Veliger Sampling Protocol

State of Wisconsin Department of Natural Resources

STANDARD OPERATING PROCEDURES

September 2021

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Methods

Zebra mussel establishment is limited by calcium concentration. Conductivity is a predictor for calcium and lakes with a conductivity of 99 umhos/cm or greater should be sampled for veligers if adult mussels are not already known to be established in the waterbody. Conductivity can be collected via a handheld meter if it is not known.

Status Guidance

To determine whether there is a reproducing established population, live adults must be collected using a plankton tow to verify an established population. See the AIS Status Guidance for criteria on assigning statuses (https://dnrx.wisconsin.gov/swims/downloadDocument.do?id=127413817).

Check with the Regional Aquatic Invasive Species Coordinator to determine whether sampling is needed and check with the AIS Monitoring Coordinator to determine whether there is sufficient funding for analysis.

https://dnr.wi.gov/lakes/contacts/Contacts.aspx?role=AIS_RE_COORD

The regional DNR AIS coordinator will track the sampling efforts on an internal network drive.

\\central\water\WQWT_PROJECTS\WY_LRS\AIS\AIS Monitoring\Verification\Plankton tows-dredges\plankton veliger tracking list.xlsx

Materials

- Boat
- Anchor
- Lake bathymetry maps
- GPS unit – optional
- 50-cm diameter and 200 cm length plankton net with 64-micron mesh (net body, ring and brindle, detachable cod-end assembly, lined with nitrex mesh) on a rope with 1-meter increments marked. Aquatic Research Instruments is one option for the plankton nets. PH 208.290.3820; email: hydrobio@aol.com; website: aquaticresearch.com.
- Spray bottle
- 250 ml and 1-liter plastic bottles
- Ethanol, 95% ethanol (190 proof ethyl ethanol)
- Sample labels: provided to regional DNR by the State Lab
- Pencil
- *Test Request – Mussel Veliger and Waterflea Analysis Form 4800-027* labslip: generated in the "*DNR AIS Monitoring -_____County (Spiny Waterflea and Zebra Mussel Veliger Tows)*" SWIMS project (Appendix A).
- Large brush and small brush
- Fresh rinse water
- Chlorine bleach
- Vinegar
- Large container to hold plankton net for bleach and vinegar solution

Field protocol for veliger tows

1. Collect 3 vertical zebra mussel veliger net tows (using the 64-micron mesh net); one from the deep hole and 2 other tows from deep areas along the downwind side of the lake.
2. Veligers are plankton and will move with the water flow and wind so sample deep, downwind portions of a lake. In lakes that stratify, veligers will often be in higher abundance above the thermocline so samples should be collected so that the ring of the net passes through the thermocline. Since most staff do not have temperature probes to determine the depth of stratification, we have included estimates for sampling depth below.
    a) if water column is **deeper than 6 meters** (~19.7 feet), then collect each tow with the ring of the net **4 meters** (~13.1 feet) below the waters' surface; or
    b) if the water column is **shallower than 6 meters** (~19.7 feet), attempt to collect a vertical tow with the ring of the net within **2.5 meters** of the bottom.
    c) If the water column is too shallow to lower the ring into the water, try to collect a horizontal tow or just collect tows from the deep hole.
3. The net must be lowered slowly to not disturb the bottom sediments and get sediment in the net. If you hit the lake bottom, rinse out the sampling equipment and go to a different area of the lake to avoid the sediment plume. Use the spray bottle to rinse the outside of the net so that samples are washed into the collection cup
4. Transfer the sample from the collection cup to the sample bottle.
5. Condense the size of the sample by decanting out as much water as possible in the field. This helps reduce the amount of ethanol that needs to be added and aids in the analyses and to obtain an accurate enumeration of the larval density.
6. Repeat the process at the other two preselected sites. Composite the samples from the three sites into one 250-ml or larger (or 1-L) bottle and receive a single enumeration for the lake.
7. Label the sample bottle(s) externally. Prepare labels prior to sampling so that you don't have to write on wet paper (Figure 1).

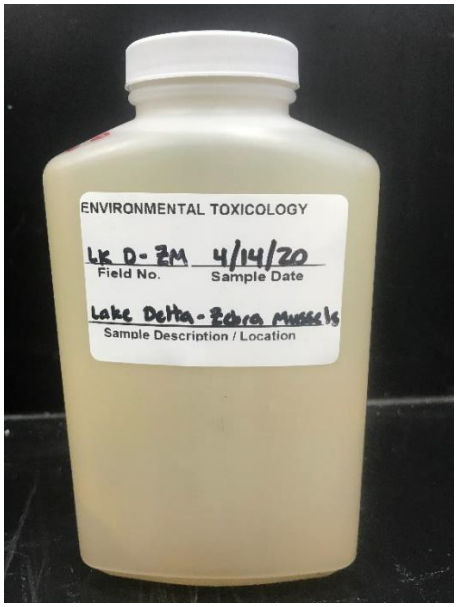


Figure 1 Example of labelled sample bottle.

8. Preserve the sample using 95% ethanol. The ratio should be 4-part ethanol to 1-part sample. *Note: If the prescribed ethanol to sample ratio (4:1) cannot be achieved after repeated condensing and decanting, then the sample should be split between two sample bottles. Label the bottle in sequential order.*

    Note: If the prescribed ethanol to sample ratio (4:1) cannot be achieved after repeated condensing and decanting, then the sample should be split between two sample bottles. Label the bottles in sequential order.

