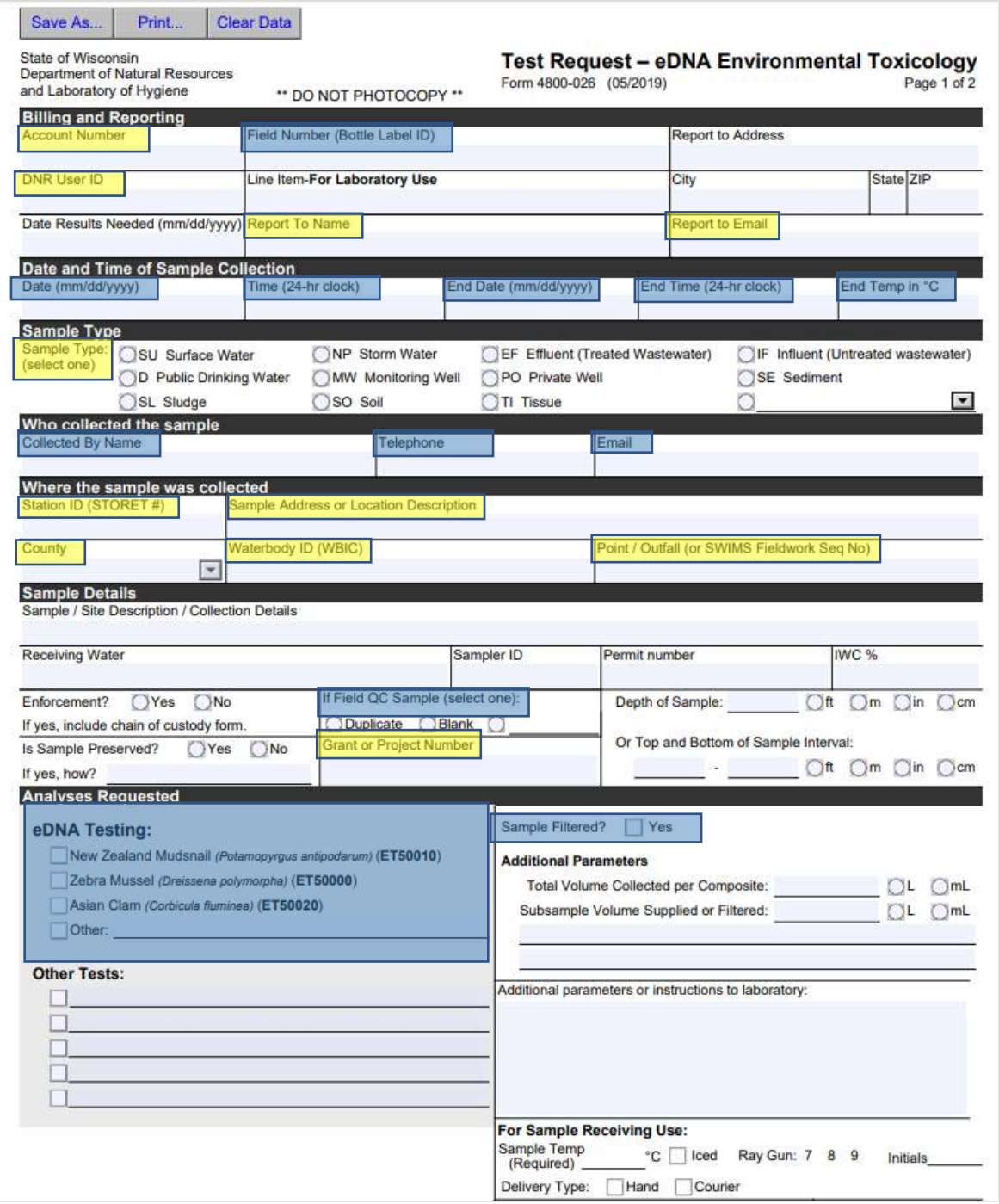
Point/Outfall (or SWIMS Fieldwork Seq No): This field will be auto generated when fieldwork event is scheduled in SWIMS (when labslip is created)

Grant or Project Number: field should include the Grant Number or the SWIMS Project Number. This is a required field.

