

# Mussel Veliger (*Dressenid*) Laboratory Analysis Protocol

State of Wisconsin Department of Natural Resources

## STANDARD OPERATING PROCEDURES

February 2019

This protocol is for analysis of invasive mussel veligers of the *Dressenid* genus. Utilizing these methods, it is not possible to distinguish between Zebra Mussels (*D. polymorpha*) and Quagga Mussels (*D. bugensis*).

### Receiving Mussel Veliger 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 Mussel Veliger samples 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 sample jar 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.

### Mussel Veliger Presence Analysis

*Based on 2008 Plymouth Lab Procedures by Nicole Chase.*

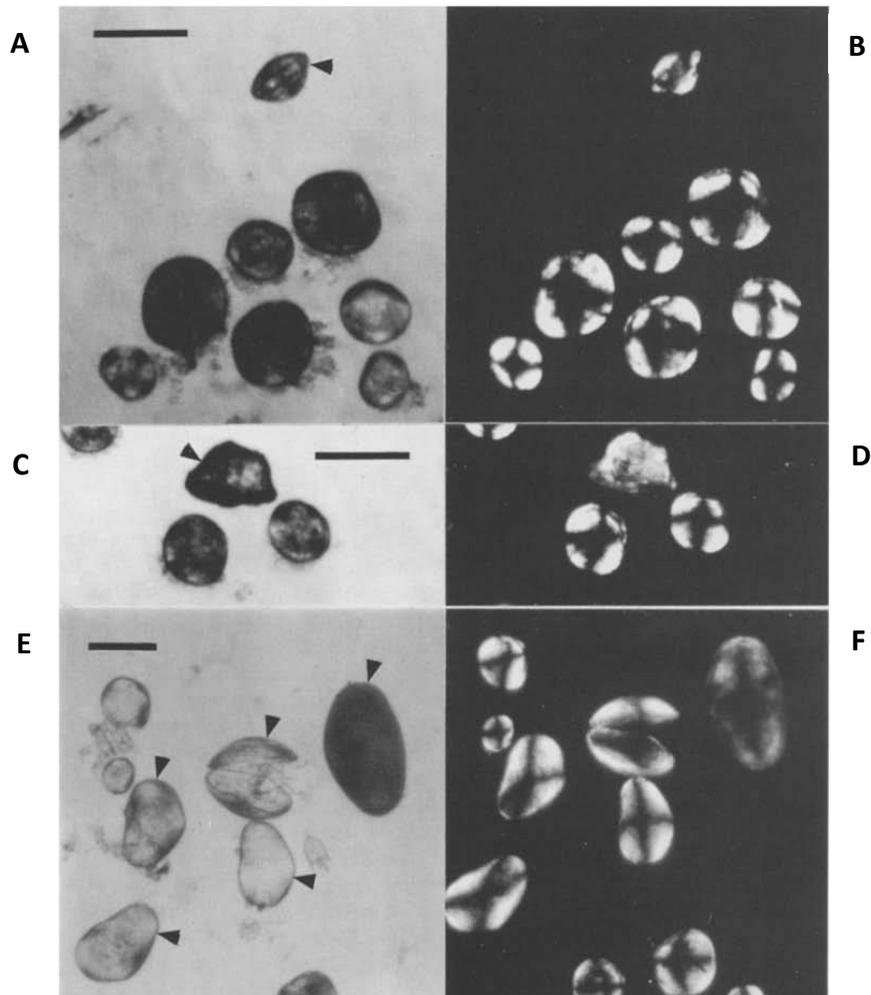
*Dressenid* mussel veliger shells are birefringent due to their crystalline calcite structure, causing them to reflect light and “glow” brightly under cross-polarized light. When viewed under cross-polarized light, the portions of the shell that are on the axes of the cross-polarized light are unable to reflect light. This causes the veligers to appear with small “maltese” crosses or x’s on them. This makes for a simple and efficient method of distinguishing them from other material in a sample.

Ostracods (a type of common zooplankton) and other bivalve larvae are birefringent and display similar characteristics to *dressenid* mussel veligers under cross-polarized light. In these cases, individuals must be distinguished from *dressenid* mussel veligers on basis of their morphology, shape, and size. It can be helpful to view individuals under normal, bright-field microscopy in addition to cross-polarized light in these situations. Ostracods are most easily distinguished from *dressenid* mussels by their size and shape. In general, ostracods will be larger in size than *dressenid* mussel veligers, though there is size overlap in early developmental stages of ostracods. Second, ostracods appear “bean-shaped” with a

curved hinge. Dressenid mussels are round or oval shaped and straight-hinged or with umbo. Legs can be seen on ostracods when viewed with bright field microscopy, while veligers lack legs.

[USACE Dreissena Comparison](#)—This document provides photographs and useful information for identification of Dreissena veligers at different larval stages. Provided by the United States Army Corps of Engineers.

The image below is helpful comparison of Zebra Mussel (*D. polymorpha*) Veligers to Ostracods and other material.



Various plankton samples seen under polarized light (a, c, & e) and under cross-polarized light (b, d, & f) (Polarized light was used instead of unpolarized light because the use of non-polarized light would have required the repetitive removal and installation of the polarizing filters.) a & b: zebra mussel veligers without extraneous material. Note that the distinctive. Maltese cross' pattern of birefringence of veligers on their sides is not evident when the veliger is viewed edge on (arrowhead) scale bar = 200  $\mu$ m. c & d: veligers and sand grain (arrowhead). Note that sand grain does not produce the 'Maltese cross' pattern. Sand grains are also usually multi-colored while veligers are always white; scale bar= 200  $\mu$ m. e & f: mixed collection of veligers and ostracods (arrowheads). Note that size ranges of veligers and ostracods overlap (e.g. star) and that empty ostracod 'shells' (lighter individuals in e) are more birefringent, and thus more similar to veligers, than whole animals due to the lack of interfering body tissue. Certain morphological features that distinguish veligers from ostracods (see Table 1) are not visible at this magnification, e.g., shell ornamentation; scale bar =200  $\mu$ m.