    Note: Transport the sample bottle(s) on ice in a cooler if you are not preserving immediately.
9. Record data on the Test Request – Mussel Veliger and Waterflea Analysis Form 4800-027.

Decontamination and Disinfection for Tows

To stop the spread of veligers and to eliminate cross-contamination, use the following procedure to clean plankton nets. You do not have to decontaminate and disinfect equipment between sample sites on the same lake. If multiple lakes are sampled in one day, DNR employees, agents and some permittees and contractors must follow the Decontamination and Disinfection Manual Code.

http://dnr.wi.gov/topic/invasives/disinfection.html

Another approach is to ask citizens on the lake to take DNR staff onto the lake with their boats. This saves time because we don't have to launch and then decontaminate the trailer and boat upon departure, and it is valuable for the citizens to learn from staff and develop positive relationships. The following are detailed options for cleaning veliger nets in order of effectiveness.

- **Option 1:** Use a completely new set of gear for each waterbody during the work day and disinfect all gear at the end of the day using one of the options below.

- **Option 2:** The gear must be soaked in vinegar to dissolve veliger shells and prevent false positives on the next waterbody. Then the gear can be sprayed with a handheld Jiffy steam cleaner or 140^{o}F hot water unit for 10 to 13 seconds so that there is a continuous stream of steam over each area of the gear. A handheld Jiffy steamer is gentler on equipment than a large steam cleaner. This method is preferred over options 3 and 4.

- **Option 3:** Soak gear in vinegar to dissolve veliger shells and prevent false positives on the next waterbody. Then soak gear in 500 ppm chlorine solution (~2.5 Tbsp chlorine/gallon water) for 10 minutes. Tow nets, metal ring, plastic netting, etc. will degrade if chlorine or virkon is not rinsed off thoroughly.

- **Option 4:** Soak gear in vinegar to dissolve veliger shells and prevent false positives on the next waterbody. Then spray gear with 500 ppm chlorine solution (~2.5 Tbsp chlorine/gallon water) for 10 minutes. This spraying method is the least effective and should only be used if other options are not available. The gear should be rinsed with clean water or water immediately. Tow nets, metal ring, plastic netting, etc. will degrade if chlorine or virkon is not rinsed off thoroughly.

** If adult zebra or quagga mussels are observed, extreme care should be taken to avoid transporting individuals. We recommend that you use a different set of equipment if you have additional lakes to sample or discontinue sampling for that day to allow for sufficient decontamination if you do not have another net. Regardless, it is a good idea to rinse your equipment in hot water and let the net and cup dry thoroughly for at least 5 days sampling, both to preserve the integrity of the net and reduce the risk of any veligers remaining viable.

Data Management

A completed ***Test Request – Mussel Veliger and Waterflea Analysis Form 4800-027*** must be submitted with each sample jar (even for samples that are split into separate jars you must have one form per jar). This labslip form is generated in SWIMS prior to sample collection. 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 2 and 3). 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 tow samples and SE Sediment for dredge 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)

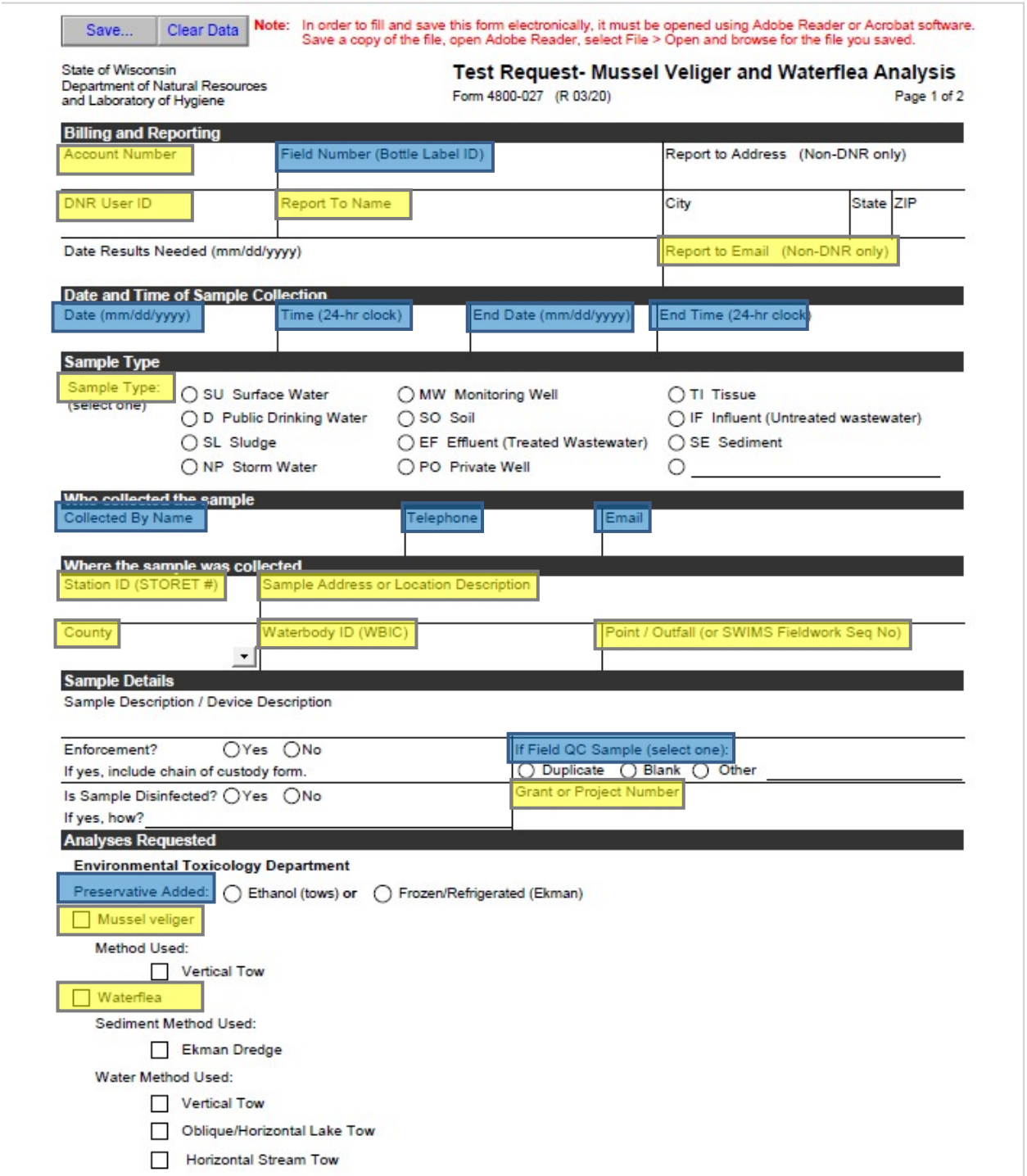
Grant or Project Number: field should include the Grant Number or the SWIMS Project Number. This is a required field.

