

Waterflea (*Bythotrephes*) Laboratory Analysis Protocol

State of Wisconsin Department of Natural Resources

STANDARD OPERATING PROCEDURES

February 2019

Receiving Waterflea Samples

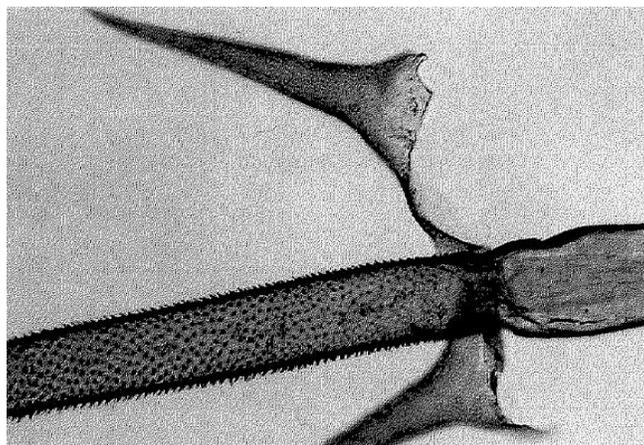
Throughout the field season, prior to sending samples for analysis, collectors should track sampling efforts in the [Plankton-Veliger Tracking List](#) on the internal DNR network drive. This list helps the laboratory analyst to estimate the number of Waterflea samples, both tow and sediment, they will receive for the year and provides the analyst with important, basic information about sampling events.

When samples are received by the laboratory analyst, they should be “checked-in.” A lab sample number should be written on the lid of each tow sample jar and on each sediment sample bag in blue sharpie, this helps to easily keep track of all samples received. Once given a lab sample number, sample background information should be filled into the [Results Log](#) for that year. When samples are successfully received and checked in, collectors should be notified by email or phone of the receipt of their samples.

Waterflea Presence Analysis-Sediment Sampling Method

Based on Waterflea monitoring protocol developed by Jake Walsh, UW-Madison Center for Limnology, May 2016. [2016 Spiny Waterflea Lab Methods-Photos](#)

Waterflea spines are present in sediment year-round and this collection method can be used any time of the year. Collected samples are frozen for preservation until thawed at time of laboratory analysis.



During analysis it can be difficult to distinguish Waterflea spines from plant material of similar shape and color. Under magnification Waterflea spines' unique texture of tiny "spikes" or "sharkskin" can be observed and be easily differentiating them from plant material. Example of texture below.

Equipment

- 150 Micron Sieve
- Two-Gallon Bucket
- Glass Petri Dish, 10 cm diameter
- Stainless Steel Spoon or Spatula
- 400 mL Glass Beaker
- Tap and DI Water
- Dissecting Microscope
- Dual Gooseneck Illuminator
- Five-Gallon Buckets (2)
- Gloves
- Liquinox



Laboratory Protocols for Waterflea Presence Analysis-Sediment Sampling Method

1. Thaw sample overnight and write Analysis # on sample bag with red sharpie.
2. Empty thawed sample into 150 Micron Sieve with two-gallon collection bucket beneath. Rinse sample material with tap water to remove the smallest particulate matter (most material should remain after rinsing).
 - In cases of samples containing large quantities of extremely fine sediments, follow these additional processing steps.
 1. Empty sample into a 500 Micron Sieve and rinse with tap water into two-gallon collection bucket below.
 2. Examine coarse material.
 3. Allow collection bucket with fine material to settle overnight.
 4. Rinse settled material through 150 Micron Sieve.
 5. Examine fine material.
3. Remove remaining sample material from sieve and place into 400 mL glass beaker.
4. Using stainless steel spoon or spatula scoop a subsample into a glass petri dish. Add DI water to disperse the material throughout the petri dish.
5. Place petri dish under dissecting microscope. Starting around 100x magnification, examine sample for spine fragments. High magnification will likely need to be used to distinguish spine fragments from similarly shaped plant material.
6. After examination of subsample is completed, rinse subsample material from petri dish into collection bucket.
7. Repeat steps 4-6 until a minimum of five subsamples has been examined. Number of subsamples examined may need to be adjusted depending on size of sample or number of bags received for a single sample.

