



# Water Flea Monitoring Protocol

*Based on water fleas monitoring protocol developed by Pieter Johnson, UW – Madison Limnology Department - April 2006.*

Annually the DNR staff selects water bodies to sample for water flea infestations. This protocol is designed to assist DNR staff in collecting samples, shipping, reporting and how the information is released for public knowledge. Using this protocol will standardize the collection techniques, improve the quality of collected samples, limit the number of contaminated samples and ensure an accurate water flea infestation database.

The Department updates the water flea listing annually after sampling results are compiled and then issues a press release listing those waters infested with water fleas and other invasive species. The Department issues a second press release and list prior to the opening of the fishing season reminding boaters to take precautions to avoid spreading spiny waterfleas and other invasive species. A current water flea infestation map can be found at this website: <http://dnr.wi.gov/invasives/aquatic.htm>

Spiny water fleas (*Bythotrephes longimanus*) are large (up to  $\frac{3}{4}$  inch), predatory crustacean zooplankton native to parts of Europe and Asia. They have been established in all of the Great Lakes since 1992 and have begun moving into inland lakes in Ontario, Minnesota, Ohio, Michigan and, most recently, Wisconsin. Outside of Mysis shrimp, spiny water fleas are considerably larger than any naturally occurring lake zooplankton. They are readily distinguished by their long tail spines, which generally support between one and three barbs. Fish hook water fleas (*Cercopagis* sp.) are another predatory crustacean zooplankton known to have invaded the Great Lakes. At this time, however, their movement into inland lakes has been limited primarily to upstate New York. Both spiny and fish hook water fleas are often first noticed by anglers and recreational lake users. These invasive water fleas have a tendency to become entangled on fish lines, anchors, downrigging cables, and other types of gear used in boating activities. Extreme care should be exercised to avoid transporting these organisms among lakes.

## Water Flea Monitoring

### Sampling Equipment:

- Boat/Anchor
- 0.5-1 meter diameter, 250-micron mesh plankton net
- Rope on net with the meter increments marked
- Vinegar/Large container to hold plankton net for vinegar bath
- 250 ml plastic bottles
- 1-liter plastic bottles
- Alcohol, 95% alcohol (190 proof ethyl alcohol)
- Lake Maps
- Sharpie/Labels for Bottles
- Water flea data collection sheet
- Cooler with ice
- Change for car wash (you may want to make a map of the car wash stations in your area)
- GPS unit



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## Sampling:

### Frequency

Three samples should be collected from a particular lake on three dates between June and September (for a total of nine samples per lake). Ideally, samples should be collected at monthly intervals after the water temperatures reach 54 degrees. The first collection dates will vary from early to late June. If you choose not to sample the same lakes on each of the three sample periods, please contact the analytical lab so that they can make the appropriate changes to the database.

### Sample Location

On each sampling date, water flea samples should be collected from three different locations in a lake. The sites should be in different bays or basins or at several of the more heavily used lake sites. The three sampling sites should be deep enough to sample, so perhaps in 15 to 20 feet of water is a good rule of thumb. Ideally, the deepest point of the lake and areas near boat landings (sites of boat traffic) or lake's outlet are the best locations to sample. Shallower lakes can be sampled as well by making more of a horizontal tow so as not to disturb the bottom of the lake. Mark on the lake map where samples were collected. These same sites should be used for each of the sample periods – if not, then submit a revised map with subsequent samples.

### Collection

1. Collect sample with a large diameter (0.5-1 meter opening) zooplankton net with a mesh size of 250 microns. Smaller nets can easily clog with small forms of phytoplankton and zooplankton and can allow spiny water fleas to elude capture.
2. A 100 meter horizontal tow is best suited for capturing water fleas. A GPS unit (to measure distance and/or rate of travel) can be used or tow the net for 120 seconds at a low boat speed (~3 km hr<sup>-1</sup>) that prevents the net from surfacing (horizontal tows are facilitated by adding weight to the zooplankton net; tie a loop in the rope approximately 0.5-1 meter in front of the net and attach a weight (e.g., a brick with a hole in it) using an additional piece of rope or cable tie). Ideally, horizontal tows should be oblique (Figure 1), sampling from the top of the thermocline to just below the water's surface. Care must be given that the net does not hit the lake 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 different area of the lake that will provide enough depth for a good tow.
3. Be sure to rinse the net from the outside of the net so that all of the material washes into the plankton collection cup. If water fleas are present, they will likely be visible immediately within the collection cup (but not always).
4. Record sampling information on the water flea collection data sheet.
5. Decant your plankton sample into a 250 ml sample bottle after each tow.