Fields Populated During Sample Collection (Highlighted in Blue)

Field Number: Should contain abbreviated lake name and sample type (i.e. LK D – SWF). This field must match the "Field Number" on the sampling container's labels. If samples must be

split in separate jars, label them with sequential numbers (i.e. LK D – SWF 2 of 2, if second sample).

Preservation (ethanol/refrigeration): Tow samples must be preserved in ethanol. Sediment samples must be frozen. There are fields relating to preservation methods in two separate sections of the labslip form, it is required to populate all these fields.



Save... Clear Data **Note:** In order to fill and save this form electronically, it must be opened using Adobe Reader or Acrobat software. Save a copy of the file, open Adobe Reader, select File > Open and browse for the file you saved.

State of Wisconsin
Department of Natural Resources
and Laboratory of Hygiene

Test Request- Mussel Veliger and Waterflea Analysis

Form 4800-027 (R 03/20) Page 1 of 2

Billing and Reporting

Account Number | Field Number (Bottle Label ID) | Report to Address (Non-DNR only)

DNR User ID | Report To Name | City | State | ZIP

Date Results Needed (mm/dd/yyyy) | Report to Email (Non-DNR only)

Date and Time of Sample Collection

Date (mm/dd/yyyy) | Time (24-hr clock) | End Date (mm/dd/yyyy) | End Time (24-hr clock)

Sample Type

Sample Type: (select one)

- ◯ SU Surface Water
- ◯ D Public Drinking Water
- ◯ SL Sludge
- ◯ NP Storm Water
- ◯ MW Monitoring Well
- ◯ SO Soil
- ◯ EF Effluent (Treated Wastewater)
- ◯ PO Private Well
- ◯ TI Tissue
- ◯ IF Influent (Untreated wastewater)
- ◯ SE Sediment
- ◯ _____

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 Description / Device Description

Enforcement? ◯ Yes ◯ No
If yes, include chain of custody form.

If Field QC Sample (select one):
◯ Duplicate ◯ Blank ◯ Other _____

Is Sample Disinfected? ◯ Yes ◯ No
If yes, how? _____

Grant or Project Number

Analyses Requested

Environmental Toxicology Department

Preservative Added: ◯ Ethanol (tows) or ◯ Frozen/Refrigerated (Ekman)

☐ Mussel veliger

Method Used:

☐ Vertical Tow

☐ Waterflea

Sediment Method Used:

☐ Ekman Dredge

Water Method Used:

☐ Vertical Tow
☐ Oblique/Horizontal Lake Tow
☐ Horizontal Stream Tow

Figure 2 Front page of *Test Request – Mussel Veliger and Waterflea Analysis Form 4800-027.* 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- Mussel Veliger and Waterflea Analysis

Form 4800-027 (R 03/20) Page 2 of 2

The purpose of this form is to track waterflea or mussel veligers during AIS monitoring.

Notice: Information on this voluntary form is collected under ss. 33.02 and 281.11, Wis. Stats. Personally, identifiable information collected on this form will be incorporated into the DNR Surface Water Integrated Monitoring System Database. It is not intended to be used for any other purposes but may be made available to requesters under Wisconsin's Open Records laws, ss. 19.32 - 19.39, Wis. Stats.