8. Once examination of sample is completed, rinse all remaining sample material and rinsewater from small collection bucket into five-gallon settling bucket. When five gallon settling bucket is full allow solid material to settle out of water overnight. Clean water can be poured down drain and settled solid material can be bagged and put in the trash or composted in a place where there is no risk of it washing into surface water.
 - If sample contains Waterflea spines, return remaining sample material to sample bag and refreeze. Store frozen sample until given permission for disposal. Draw a ⊕ symbol on sample bag in red sharpie.
9. Clean 150 micron sieve, glass petri dish, and 400 mL beaker with water and Liquinox between samples to ensure samples are not cross contaminated.
10. Record results in the [Results Log](#).

Waterflea Presence Analysis-Tow Sampling Method

Based on 2008 Plymouth Lab Procedures by Nicole Chase.

This method of Waterflea Presence Analysis can be carried out using samples collected by oblique, vertical, or horizontal tow methods in the field. Adult Waterfleas are generally present in the water column during the months of August and September and are generally present earlier in the northern part to the state.

Equipment

- 10 mL Wide Bore Glass Pipet, such as Pyrex #7087 or another wide-tip serological pipet
- Valved Aspirator Bulb
- Glass Petri Dish, 10 cm diameter
- 400mL Glass Bleaker
- Dissecting Microscope
- Dual Gooseneck Illuminator
- Liquinox

Laboratory Protocols for Waterflea Presence Analysis-Tow Sampling Method

1. Write Analysis # on lid of sample jar in red sharpie.
2. Using 10 mL wide bore glass pipet fitted with a valved aspirator bulb, withdraw 10 mL of sample directly from material settled on the bottom of the sample jar. Empty pipet into a glass petri dish.
 - Volume of sample withdrawn may need to be adjusted depending on how “thick” sample material is.
3. Place glass petri dish under dissection microscope. Examine sample for presence of Waterflea Individuals and Spine Fragments.
4. Rinse sample from glass petri dish into 400 mL glass beaker, using ethanol from top of sample jar.
5. Repeat steps 2-4 until a total of 50 mL has been examined or until a Waterflea Individual has been identified.

6. When examination is completed, return sample material from 400 mL beaker to sample jar. If Waterflea Individuals or Spine Fragments are present, draw a ⊕ symbol on lid of sample jar in red sharpie.
7. Clean 10 ml pipet, glass petri dish, and glass beaker with water and Liquinox between samples to ensure samples are not cross contaminated.
8. Record results in the [Results Log](#).

Waterflea Enumeration Analysis-Tow Sampling Method

This method of Waterflea Enumeration Analysis can be carried out using samples collected by vertical or horizontal tow methods in the field. Samples collected using the oblique tow method should not be used in enumeration analyses.

Equipment

- 10 mL Wide Bore Glass Pipet, such as Pyrex #7087 or another wide-tip serological pipet
- Valved Aspirator Bulb
- Glass Petri Dish, 10 cm diameter (gridded recommended)
- 400mL or 600 mL Glass Bleaker
- Dissecting Microscope
- Dual Gooseneck Illuminator
- Clicker Counter
- Liquinox

Laboratory Protocols for Waterflea Enumeration Analysis-Tow Sampling Method

1. Carefully pour off extra volume of from sample jar to condense amount of ethanol in relation to the material settled at bottom of sample jar into 400-600 mL glass beaker.
2. Gently agitate sample to distribute material throughout.
3. Using 10 mL wide boar glass pipet fitted with a valved aspirator bulb, remove ~10 mL of sample from the sample jar and place on glass petri dish.
4. Place glass petri dish under dissecting microscope and count the number of Waterflea Individuals with the assistance of a clicker counter.
 - Note: For enumeration analysis do not count Waterflea Spine Fragments.
5. When count is complete thoroughly rinse material from glass petri dish into the 400-600mL glass beaker with reserve ethanol.
6. Repeat steps 2-5 until all condensed sample material has been counted.
7. Return sample in 400-600 mL glass beaker to sample jar.
8. Clean 10 mL pipet, glass petri dish, and glass beaker with water and Liquinox between samples to ensure samples are not cross contaminated.
9. Record Waterflea Individual count into [SWF Density Calculation Template](#).
10. Record density result in the [Results Log](#).