6. Condense the size of the sample by filtering out as much water as possible in the field. This helps reduce the amount of alcohol that needs to be added and aids in the analyses as well.
7. Preserve the sample using 95% alcohol. The ratio should be 4 parts alcohol to 1 part sample. Note: If the prescribed alcohol to sample ratio (4:1) can not be achieved after repeated condensing and decanting, then the sample should be split between two sample bottles. Label each with the same information (as specified under “Processing the Sample – Field”), and label one as “Split 1 of 2” and the other as “Split 2 of 2”.
8. 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 and receive a single enumeration for the lake. Note: If samples are 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.
9. Transport the sample bottle(s) on ice in a cooler.
10. Local laboratory identification of waterfleas can be conducted by the region water quality biologist. Spiny and Fishhook Waterflea Watch and Wild cards are excellent resources for identification. Confirmation samples may be mailed to the DNR Southeast Region analytical lab, (Jim Steinke, via e-mail at [james.steinke@wisconsin.gov](mailto:james.steinke@wisconsin.gov) or 920-892-8756 ext. 3035). Provide your contact information as well so that the lab can get in contact with you. The analytical lab will e-mail back to you a receipt of the samples and the results when they are completed.

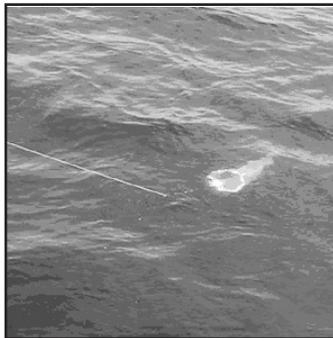


Figure 1. Water flea plankton net tow pulled behind a slow-moving boat.

## Processing the Sample – Field

Attach a label to each sample bottle and include the following information. Be sure to write legibly and with indelible ink (e.g. Sharpie) – do not use a ball point pen, as the ink is soluble in alcohol.

Label sample bottles with the following information:

- Sampler’s name
- Sampler’s phone number - important, as the analytical lab may need to contact you regarding the sample



- Lake name
- WBIC
- County
- TRS
- optional - Sample site Latitude/Longitude locational data using a GPS unit
- Site number
- Net opening diameter (0.5m or 0.3m)
- Sample date
- Number of tows
- Depth of the tows
- Preservatives added

## Shipping Samples:

Water flea 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). Any staff preparing specimens for shipment MUST attend a hazardous training workshop. These workshops are offered annually at the spring watercraft inspector meeting. The certificate of completion is good for three years.

It is mandatory that we use the State Spee-Dee Delivery contract to send samples within Wisconsin, unless they are not available in your region. All veliger samples should be shipped monthly to Plymouth. Do not store all of your samples until the end of the season. Here are websites for Spee-Dee: <http://www.speedeedelivery.com/walkin-wi.html>  
<http://www.speedeedelivery.com/OnCallLetter.pdf>

## Decontamination Procedures:

When multiple lakes are sampled on the same day, the net, boat and all other sampling equipment must be decontaminated between lakes. Decontaminating will eliminate cross contamination and reduce the risk of transporting invasive species from lake to lake. You do not have to decontaminate equipment between sample sites on the same lake. If multiple lakes are sampled in one day, it is recommended to sample any lakes that are not on the watch or infestation lists before sampling lakes on those lists, to minimize the potential for transport

- Remove any organic material from sampling gear. There are several options for disinfecting smaller gear. *Dissolved oxygen probes and other sensitive electronic sampling gear may be damaged by disinfection solution and should only be rinsed with clean water.* For other gear used in water choose one of the following options:

Option one: The gear can be sprayed with the disinfection solution and a wet surface maintained for the appropriate contact time. The gear should be rinsed with clean water or water from the next waterbody before it is used again.