OPTIONAL FIELD PARAMETERS

Secchi Depth (feet or meters) ft or m ___ ___ . ___ Flow (cfs) ___ ___ ___ ___ ___ ___ . ___

Secchi Depth Hit Bottom? ◯ Yes ◯ No

Cloud Cover (%) ___ ___ ___

Cond (µS/CM@25°C) ___ ___ ___ ___

Monitoring Results

Diameter of plankton net opening ◯ 30cm ◯ 50cm ◯ Other _____

Length of plankton net: ◯ 250cm ◯ Other _____

Site 1 : Latitude (optional): _____ Longitude (optional): _____ Preservative: Ethanol (tows) ◯ or frozen/refrigerated (Ekman) ◯

Depth sampled (if vertical tow) _____ ft/m

Site 2 : Latitude (optional): _____ Longitude (optional): _____ Preservative: Ethanol (tows) ◯ or frozen/refrigerated (Ekman) ◯

Depth sampled (if vertical tow) _____ ft/m

Site 3 : Latitude (optional): _____ Longitude (optional): _____ Preservative: Ethanol (tows) ◯ or frozen/refrigerated (Ekman) ◯

Depth sampled (if vertical tow) _____ ft/m

Have you consolidated all your plankton samples into one composite bottle? ◯ Yes ◯ No ◯ NA

If physical specimens of Waterflea or Dressenid mussel are observed, the collector must enter this as a fieldwork event. Use an early detection form if observed during an early detection survey. Use an incident report form if this was not during an early detection survey.

Brief Sampling Instructions:

1. Detailed collection protocols can be found at:
   - Mussel veliger: https://dnrx.wisconsin.gov/swims/downloadDocument.do?id=159656867
   - Waterflea: https://dnrx.wisconsin.gov/swims/downloadDocument.do?id=159656863.
2. **Lab Account Number** 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 (608-264-8589).
3. **Field Number** is the label on the sampling container (see SOP). Remember, one form must be completed for each composite sample.
4. **Sample type**: select SU Surface Water for tow samples and SE Sediment for dredge samples.
5. Point/Outfall (or **SWIMS Fieldwork Seq No**) will be auto-generated when fieldwork event is scheduled in SWIMS. Not required field.
6. **Grant or Project Number**: field should include the Grant Number or the SWIMS Project Number. This is a required field.
7. **Preservation** (ethanol/refrigeration):
   - Tow samples must be preserved in ethanol.
   - Sediment samples must be frozen.

Brief Shipping Instructions:

1. Deliver samples in person to the laboratory (2601 Agriculture Drive, Madison, WI 53718; 7:45AM-4:30PM weekdays).
2. Pack double bagged samples (keeping them upright) and lab request forms into an appropriate, leak-proof shipping container (i.e. Styrofoam or plastic cooler). Packing materials (e.g. styrofoam, newspapers, bubble-wrap, etc.) should be added to the cooler to prevent breakage. See field protocol for a photo example.
3. Add ice to frozen sediment samples (in sealed bags) or freezer ice packs.
4. Securely tape the shipping cooler closed.

For questions about sample collection contact the AIS Monitoring Lead at (608) 261-6450.

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

Figure 3 Back page of Test Request – Mussel Veliger and Waterflea Analysis Form 4800-027. Optional Field Parameters will be entered into SWIMS by the Wisconsin State Lab of Hygiene. Monitoring Results are mandatory for quantitative results, but this data will not

When you receive waterflea 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

All samples must be submitted by October 18.

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 veliger samples should be delivered monthly to Wisconsin State Laboratory of Hygiene. Do not store all your samples until the end of the season.

Please deliver samples to:

Wisconsin State Laboratory of Hygiene
c/o Environmental Toxicology Department
2601 Agriculture Drive
Madison, WI 53718

7:45 am – 4:30 pm weekdays

If you are delivering more than 10 samples at once, please notify the Wisconsin State Lab of Hygiene first 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.

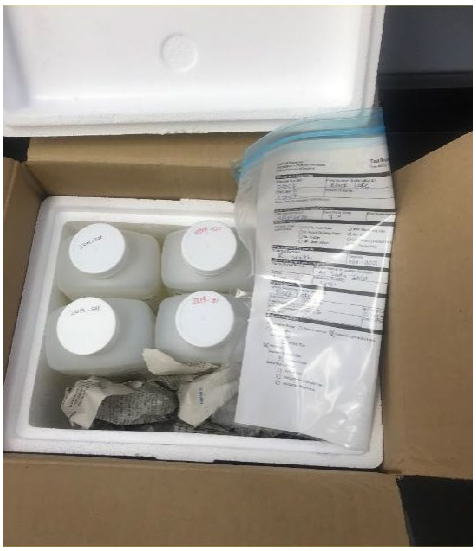


Figure 4 Example of plankton tows with bagged datasheet and packaging material.

Delivery of Tow Samples

Veliger samples, preserved with ethanol (4 parts ethanol: 1-part sample), are considered hazardous materials because of their flammability (the flash point of a 4:1 ethanol/water solution is approximately 72^{o} F). 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, as listed below. 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.

While we prefer in-person delivery, anyone preparing samples (preserved in ethanol) for shipment MUST be certified in hazardous shipping. Training information is on the DNR intranet: http://intranet.dnr.state.wi.us/int/es/science/ls/Shipping/Training/. The certificate of completion is good for three years. Figure 5 includes an example of a package prepared for hazardous shipping that will be learned during the hazardous shipping training.