Calculations for the Enumeration of Waterflea Horizontal Tow

Below is an explanation of equations used to calculate average densities of Waterfleas in a water body based from enumeration of horizontal tow samples. These calculations are pre-written into the [SWF Density Calculation Template](#).

Step 1:

$$S_A * A_A = F_A$$

$$S_B * A_B = F_B$$

$$S_C * A_C = F_C$$

Step 2:

$$F_A * T_A = V_A$$

$$F_B * T_B = V_B$$

$$F_C * T_C = V_C$$

Step 3:

$$C_A / V_A = D_A$$

$$C_B / V_B = D_B$$

$$C_C / V_C = D_C$$

Step 4:

$$(D_A + D_B + D_C) / 3 = D_{Average}$$

S = Velocity (meter/second or feet/second)

A = Area of Net (meter² or feet²)

F = Flow (meter³/second or feet³/second)

T = Time (seconds)

V = Volume (meter³ or feet³)

C = Number of SWF individuals

D = Density (individuals/meter³ or individuals/feet³)

Waterflea Laboratory Analysis Results Reporting

Reporting Results in DNR SWIMS Database

Prior to sending waterflea samples in for laboratory analysis, sample collectors should create SWIMS Fieldwork Events for their samples and enter required field data into the Waterflea Monitoring Report (3200-128) form. In cases where collectors have failed to create SWIMS Fieldwork Events for their samples, the [Regional Aquatic Invasive Species Coordinator](#) should be notified by email (copy the Aquatic Invasive Species Monitoring Lead, [Maureen Ferry](#)). Samples should not be analyzed until collectors have created a SWIMS Fieldwork Event for their samples and entered the appropriate field data into the Waterflea Monitoring Report (3200-128) form. In cases where samples are submitted by partners without access to the SWIMS database, laboratory analysts should create a Fieldwork Event and enter data using information available to them (bottle labels, datasheets, tracking spreadsheet records, etc.). A missing Fieldwork Event should be noted in the [Results Log](#).

Waterflea Presence Analysis results for sediment samples should be entered into the sample's SWIMS Fieldwork Event utilizing the Waterflea Monitoring Report (3200-128) form with some modification. This form does not include a section to record Ekman Grab/Sediment Sample as the sampling method, so this must be recorded within the comment section for the Fieldwork Event. The volume of sample

analyzed will also not be entered into the form for these samples, and instead the number of subsamples examined will be recorded within the comment section for the Fieldwork Event. When entering these results and other comments into SWIMS, the laboratory analyst should include their full name with the comment.

Waterflea Presence Analysis results for tow samples should be entered into the sample's SWIMS Fieldwork Event utilizing the Waterflea Monitoring Report (3200-128) form.

Waterflea Enumeration Analysis results for tow samples should be entered into the sample's SWIMS Fieldwork Event comments. Currently, no form exists in the SWIMS database to capture this type of data. When entering these results and other comments into SWIMS, the laboratory analyst should include their full name with the comment.

Reporting Results to Collectors

Both Waterflea Presence Analysis and Enumeration Analysis should be recorded in the appropriate columns within the [Results Log](#). When a positive result occurs during Waterflea Presence Analysis, this result should be immediately reported to the [Regional Aquatic Invasive Species Coordinator](#) for the sample, the Aquatic Invasive Species Monitoring Lead ([Maureen Ferry](#)), and the Aquatic Invasive Species Response Coordinator ([Kyle Mosel](#)). When laboratory analysis is completed for the year, the results (in the form of the [Results Log](#)) should be reported to the Aquatic Invasive Species Monitoring Lead, [Maureen Ferry](#). The results are then passed on for distribution to the [Regional Aquatic Invasive Species Coordinators](#). If contacted by collectors before dispersal of results, direct questions to the Regional Aquatic Invasive Species Coordinators.