Fields Populated During Sample Collection (Highlighted in Blue)

Field Number: Should contain abbreviated stream name and sample type and which set of replicates (i.e., STRM D – NZMS, 1; STRM D – NZMS 2, STRM D – NZMS 3). This field must match the "Field Number" on the sampling container's labels.

Preservation (ethanol/refrigeration).


Save As... Print... Clear Data

State of Wisconsin
Department of Natural Resources
and Laboratory of Hygiene

** DO NOT PHOTOCOPY **

Test Request – eDNA Environmental Toxicology
Form 4800-026 (05/2019) Page 1 of 2

Billing and Reporting
Account Number | Field Number (Bottle Label ID) | Report to Address
DNR User ID | Line Item-**For Laboratory Use** | City | State | ZIP
Date Results Needed (mm/dd/yyyy) | Report To Name | Report to Email

Date and Time of Sample Collection
Date (mm/dd/yyyy) | Time (24-hr clock) | End Date (mm/dd/yyyy) | End Time (24-hr clock) | End Temp in °C

Sample Type
Sample Type: (select one)
SU Surface Water | NP Storm Water | EF Effluent (Treated Wastewater) | IF Influent (Untreated wastewater)
D Public Drinking Water | MW Monitoring Well | PO Private Well | SE Sediment
SL Sludge | SO Soil | TI Tissue

Who collected the sample
Collected By Name | Telephone | Email

Where the sample was collected
Station ID (STORET #) | Sample Address or Location Description
County | Waterbody ID (WBIC) | Point / Outfall (or SWIMS Fieldwork Seq No)

Sample Details
Sample / Site Description / Collection Details
Receiving Water | Sampler ID | Permit number | IWC %
Enforcement? Yes No
If yes, include chain of custody form.
Is Sample Preserved? Yes No
If yes, how?
If Field QC Sample (select one): Duplicate Blank
Grant or Project Number
Depth of Sample: ft m in cm
Or Top and Bottom of Sample Interval: ft m in cm

Analyses Requested
eDNA Testing:
New Zealand Mudsnail *(Potamopyrgus antipodarum)* (**ET50010**)
Zebra Mussel *(Dreissena polymorpha)* (**ET50000**)
Asian Clam *(Corbicula fluminea)* (**ET50020**)
Other:

Other Tests:

Sample Filtered? Yes

Additional Parameters
Total Volume Collected per Composite: L mL
Subsample Volume Supplied or Filtered: L mL

Additional parameters or instructions to laboratory:

For Sample Receiving Use:
Sample Temp (Required) °C Iced Ray Gun: 7 8 9 Initials
Delivery Type: Hand Courier

Figure 3 *Front page of Test Request eDNA Environmental Toxicology Analysis Form 4800-026. Hight lighted parameters are mandatory. Parameters in yellow highlights are completed prior to sampling and blue are completed during sampling. Data will be entered into SWIMS by the Wisconsin State Lab of Hygiene.*

Test Request – eDNA Environmental Toxicology

Form 4800-026 (05/2019) Page 2 of 2

Field Parameters - Optional **Only fill out if directed by your project coordinator.**

Temperature - Sample (°C)		Gage Height (ft)	
Temperature - Ambient Air (°C)		Flow (cfs)	
DO (mg/l)		Flow (MGD)	
% Saturation		Depth to Groundwater (ft or m)	
pH (su)		Turbidity (NTU)	
Secchi Depth (feet or meters) (ft or m)		Transparency Tube (cm)	
Secchi Depth Hit Bottom?	☐ Yes ☐ No	Nitrates (mg/l)	
Cloud Cover (%)			
Cond (µS/CM@25°C)			

Brief Sampling and Shipping Instructions:

1. **Important: Please plan on either delivering samples in person to the laboratory (2601 Agriculture Drive, Madison, WI; 7:45AM-4:30PM weekdays) or shipping samples out for the next day delivery on ice.
2. The detailed eDNA collection protocol can be found at: https://dnr.wi.gov/water/wsSWIMSDocument.ashx?documentSeqNo=194373988
3. Each sample should include external labels with:
   - The water body identification code and/or station identification code
   - Latitude, longitude
   - Collector's name and date
4. Samples should be stored upright in a rack to avoid spilling and DNA contamination. Samples in racks should be stored in 1 gallon Ziploc bags to keep dry, and transported on ice or with freezer ice packs. eDNA samples can be stored frozen (-20°C or less is best) for up to 2 months.
5. Samples should be hand-delivered or shipped on ice to WI State Lab of Hygiene.
6. Complete the test request form(s), using a separate form for each sample collected.
7. Pack bagged samples (keeping them upright) and lab request forms into an appropriate, leak-proof shipping container. It is highly recommended that appropriate packing materials (e.g. styrofoam, newspapers, bubble-wrap, etc…) be added to the container in order to prevent breakage.
8. Add ice (in sealed bags) or freezer ice packs. Securely tape the shipping container closed.
9. Prior to delivering samples, please notify the lab by email and/or phone when you will be sending samples and when they can expect to receive them.
10. Ship samples for next day delivery, whenever possible (e.g. FedEx or UPS). Do not ship samples on Fridays or Saturdays because the lab does not accept the samples on weekends.

Shipping Address:

Wisconsin State Lab of Hygiene
Attn: Sample Receiving
2601 Agriculture Drive
Madison, WI 5371

If you have test related questions, please contact the WSLH Environmental Toxicology Department at 608-224-6230

The **Account Number** must be completed in order for the samples to be billed to the correct funding source. If you are unsure what the proper account number is refer to http://intranet/int/es/science/ls/Account.htm or contact the DNR Laboratory Coordinator or the State Laboratory of Hygiene.

The **Lake Grant or Project Number field** should include the Lake Planning Grant Number or the Project Number.

Sample Depth – If you sample in a lake, this is required.

Field Parameters – If you do fill this out, the data will go into SWIMS automatically, as long as the STORET# has been entered. Please do not re-enter.

Figure 4 *Back page of Test Request - eDNA Analysis Form 4800-026.0 Optional Field Parameters will be entered into SWIMS for the Wisconsin State Lab of Hygiene. Monitoring Results are mandatory for quantitative results, but this is data will not be entered into SWIMS, but a scanned copy will be available in the PDF that is saved to SWIMS.*

When you receive results, you will need to search for the SWIMS fieldwork event if you want to check the parameters collected. Theses parameters will also be available in the report for the State Lab.

When looking at the General tab in LDES, you will see a field for Sample/Labslip ID. You can search for this in SWIMs by:

- Clicking search fieldwork events
- Pasting the sample/labslip ID from LDES into the Sample/Labslip ID field into SWIMs
- Click submit

Sample Submission

The lab must be notified as soon as possible that eDNA samples will be submitted (within 2 weeks of submission).

It is preferred that samples are delivered in person to the Wisconsin State Laboratory of Hygiene. It is best to deliver samples to the regional DNR AIS coordinator to submit or to the Statewide AIS Monitoring Lead at the statewide meetings. If unable to deliver samples in person, you must follow shipment instructions according to sample type. All eDNA samples should be delivered monthly to the Wisconsin State Laboratory of Hygiene. Do not store all your samples until the end of the season.

Please deliver samples to:

Dagmara Antkiewicz
Environmental Toxicology, Rm. 205
Wisconsin State Lab of Hygiene
2601 Agriculture Drive
Madison WI 53718

phone 608.224.6230

7:45 am – 4:30 pm weekdays

You **must** notify the Wisconsin State Lab of Hygiene at:

Email: Biomonitoring@slh.wisc.edu
Phone: 608-224-6230

Note: This does not include samples that you provide to the regional DNR AIS Coordinator or Statewide AIS Monitoring Lead for submission to the State Lab of Hygiene on your behalf.

Delivery preparation

Pack labeled bottles (keeping them upright) with lab request forms into an appropriate, leak-proof shipping container (i.e., Styrofoam or plastic cooler) (Figure 4). Packing materials (e.g., Styrofoam, newspapers, bubble-wrap, etc.) should be added to the shipping container to prevent breakage. Securely tape the shipping cooler closed.

Figure 6 Example of environmental DNA samples with bagged datasheet and packaging material.

Delivery of eDNA samples

Ethanol solutions are classified as flammable liquids by the US Department of Transportation and the shipment of such materials is governed by US DOT's regulations - with a couple of exceptions. Transporting hazardous materials, including ethanol, is allowed in State of Wisconsin vehicles, without the need to comply with any US DOT regulations. Thus, it is permissible to transport these samples by state vehicle. Additionally, these eDNA samples are generally within the small quantify threshold below 30 ml of ethanol per vial/bag. Once the bag/vial exceeds 30 ml, it meets the limited quantity threshold and use the black and white label.