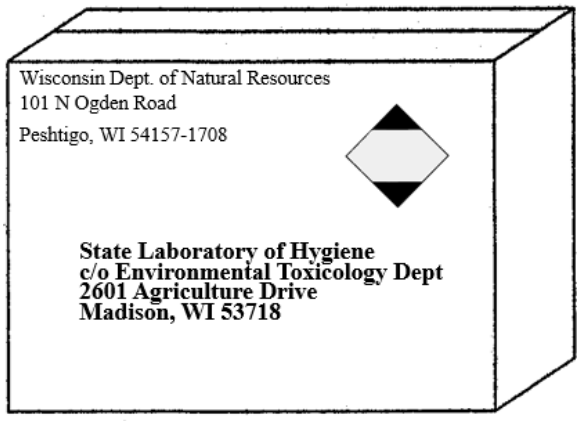


Figure 5 Example of package prepared for hazardous shipping. Everyone shipping must have Hazardous shipping certification training.

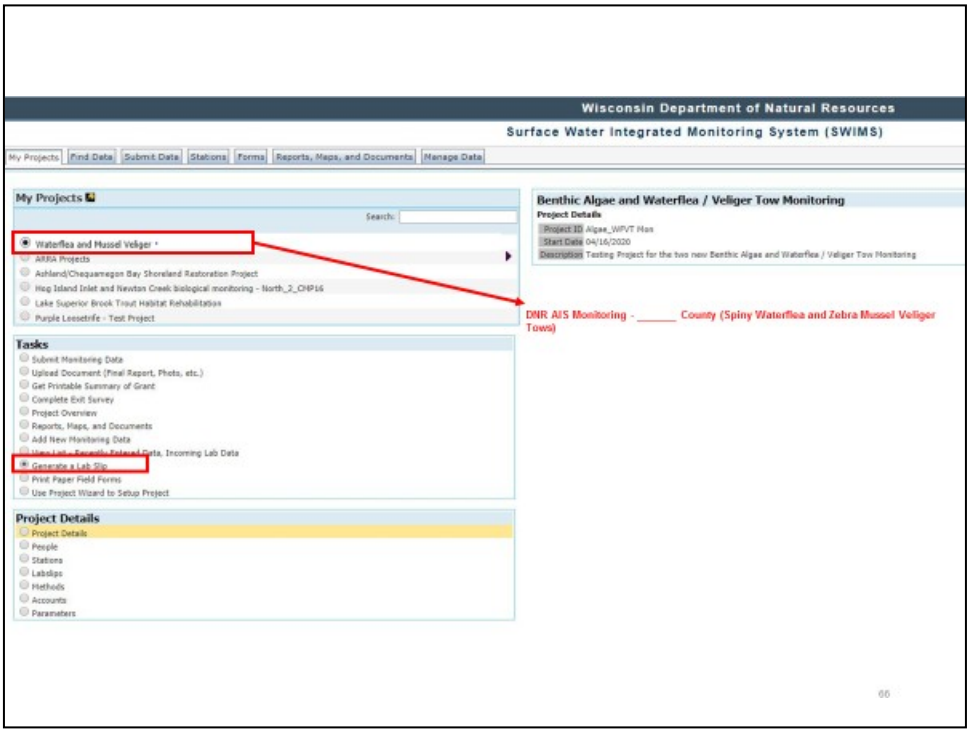
It is mandatory that DNR use the State Spee-Dee Delivery contract to send samples within Wisconsin, unless they are not available in your region. Here are websites for Spee-Dee:

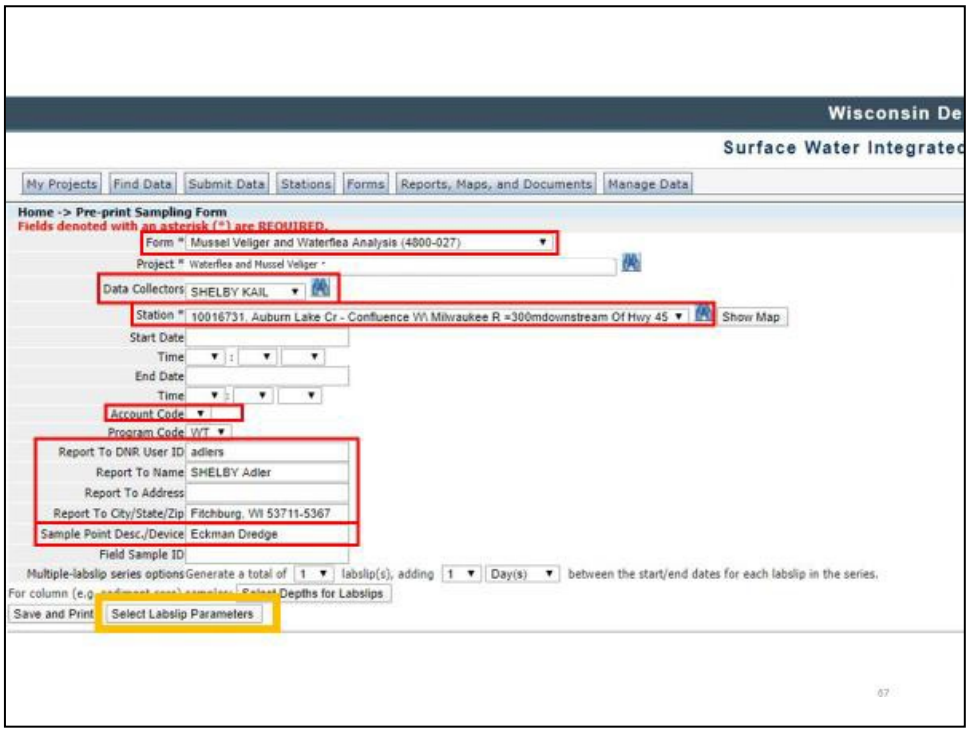
http://www.speedeedelivery.com/walkin-wi.html
http://www.speedeedelivery.com/OnCallLetter.pdf

