Waterflea Sampling Protocol

State of Wisconsin Department of Natural Resources

STANDARD OPERATING PROCEDURES

September 2021

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Methods

There are qualitative and quantitative methods for waterflea sampling. Qualitative methods are used to determine presence during early detection monitoring and quantitative methods are used to determine abundance during long-term trend sampling.

QUALITATIVE:

Waterflea Ekman Sediment Sample

This method is used for determining presence only. Waterflea spines are present year-round in sediment. Therefore, this method can be used any time of year. Although to verify an established population, live adults must be sampled using tows.

Waterflea Oblique/Horizontal Tow

This method is used for determining presence and not a quantitative estimate. However, this method can be used to verify whether there is an established population. Adult waterfleas are generally present in the water column during Late Summer/Early Fall, this timing is largely driven by cooling water temperature. In general, waterflea are present in the water column during August in the northern region of the state. In general, waterflea are present in the water column during September-October in the southern region of the state. Once adult waterfleas are detectable in a waterbody with a known population, it can be assumed they will be detectable in other waterbodies in that region.

QUANTITATIVE:

Waterflea Vertical Lake Tow

Vertical waterflea tows will be used on pre-determined lakes for long-term-trend analysis. Biologists may consult the AIS Monitoring Coordinator if they want to collect quantitative vertical tows to estimate abundance.

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 (<u>https://dnrx.wisconsin.gov/swims/downloadDocument.do?id=127413817</u>).

A plankton tow is preferred to detect live animals and for easier analysis. If you only have an Ekman dredge, then use the Ekman. If you only have a plankton net, you can only sample with the net during August (in the north) or September (in the south) through October. If you have both, use the plankton net during August (in the north) or September (in the south) through October and Ekman outside that time.

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 enough 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 towsdredges\plankton veliger tracking list.xlsx

Waterflea Ekman Sediment Sampling (Qualitative)

Based on waterflea monitoring protocol developed by Jake Walsh, UW-Madison Center49 for Limnology, May 2016.

Equipment

- Boat
- Lake bathymetry maps
- GPS unit optional
- Ekman grab WildCo is one option for the Ekman grab and bucket, though there might be more affordable sources <u>https://shop.sciencefirst.com/wildco/standard-ekman-grab/5997-ekman-grab-standard-6x6x6-ekman-grab-only-ss.html</u>
- Messenger <u>https://shop.sciencefirst.com/wildco/messengers/6653-stainless-steel-split-messenger-ss-11-once.html</u>
- 100 ft Braided polyester line <u>https://shop.sciencefirst.com/wildco/line-cable-and-chain/6680-braided-polyester-line-3-16in-polyester-100ft.html</u>
- Spray bottle
- Two State Lab of Hygiene provided plastic bags (double bag)
- Sample labels: provided to regional DNR by the State Lab
- Pencil
- Cooler with ice
- 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
- Steam or hot water unit

Field protocols for Ekman grab

- 1. Anchor at the deep hole of the lake.
- 2. "Set" the Ekman grab. Press the button on the top of the Ekman to depress the fully enclosed pin. Start by placing the loop over the pin that is fully enclosed. Then, release the top button so that the enclosed pin rises and holds the wire in place. Finally, place the second loop over the exposed pin. The Ekman is very powerful, so keep anything valuable (e.g., fingers) out of the mouth of the trap.
- 3. Hold the messenger in your off hand and the Ekman and rope in your dominant hand.
- 4. Lower the Ekman to ~1 m (exact distance doesn't matter) off the lake bottom. Wait until the Ekman has stabilized horizontally and is perpendicular to the lake bottom and surface of the water. Gently lower the Ekman the remaining distance. Pick up all slack on the line so that the rope is taut (without pulling the Ekman off the sediment).
- 5. Drop the messenger and wait for the feel on the line and sound of the "click" of the trap closing shut. Make sure the line is taut so that the Ekman door trap is triggered.
- 6. Open the top of the Ekman dredge and use a small cup or Ziploc bag to scoop out 1-2 cups of material into the one of the bags. Do this over the side of the boat to prevent releasing sediment into the boat and decrease your risk of spread. If there is no sediment in the Ekman dredge, the messenger might not have released the Ekman door so you will need to try collecting another sample and make sure the line is taught. On occasion, something will get

trapped in the grab (between the two scooping plates) and sediment will be washed out of the Ekman as you pull up the water... so repeat 1-6 as necessary.