For questions about sample collection contact the AIS Monitoring Lead at (608) 381-3231. If you have test related questions, contact the WSLH Environmental Toxicology Department at (608) 224-6230.

Figure 7 Example of package prepared for hazardous shipping. Everyone shipping must have Hazardous shipping certification training.

It is mandatory that DNR use the State Spee-Dee Delivery contract to send samples within

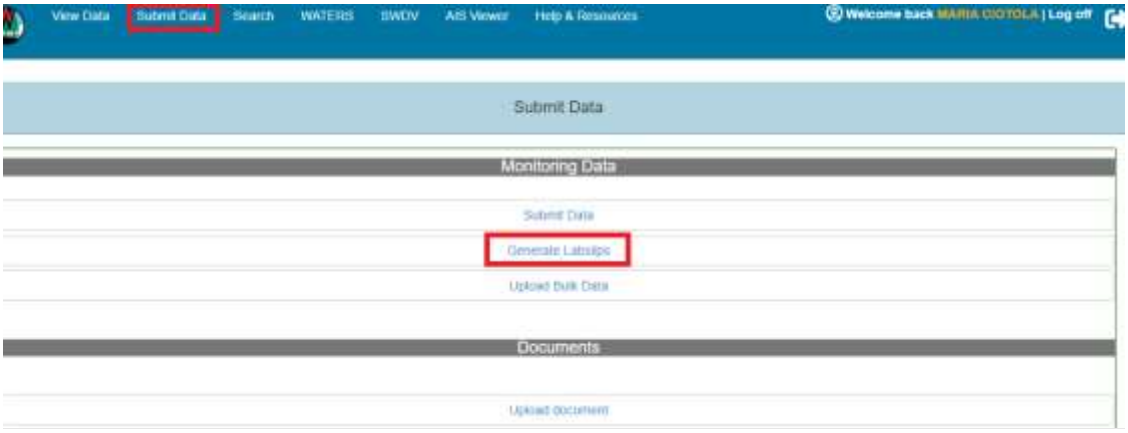
Wisconsin, unless they are not available in your region. Here are websites for Spee-Dee:

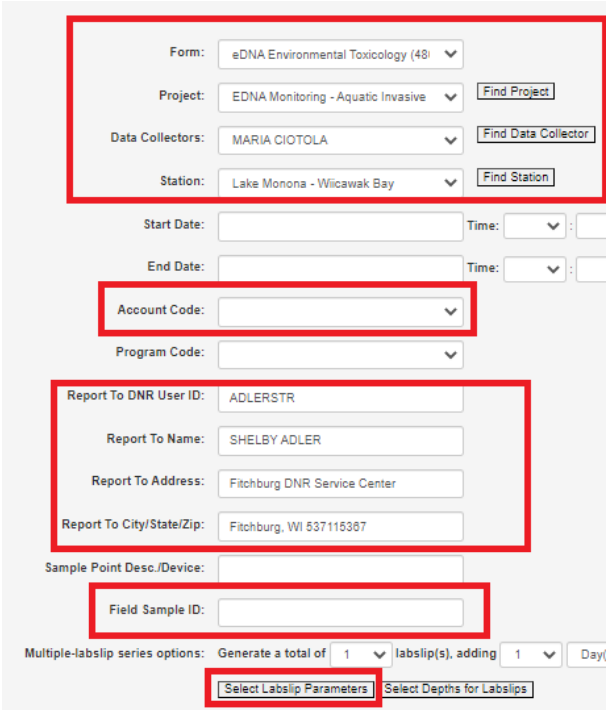
http://www.speedeedelivery.com/walkin-wi.html
http://www.speedeedelivery.com/OnCallLetter.pdf