Johnson, E Ladd. 1995

## 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
- 400 mL Glass Beaker
- Dissecting Microscope equipped with Cross-Polarized Light Filter
- Liquinox

## Laboratory Protocols for Mussel Veliger Presence Analysis

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 dissecting microscope equipped with cross-polarized light filter. Examine sample for presence of dresenid mussel veligers.
  - Note presence of ostracods.
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 zebra mussel veliger is identified.
  - In cases where sample material provided is <50mL, examine entire sample and make note.
  - In cases where you are looking for veligers after adult Zebra Mussels (*D. polymorpha*) have been found in a waterbody, examine all the settled material if possible.
6. When examination is completed, return sample material from 400 mL beaker to sample jar. If dresenid mussel veligers are present, draw a ⊕ symbol on lid of sample jar in red sharpie.
7. Clean 10 ml pipet, glass petri dish, and 400 mL beaker with water and Liquinox between samples to ensure samples are not cross contaminated.
8. Record results in the [Results Log](#) .

## Mussel Veliger Enumeration Analysis

*Based on 2008 Plymouth Lab Procedures by Nicole Chase.*

[Dreissena Veligers/Ostracods Images--Laliberte](#)—This document provides useful image comparisons of zebra mussel veligers and ostracods in a gridded Sedgewick-Rafter Cell. Images provided by Gina LaLiberte, WI DNR.

## Equipment

- 10 mL Wide Bore Glass Pipet, such as Pyrex #7087 or another wide-tip serological pipet
- Valved Aspirator Bulb
- Gridded Sedgewick – Rafter Cell Slide
- Glass Slide Cover
- 400mL or 600 mL Glass Bleaker

- Dissecting Microscope
- Clicker Counter
- Graduated Cylinder
- Liquinox

### Laboratory Protocols for Mussel Veliger Enumeration Analysis

1. Draw off and reserve most of the ethanol from the sample jar in a 400-600mL glass beaker to concentrate the sample material. Do this by pouring off majority of ethanol, allowing to resettle, drawing off most of remaining ethanol with 10 mL pipet or drawing off ethanol only using 10 mL pipet.
  - When mixing sparse settled material throughout a 250mL-1000mL sample it becomes highly unlikely that a veliger will be encountered in the 1 mL aliquots. When this occurs, estimated veliger density may become zero despite their presence. Therefore, it is important to concentrate sample material before enumeration.
2. Gently agitate sample to distribute settled material throughout. Using 10 mL wide bore glass pipet fitted with a valved aspirator bulb, remove a 1mL of aliquot of sample from the center of the jar and place on gridded Sedgewick-Rafter Cell Slide and cover with Glass Slide Cover.
3. Place gridded Sedgewick-Rafter Cell Slide under the dissecting microscope and count individual dresenid mussel veligers present, utilizing clicker counter.
4. When count is completed gently rinse and dry Sedgewick-Rafter Cell Slide in sink. Do Not rinse 1 mL aliquots back into sample jar after completing count.
5. Repeat steps 2-4 until ten 1 mL aliquots have been examined.
6. Measure volume of remaining concentrated sample with graduated cylinder and add 10 mL (volume of ten 1 mL aliquots). Record this as the volume of sample (V<sub>s</sub>). Return reserve ethanol to sample jar.
7. Clean 10 ml pipet, glass petri dish, graduated cylinder, and glass beaker with water and Liquinox between samples to ensure samples are not cross contaminated.
8. Record counts and sample volume in [Veliger Enumeration Calculation Template](#).
9. Record density results in the [Results Log](#).

### Calculations for the Enumeration of Mussel Veligers

*Adapted from Wetzel and Likens (1979).*

Below is an explanation of the equations used to calculate average densities of Mussel Veligers in a waterbody from samples enumerated using a Sedgewick-Rafter cell in the laboratory. These calculations are pre-written into the [Veliger Enumeration Calculation Template](#).

Calculating Density of Veligers (n)

$$n = \frac{[(N * 1000) V_s]}{V_f}$$

**n** = number of mussel veligers per liter

**N** = average number of veligers per 1 mL aliquot, +/- standard deviation of mean

$V_s$  = volume of sample in liters

$V_f$  = volume of sample filtered in liters

#### Calculating Volume of Sample Filtered ( $V_f$ )

$$V_f = 1000 * [(\pi r^2) * D]$$

$V_f$  = volume of sample filtered in liters

$r$  = radius of tow net in meters

$D$  = depth of tow in meters

## Mussel Veliger Laboratory Analysis Results Reporting

### Reporting Results in DNR SWIMS Database

Prior to sending mussel samples in for laboratory analysis, sample collectors should create SWIMS Fieldwork Events for their samples and enter required field data into the Zebra Mussel Veliger Tow Monitoring Report (3200-135) 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 Zebra Mussel Veliger Tow Monitoring Report (3200-135) 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](#).

Mussel Veliger Presence Analysis results should be entered into the sample's SWIMS Fieldwork Event utilizing the Zebra Mussel Veliger Tow Monitoring Report (3200-135) form.

Mussel Veliger Enumeration Analysis results should be entered in the comments section of the sample's SWIMS Fieldwork Event. 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 Mussel Veliger Presence Analysis and Enumeration Analysis should be recorded in the appropriate columns within the [Results Log](#). When a positive result occurs during Mussel Veliger 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.

## Citations

Wetzel, R. G., & Likens, G. E. (1979). *Limnological analyses*, 357.