 Be sure to double bag the sediments and include an "External Label" which is inside the outer/second bag. in a cooler. Samples should be refrigerated or frozen and <u>not preserved in</u> <u>ethanol</u> (Figure 1).



Figure 1 example of double bagged sample with label.

8. Record data on the Test Request – Mussel Veliger and Waterflea Analysis Form 4800-027.

Decontamination and Disinfection for Ekman grab and bucket

To stop the spread of waterflea and to eliminate cross-contamination, use the following procedure to disinfect the Eckman dredge. 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

The following are instructions for cleaning waterflea sampling equipment.

- 1. Remove spines to prevent false positives on the next lake:
 - a. Rinse and scrub equipment thoroughly with lake water vigorously and thoroughly while on the lake. Use a larger brush for larger flat surfaces (e.g., the bucket, the mesh, some of the Ekman) and a smaller brush for more complicated nooks and crannies (e.g., joints in the Ekman). Spines are functionally like Velcro on mesh of just about any size, so it's important to do a thorough job cleaning the mesh of the filter bucket.
 - b. Contain all equipment so no residual water can escape. Rinse and scrub equipment back in the garage with a hose or clean tap water. Use a larger brush and a smaller brush.
 - c. Ekman dredge and bucket can be pressure washed with hot water or steamed for 1 minute at a temperature higher than 122°F.
- 2. To kill any remaining resting eggs after removing spines:

- a. Dry equipment for 6 hours at $63^{\circ}F$ or for at least 5 days.
- b. Ekman dredge and bucket can be pressure washed with hot water or steamed for 1 minute at a temperature higher than 122°F.
- c. Chlorine is not effective to kill resting eggs.

Waterflea Oblique/Horizontal Tows (Qualitative)

Based on waterflea monitoring protocol developed by Pieter Johnson, UW-Madison Center for Limnology, April 2006.

Materials

- Lake maps
- GPS unit (optional)
- 0.5 to 1-meter diameter, 253-micron mesh plankton net w/ rope
- Spray bottle
- 250 ml and/or 1 L plastic bottles
- Ethanol 95% (190 proof)
- Sample label https://dnrx.wisconsin.gov/swims/downloadDocument.do?id=158680468
- 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.
- Large brush and small brush
- Fresh rinse water
- Steam or hot water unit

Field protocols for waterflea oblique/horizontal tow

Conduct 3 oblique (sampling from the top of the thermocline to just below the water's surface) tows with zooplankton net. In shallow lakes where it is difficult to conduct oblique tows, conduct 3 horizontal tows with zooplankton net.

 <u>Oblique Tow</u>: Lower zooplankton net until top of net is at the top of the thermocline. For consistent tow sampling effort, tow the net for 2 minutes (120 seconds) driving backwards at a low boat speed (~3 km/hr). While the slow speed helps prevent the net from surfacing, weight can also be added to the zooplankton net (tie a loop in the rope approximately 0.5 to 1 meter in front of the net and attach a brick with a hole in it) using an additional piece of rope or cable tie.

Note: Care must be given that the net does not hit the lake bottom. When this happens, the sample will contain muddy water, which is very difficult or impossible to analyze. If you hit the lake bottom, rinse out the sampling equipment and go to a deeper area of the lake to perform a good tow.

<u>Horizontal Tow</u>: Lower zooplankton net to a depth where the top of the net is fully submerged, and net will not hit the bottom of the lake. Drive backwards at a low boat speed (~3 km/hr) for 2 minutes (120 seconds). Raise zooplankton net.