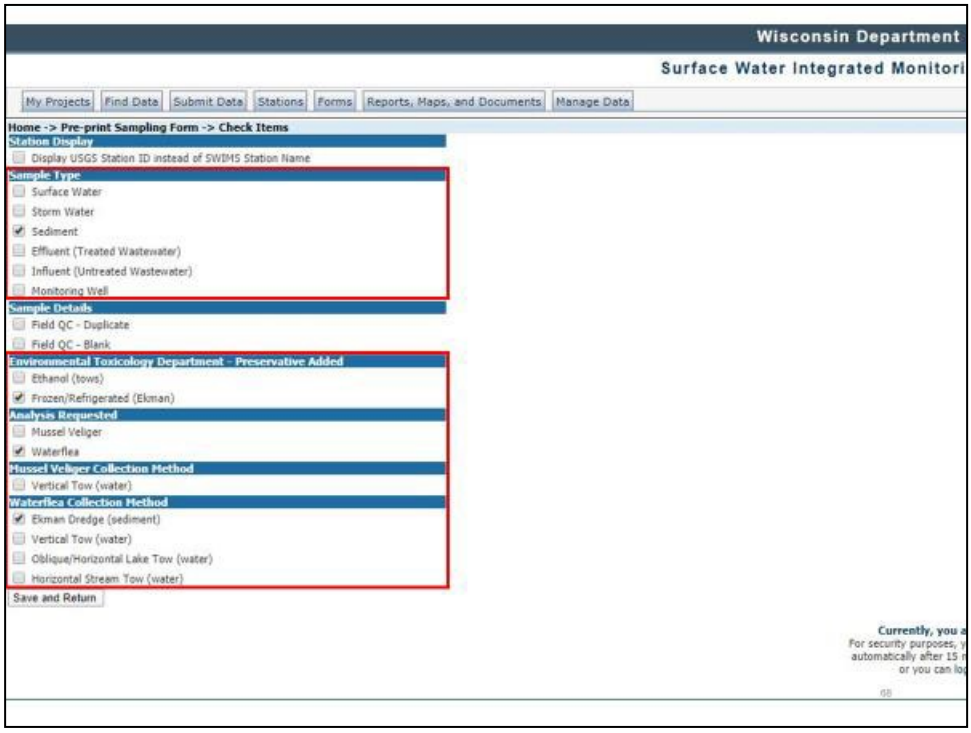
Note: If samples are to be shipped by common carrier, size restrictions may apply to the sample containers. The maximum size allowed under the US DOT regulations for plastic containers is 1 liter – check with the shipper for any additional restrictions prior to sampling so that samples are collected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

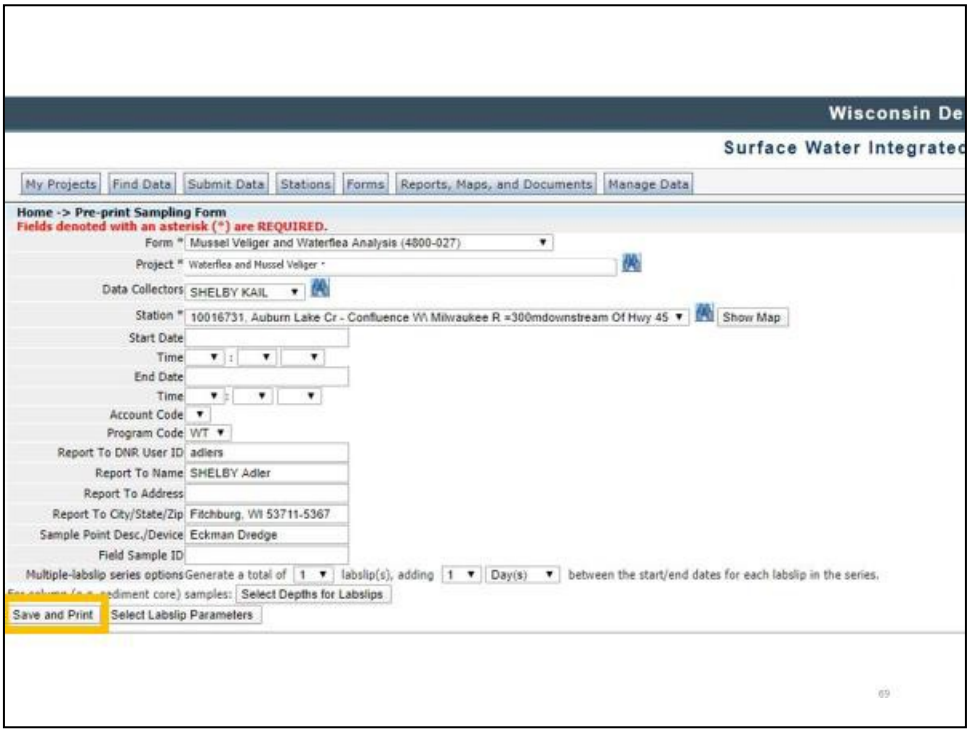
For questions about sample collection contact the AIS Monitoring Lead at (608) 261-6450. If you have test related questions, contact the WSLH Environmental Toxicology Department at (608) 224-6230.