Option two: Fill a tub with disinfection solution and place all equipment in the tub for the appropriate contact time. The gear should be rinsed with clean water or water from the next waterbody before it is used again.



Option three: 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 option one or two.

\*\* If water fleas 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 24-48 hours after a day of sampling, both to preserve the integrity of the net and reduce the risk of any resting eggs remaining viable.

- You should wash the boat between lakes following the DNR boat cleaning procedures. Please refer to the agency guidelines at the beginning of this Section of the handbook.
- Another approach that has been quite effective in some areas is to benefit from citizens that offer to take our technical 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.

## Listing Guidance

The Wisconsin Department of Natural Resources is monitoring some of our inland waters for the presence of water fleas. This guidance document provides listing recommendation criteria for when to place a water body onto the Infested List. A review team consisting of the statewide and regional AIS staff makes the final determination for listing a waterbody.

DNR lists a waterbody as infested for water fleas when we have data indicating that there is an established reproducing population. Lakes that are hydraulically connected to waterbodies infested for water fleas are placed on to the Water Flea Watch List. Nearby waterbodies that are not hydraulically connected may also be put on to the Watch list based recommendation by the area biologist and decision of the Exotic Species Coordinator and review team.

## Notification/Releasing Water Flea Information

### Standard Notification Sequence:

Following the Water flea listing criteria cited above, the regional biologist and the statewide coordinator have concurred that the waterbody should be listed. The regional biologist informs the Public Information Officer (PIO), wardens, fishery and water resources staff, volunteer monitors, and management at the regional level about the sighting. The regional staff, in consultation with the statewide coordinator, determines if a press release is needed. Prior to issuing a press release, the regional biologist notifies the local entities affected by the sighting (lake association or district and industries or water utilities). Attached, as an addendum to this report, is a sample press release that can be used as a template. After the press release is issued, the regional biologist coordinates any follow-up actions that are necessary including posting signs, additional monitoring, or information and education (I&E)/outreach efforts.

**The purpose of this form is to track the presence/absence of spiny or fishhook water fleas collected using a plankton net 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 (SWIMS) 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.

Primary Data Collector			
Name	Phone Number	Email	
Monitoring Location			
Waterbody Name	WBIC	County	Township Name
Date and Time of Monitoring			
Start Date	Start Time	End Date (= Start Date)	End Time
Monitoring Results			
Method used: <input type="checkbox"/> horizontal tows (near surface) <input type="checkbox"/> oblique tows (thermocline to surface) <input type="checkbox"/> vertical tows (bottom to surface)			
Diameter of plankton net opening 30cm 50cm other _____ (circle one)			
Site 1: Latitude (optional): _____	Longitude (optional): _____	<input type="checkbox"/> Preservative Added	
Secchi depth (m) _____ (optional)	Depth sampled (if vertical or oblique tow) _____ ft/m circle one		
Site 2: Latitude (optional): _____	Longitude (optional): _____	<input type="checkbox"/> Preservative Added	
Secchi depth (m) _____ (optional)	Depth sampled (if vertical or oblique tow) _____ ft/m circle one		
Site 3: Latitude (optional): _____	Longitude (optional): _____	<input type="checkbox"/> Preservative Added	
Secchi depth (m) _____ (optional)	Depth sampled (if vertical or oblique tow) _____ ft/m circle one		
<input type="checkbox"/> Have you consolidated all of your samples into one composite bottle?			
<input type="checkbox"/> Have you sent your samples to the DNR Plymouth Service Center?			
During this monitoring trip, did you find what you suspect are Spiny or Fishhook Waterfleas in this waterbody?			<input type="checkbox"/> Yes <input type="checkbox"/> No
Voucher Sample			
If you found Spiny or Fishhook Water fleas, did you collect a voucher specimen and bring it to your local DNR office? If so, which office?			
<input type="checkbox"/> Rhinelander	<input type="checkbox"/> Spooner	<input type="checkbox"/> Green Bay	<input type="checkbox"/> Oshkosh <input type="checkbox"/> Did not take sample to a DNR office
<input type="checkbox"/> Fitchburg	<input type="checkbox"/> Waukesha	<input type="checkbox"/> Eau Claire	<input type="checkbox"/> Superior <input type="checkbox"/> Other Office: _____