Note: If lake is shallow and macrophytes are too dense to conduct a horizontal tow, attempt conducting a vertical tow. Avoid collection of macrophytes in tow.

2. Use the spray bottle to rinse the outside of the net so that samples are washed into the collection cup. Condense the size of the sample by filtering and 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 as well.

Note: If waterfleas are present, they might be visible immediately within the collection cup (but not always).

- 3. Transfer the sample from the collection cup into a 250mL sample bottle.
- 4. Repeat the process at the other two pre-selected sites. Composite the samples from the three sites into one 250-ml or larger (1-liter) bottle to obtain a single sample for the lake.
- 5. Label the sample bottle with external labels. Prepare labels prior to sampling so that you don't have to write on wet paper.
- 6. Preserve the sample immediately using 95% ethanol. The ratio should be 4 parts ethanol to 1part 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 bottles in sequential order.

Note: Transport the sample bottle(s) on ice in a cooler if you are unable to preserve immediately.

7. Record data on the Test Request – Mussel Veliger and Waterflea Analysis Form 4800-027.

Waterflea Vertical Lake Tow (Quantitative)

Based on waterflea monitoring protocol developed by Jake Walsh, UW-Madison Center for Limnology, and Carol Warden, DNR/UW Trout Lake.

Materials

- Lake maps
- GPS unit (optional)
- 0.5 to 1-meter diameter, 253-micron mesh plankton net
- Metered Rope
- Spray bottle
- 250 ml and/or 1 L plastic bottles
- Ethanol 95% (190 proof)
- Sample label <u>https://dnrx.wisconsin.gov/swims/downloadDocument.do?id=158680468</u>
- 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.
- Large brush and small brush
- Fresh rinse water
- Steam or hot water unit

Field protocols for vertical waterflea tow

Conduct 3 vertical tows from the deep hole.

1. Lower the zooplankton net to 2 meters above the waterbody bottom and slowly raise to surface. Record net depth on labslip and labels.

Note: Care must be given that the net does not hit the waterbody bottom. When this happens, the sample is of muddy water, which is very difficult or impossible to analyze. If

you hit the lake bottom, rinse out the sampling equipment and go to a deeper area of the lake that will provide enough depth for a good tow.

 Use the spray bottle to rinse the outside of the net so that samples are washed into the collection cup. Condense the size of the sample by filtering and 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 as well.

Note: If waterfleas are present, they might be visible immediately within the collection cup (but not always).

- 3. Transfer the sample from the collection cup into a 250 mL sample bottle.
- 4. Repeat the process two more times. These samples will not be composited.
- 5. Label the sample bottle with an external label. Prepare labels prior to sampling so that you don't have to write on wet paper (Figure 2).

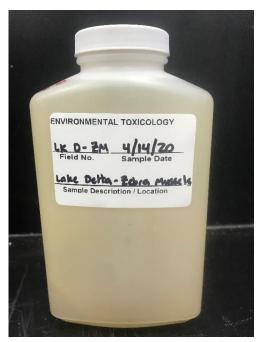


Figure 2 Example of labelled sample bottle.

6. Preserve the sample using 95% ethanol. The ratio should be 4 parts 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 bottles in sequential order.

Note: Transport the sample bottle(s) on ice in a cooler if you are unable to preserve immediately.

7. Record data on the Test Request – Mussel Veliger and Waterflea Analysis Form 4800-027.

Decontamination and Disinfection for Tows

To stop the spread of spiny waterfleas and to eliminate cross contamination, use the options below 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 must be followed.

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

Another approach is to request 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 between two lakes on one day:

- **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 can be sprayed with a handheld Jiffy stream cleaner or hot-water unit for 10 to 13 seconds so that a continuous stream of steam is in contact with the gear. This method is preferred.

** If waterfleas are observed, extreme care should be taken to avoid transporting individuals. We recommend that you use a different net 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 after sampling, both to preserve the integrity of the net and reduce the risk of any resting eggs remaining viable.