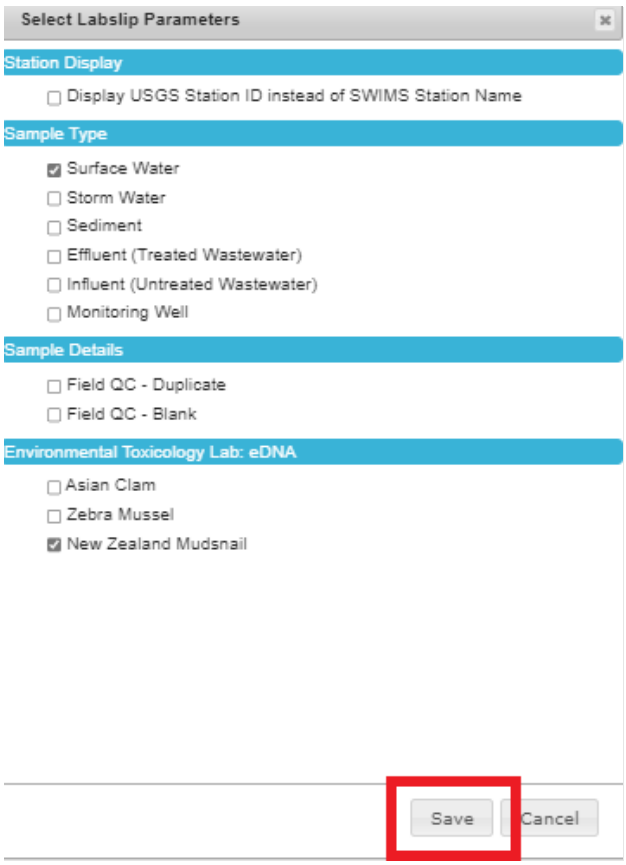
Note: If samples are to be shipped by common carrier, size restrictions may apply to the sample containers. The maximum size allowed under the US DOT regulations for plastic containers is 1 liter – check with the shipper for any additional restrictions prior to sampling so that samples are collected in appropriately sized bottles.

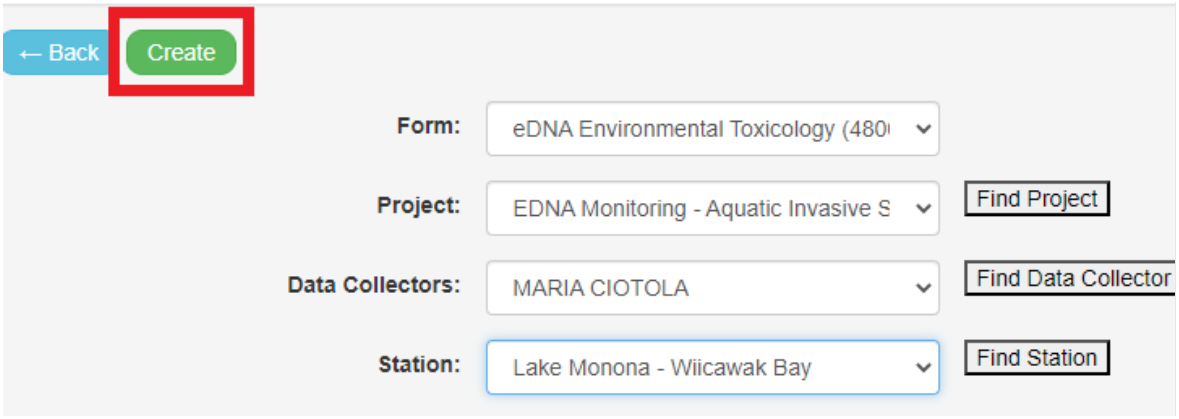
For questions about sample collection contact the AIS Monitoring Lead at (608) 381-3231. If you have test related questions, contact the WSLH Environmental Toxicology Department at (608) 224-6230.

APPENDIX: Instruction for Generating eDNA Labslips






Select Labslip Parameters
Station Display
Display USGS Station ID instead of SWIMS Station Name
Sample Type
Surface Water
Storm Water
Sediment
Effluent (Treated Wastewater)
Influent (Untreated Wastewater)
Monitoring Well
Sample Details
Field QC - Duplicate
Field QC - Blank
Environmental Toxicology Lab: eDNA
Asian Clam
Zebra Mussel
New Zealand Mudsnail
Save
Cancel


← Back
Create
Form:
eDNA Environmental Toxicology (480
Project:
EDNA Monitoring - Aquatic Invasive S
Find Project
Data Collectors:
MARIA CIOTOLA
Find Data Collector
Station:
Lake Monona - Wiicawak Bay
Find Station