*If you find Spiny or Fishhook Water Fleas*

Please bring a copy of this form, along with a voucher specimen and if possible, a map showing where you found the suspect waterfleas to your regional Citizen Lake Monitoring Coordinator at the DNR. All initial discoveries should be placed in rubbing alcohol until verification by an expert is obtained.

*If you don't Find Spiny or Fishhook Water Fleas*

If you submit your data online, that is all you need to do. Otherwise, please mail a copy to your regional DNR Citizen Lake Monitoring coordinator. <http://dnr.wi.gov/lakes/contacts>

For DNR staff to fill out	
Volume of sample that was analyzed (ml)	Date analyzed
Name of plankton sample analyst:	
Name of person or museum who identified the voucher specimen	
Was the specimen confirmed as...?	
Spiny Waterflea? <input type="checkbox"/> Yes <input type="checkbox"/> No	Fishhook Waterflea? <input type="checkbox"/> Yes <input type="checkbox"/> No
Have you entered the results of the voucher in SWIMS? <input type="checkbox"/> Yes <input type="checkbox"/> No	
DNR staff: Please enter voucher information for new AIS findings into SWIMS under the Incident Report Project for your county (Choose Incident Report Form in SWIMS). Enter date of sampling for "Start Date", Person who identified specimen as "Data Collector", and Monitoring location as "Station".	



## Press Release Template for Waterbodies to be Placed on the Water Flea Infested List

\_\_\_ (numbers) Water fleas were recently found in \_\_\_ (waterbody) in \_\_\_(county). The adults were detected as part of routine monitoring on \_\_\_ waterbody OR were discovered as part of a general lake/river survey (i.e., examining shores, and boat landings).

“The number of Water fleas found indicate there is a reproducing population in \_\_\_ (waterbody)” according to \_\_\_\_\_ from the \_\_\_ Regional office. “The \_\_\_(waterbody) will be placed on DNR’s ‘Infested List’.

Placing \_\_\_ waterbody on the ‘Infested List’ means DNR will post the ‘Exotic Species Advisory’ signs with Water flea decals at all the landings to notify the public that \_\_\_ waterbody is infested. The DNR will also work with local units of government to increase public awareness efforts and outreach efforts to control the further spread of spiny water fleas. It also affords volunteers the opportunity to become more involved by helping monitor \_\_\_ waterbody for Water fleas and/or through watercraft inspection efforts.

Since 1992, the Great Lakes have been infested with Water fleas and now the spiny water fleas are moving into inland lakes in Ontario, Minnesota, Ohio, Michigan and most recently, Wisconsin. Spiny water fleas are a small shrimp like animal that grows to an average of 0.4 inches in length and feeds on other small animals. Although spiny water fleas are tasty to fish, it is protected from small fish predators by an unusually long tail spine. Fish feeding on spiny water fleas have great difficulty ingesting the tiny zooplankton. Spiny water fleas can have a profound effect on the fish population because it feeds on the zooplankton that small fish depend on for survival.

“There are prevention steps that everyone should take when boating, fishing and otherwise enjoying the water that can help prevent the spread of invasive species,” says \_\_\_\_\_.

Before moving your boat/equipment to a new waterbody:

- Inspect and remove plants, animals, and mud from your boat and equipment;
- Drain all water from your boat’s live wells, bilge, motor, etc.;
- Don’t move live fish away from a waterbody;
- Dispose of your unwanted bait in the trash. Use leftover minnows only under certain conditions;
- Rinse your boat and equipment with high-pressure and/or hot water OR
- Dry your boat and equipment thoroughly for at least 5 days.

FOR MORE INFORMATION CONTACT: Regional ANS Coordinator and/or regional biologist (two contacts are preferred)