** Chlorine is not effective to kill resting eggs.

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) (Figures 3 and 4). The following are some definitions for fields to complete on the form:

<u>Account Number</u>: 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 autopopulate when "Station ID (STORET #) is entered.

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

<u>Grant or Project Number</u>: 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).

<u>Preservation</u> (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.

State of Wisconsin Department of Natural Resource Ind Laboratory of Hygiene		Form 4800-027 (R 03/20)	Veliger and	Waterflea Analysis Page 1 of 2
Billing and Reporting				
Account Number	Field Number (Bottle Label	ID)	Report to Addres	s (Non-DNR only)
ONR User ID	Report To Name		City	State ZIP
Date Results Needed (mm/do	1/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 c	lock
Sample Type				
Sample Type: O SU Sur (selectione) O D D total		Monitoring Well	O TI Tissue	
-	c Drinking Water OSOS	oil ffluent (Treated Wastewater)	-	Intreated wastewater)
O SL Slud		rivate Well	O SE Sedimen	L
ů,		IIvate wen	<u> </u>	
Who collected the sample Collected By Name	Telepho	ne Email		
Solicolog by Hame	Telepho			
Where the sample was col	lected			
Station ID (STORET #)	Sample Address or Location I	Description		
County	Waterbody ID (WBIC)	Point / C	Outfall (or SWIMS I	Fieldwork Seq No)
Zamala Dataila				
Sample Details Sample Description / Device	Description			
Enforcement? OYe	es ()No	If Field QC Sample (s	elect one):	
f yes, include chain of custor	ly form.	O Duplicate O BI		
s Sample Disinfected? OYe	es ONo	Grant or Project Num	ber	
f yes, how?				
Analyses Requested	Department			
Preservative Added: C		-Financial (Element)		
	thanol (tows) or O Frozen/Re	efrigerated (Ekman)		
Mussel veliger				
Method Used:				
Vertical Tov	N			
Waterflea				
Sediment Method Used:				
Ekman Dre	dge			
Water Method Used:				
Vertical Tov	N			
Oblique/Ho	rizontal Lake Tow			
Horizontal	Stream Tow			
Honzontai				

Figure 3 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

Page 2 of 2

Form 4800-027 (R 03/20)

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)		Flow (cfs)		
Secchi Depth Hit Bottom?	O Yes ONo			
Cloud Cover (%)				
Cond (µS/CM@25°C)				
Monitoring Results				
Diameter of plankton net opening	30cm ()50cm ()Other			
Length of plankton net:	250cm () Other			
Site1 : Latitude (optional):	Longitude (optional):	Preservati	ve: Ethanol (tows) or f	rozen/refrigerated (Ekman)
Depth sampled (if vertical tow)	ft/m		0	0
Site 2 : Latitude (optional):	Longitude (optional):	Preservati	ve: Ethanol (tows) or f	rozen/refrigerated (Ekman)
Depth sampled (if vertical tow)	ft/m		0	0
Site 3 : Latitude (optional):	Longitude (optional):	Preservati	ve: Ethanol (tows) or f	rozen/refrigerated (Ekman)
Depth sampled (if vertical tow)	ft/m		0	0
Have you consolidated all your plankt	on samples into one compo	site bottle? OYes	s ()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: <u>https://dnrx.wisconsin.gov/swims/downloadDocument.do?id=159856887</u>
 Waterflea: <u>https://dnrx.wisconsin.gov/swims/downloadDocument.do?id=159856883</u>
- Lab Account Number is tied to the funding source. If you are unsure what the proper account number is refer to <u>http://intranet/int/es/science/ls/Account.htm</u> or contact the DNR Laboratory Coordinator (608-264-8589).
- 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):
 - <u>Tow samples</u> 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).
- 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 4 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 be entered into SWIMS, but a scanned copy will be available in the PDF that is saved to SWIMS.

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 waterflea 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:

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

7:45 am - 4:30 pm weekdays

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.

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

Email: <u>Biomonitoring@slh.wisc.edu</u> 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.