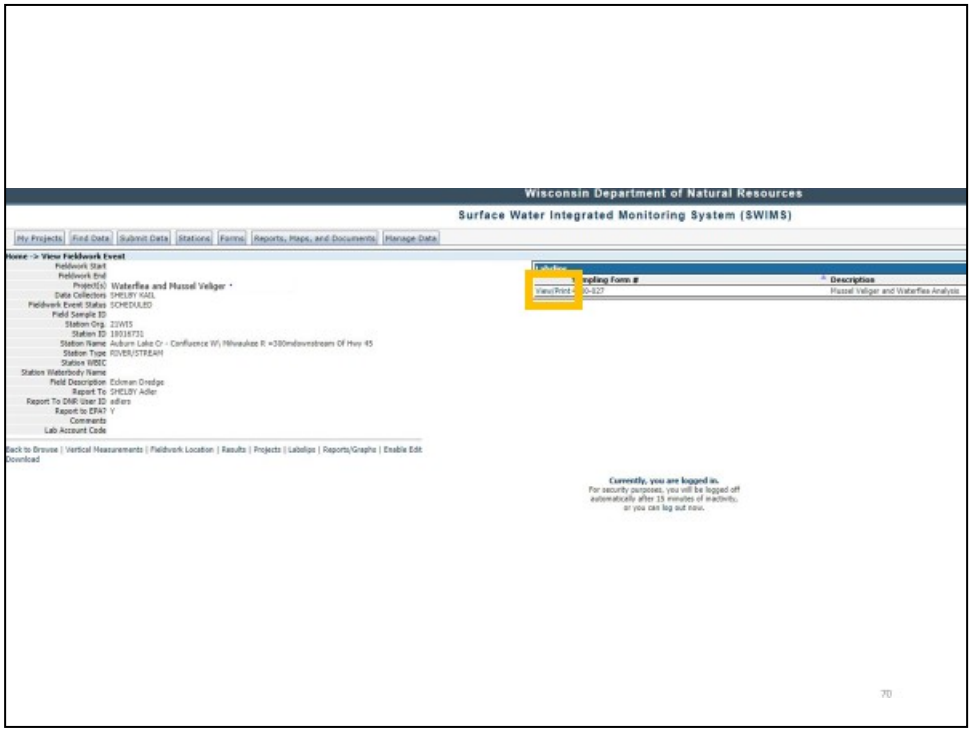
Appendix A Instructions for Generating Waterflea and Mussel Veliger SWIMS Labslips