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.

Delivery Preparation

Pack double bagged labeled frozen sediment samples (keeping them upright) and labeled bottles (keeping them upright) with lab request forms into an appropriate, leak-proof shipping container (i.e. Styrofoam or plastic cooler) (Figure 5 and 6). Packing materials (e.g. styrofoam, newspapers, bubble-wrap, etc.) should be added to the cooler to prevent breakage. Add ice (in sealed bags) to frozen sediment samples or freezer ice packs. Securely tape the shipping cooler closed.

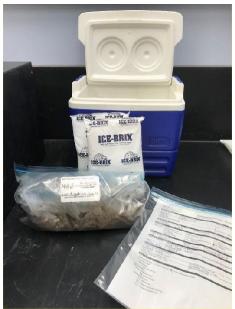


Figure 5 Example of double bagged sediment sample with external label, bagged datasheet, cooler packs and cooler.



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

Delivery of Tow Samples

Waterflea tow samples, preserved with ethanol (4 parts ethanol: 1-part sample), are hazardous materials because of their flammability (the flash point of a 4:1 ethanol/water solution is approximately 72° 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 tow 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 7 includes an example of a package prepared for hazardous shipping that will be learned during the hazardous shipping training.

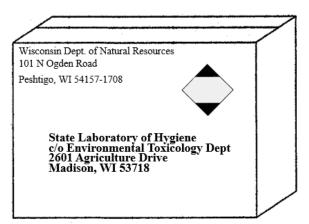


Figure 7 Example of package prepared for hazardous shipping. Everyone shipping must have Hazardous shipping certification training.

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) 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 of Natural Resources
	Surface Water Integrated Monitoring System (SWIMS)
Projects Find Data Submit Data Stations Forms Reports, Maps, and Documents Ma	inage Data
Hy Projects 🖬 Search:	Benthic Algae and Waterflea / Veliger Tow Monitoring Project Details
Waterflea and Hussel Veliger ABPA Projects	Protect ID Alges_WVFT Hos Date these Ov (V4/CO20 Beautypice) Testing Project for the two new Besthic Algae and Waterfies / Valger Tew Hontong
Ashfand(Chequamegen Bay Shoreland Rastoration Project Hing Island Inlet and Hievatan Creek Sitelegical monitoring - North, 2_CNP16 Lake Superior Prove Trust Hisbits (Hebbits)	
Purple Loosetrife - Test Project	DNR AIS Monitoring County (Spiny Waterflea and Zebra Mussel Velige Tows)
Subset Neutraining Data United Sources (from Rearch, Priets, etc.) Ger Privable Summary of Grant Ger Complete Dirit Summary Project Summary Project Summary Rearch, Neues, and Sourcements Add How Handwalks (Data Generate, Lub, Rip	
Project Details Project Details Project Projec	
Labsips Hebroids Accounts Promotions	

						Wisconsin I
			_			Surface Water Integrat
My Projects	Find Data 5	Submit Data Stations	Forms	Reports, Maps, and Documents	Manage Data	
	print Sampling					
Fields denote		isk (*) are REOUIRED. Mussel Veliger and Waterfi	en Annius	is (4800-027)		
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	Wisconsin Department of Natural Resou	rces
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State of Wisconsin Department of Natural Reso and Laboratory of Hygiene	ources		est Request orm 4800-027 (R.0		ger and Wate	orflea	Analysis Page 1 of 2
Billing and Reporting							
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Where the sample was staten ID (STORET #)	collected	and the second second	_				
10016731		V Location Descriptio CR - CONFLUENCE		-300MDOWNSTRE	AM OF HWY 45		
County 20-Fond du Lac	Waterbody ID (Wit	BIC)		Point / Outfall (or SV 63861306	VIMS Fieldwark Seq	No)	
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If yes, include chain of cust	Yes ONo		0.0000000000000000000000000000000000000	OBlank O Othe			
Is Sample Disinfected?			Grant or Proje				
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