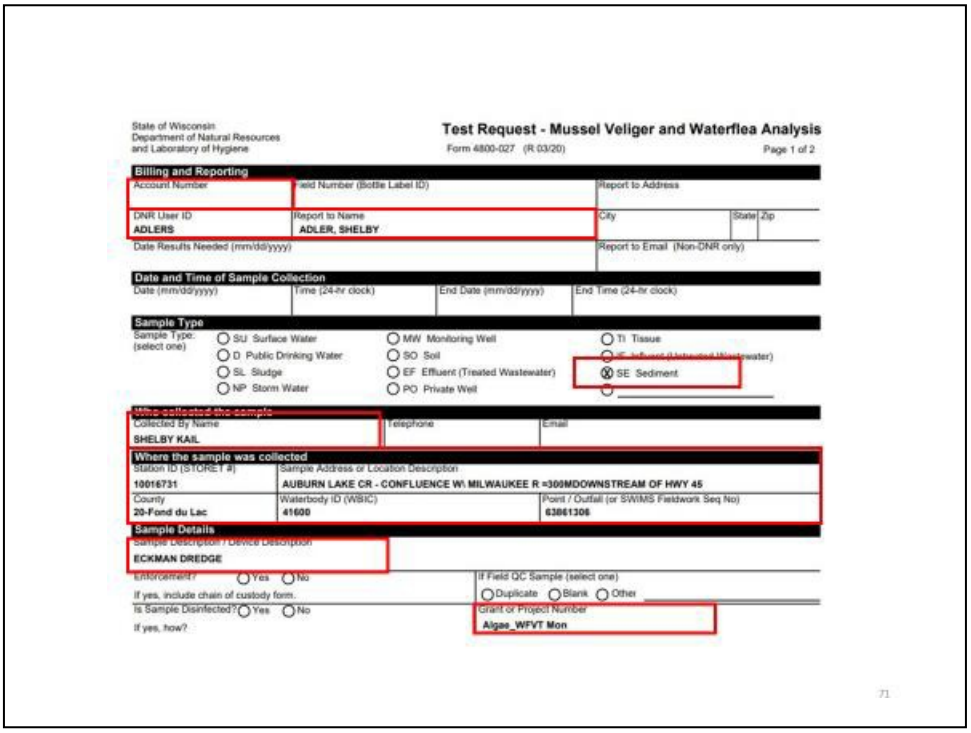





Wisconsin Department
Surface Water Integrated Monitori
My Projects
Find Data
Submit Data
Stations
Forms
Reports, Maps, and Documents
Manage Data
Home -> Pre-print Sampling Form -> Check Items
Station Display
Display USGS Station ID instead of SWIMS Station Name
Sample Type
Surface Water
Storm Water
Sediment
Effluent (Treated Wastewater)
Influent (Untreated Wastewater)
Monitoring Well
Sample Details
Field QC - Duplicate
Field QC - Blank
Environmental Toxicology Department - Preservative Added
Ethanol (tows)
Frozen/Refrigerated (Ekman)
Analysis Requested
Mussel Veliger
Waterflea
Mussel Veliger Collection Method
Vertical Tow (water)
Waterflea Collection Method
Ekman Dredge (sediment)
Vertical Tow (water)
Oblique/Horizontal Lake Tow (water)
Horizontal Stream Tow (water)
Save and Return


Wisconsin De
Surface Water Integrated
My Projects
Find Data
Submit Data
Stations
Forms
Reports, Maps, and Documents
Manage Data
Home -> Pre-print Sampling Form
Fields denoted with an asterisk (*) are REQUIRED.
Form * Mussel Veliger and Waterflea Analysis (4800-027)
Project * Waterflea and Mussel Veliger
Data Collectors SHELBY KAIL
Station * 10016731, Auburn Lake Cr - Confluence W\ Milwaukee R =300mdownstream Of Hwy 45
Show Map
Start Date
Time
End Date
Time
Account Code
Program Code WT
Report To DNR User ID adlers
Report To Name SHELBY Adler
Report To Address
Report To City/State/Zip Fitchburg, WI 53711-5367
Sample Point Desc./Device Eckman Dredge
Field Sample ID
Multiple-labslip series options Generate a total of 1 labslip(s), adding 1 Day(s) between the start/end dates for each labslip in the series.
Select Depths for Labslips
Save and Print
Select Labslip Parameters


Wisconsin Department of Natural Resources
Surface Water Integrated Monitoring System (SWIMS)
My Projects | Find Data | Submit Data | Stations | Forms | Reports, Maps, and Documents | Manage Data
Home -> View Fieldwork Event
Fieldwork Start
Fieldwork End
Project(s) Waterflea and Mussel Veliger *
Data Collectors SHELBY KAIL
Fieldwork Event Status SCHEDULED
Field Sample ID
Station Org. 21WIS
Station ID 10016731
Station Name Auburn Lake Cr - Confluence W\ Milwaukee R =300mdownstream Of Hwy 45
Station Type RIVER/STREAM
Station WBIC
Station Waterbody Name
Field Description Eckman Dredge
Report To SHELBY Adler
Report To DNR User ID adlers
Report to EPA? Y
Comments
Lab Account Code
Back to Browse | Vertical Measurements | Fieldwork Location | Results | Projects | Labslips | Reports/Graphs | Enable Edit
Download
Description
Mussel Veliger and Waterflea Analysis
Currently, you are logged in.
For security purposes, you will be logged off automatically after 15 minutes of inactivity, or you can log out now.
70


State of Wisconsin
Department of Natural Resources
and Laboratory of Hygiene
Test Request - Mussel Veliger and Waterflea Analysis
Form 4800-027 (R 03/20)
Page 1 of 2
Billing and Reporting
Account Number
Field Number (Bottle Label ID)
Report to Address
DNR User ID
ADLERS
Report to Name
ADLER, SHELBY
City
State
Zip
Date Results Needed (mm/dd/yyyy)
Report to Email (Non-DNR only)
Date and Time of Sample Collection
Date (mm/dd/yyyy)
Time (24-hr clock)
End Date (mm/dd/yyyy)
End Time (24-hr clock)
Sample Type
Sample Type: (select one)
SU Surface Water
D Public Drinking Water
SL Sludge
NP Storm Water
MW Monitoring Well
SO Soil
EF Effluent (Treated Wastewater)
PO Private Well
TI Tissue
SE Sediment
Who collected the sample
Collected By Name
SHELBY KAIL
Telephone
Email
Where the sample was collected
Station ID (STORET #)
10016731
Sample Address or Location Description
AUBURN LAKE CR - CONFLUENCE W\ MILWAUKEE R =300MDOWNSTREAM OF HWY 45
County
20-Fond du Lac
Waterbody ID (WBIC)
41600
Point / Outfall (or SWIMS Fieldwork Seq No)
63861306
Sample Details
Sample Description / Device Description
ECKMAN DREDGE
Enforcement? Yes No
If yes, include chain of custody form.
Is Sample Disinfected? Yes No
If yes, how?
If Field QC Sample (select one)
Duplicate Blank Other
Grant or Project Number
Algae_WFVT Mon
71

Analyses Requested

Environmental Toxicology Department - Preservative Added

Preservative Added: ○ Ethanol (tows) or ⊗ Frozen/Refrigerated (Ekman)

Analysis Requested: ☐ Mussel veliger ☒ Waterflea

Mussel Veliger Collecion Method:

- ☐ Vertical Tow

Waterflea Collection Method:

- ☒ Ekman Dredge (sediment)
- ☐ Vertical Tow (water)
- ☐ Oblique/Horizontal Lake Tow (water)
- ☐ Horizontal Stream Tow (water)

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