

Protocol

Our recommended plankton tow protocol focuses on collecting a sample from a large volume of water to capture target dreissenid veligers. The plankton tow net allows the filtering of thousands of liters of water through fine pore size mesh in a dolphin bucket, which captures planktonic larvae. The material in the dolphin bucket is then preserved and transferred to the MT Fish, Wildlife & Parks lab in Helena for microscopy analyses.

The specifics of the protocol depend on site characteristics. We recommend the use of a horizontal plankton tow sample to improve the probability of detecting rare species from large lentic sites. This version of the protocol details the horizontal plankton tow technique, which can be performed from the convenience of a boat dock, shoreline, or boat.

A. Sampling Location and Notes

Sample site GPS coordinates should be gathered or navigated to as part of a consistent sampling plan for future use in monitoring transparency, result reporting, and map production. Record these in your notes. Detailed notes about the sampling times, location, weather, and conditions should be recorded when possible. Photos are highly encouraged.

B. Decontamination

This method uses vinegar to dissolve veliger shells. When possible, we encourage users to decontaminate sampling gear before arriving at the sampling site. This will help to save time, conserve supplies and minimize mistakes. If you must do this in the field, please do it away from the study site and budget enough time to do this thoroughly. To prevent equipment failure and false negatives, immediately rinse or soak equipment thoroughly after the decontamination.

1. Soak all reusable supplies in a 50% vinegar (acetic acid) solution for at least 4 hours, mixing often. Acetic acid is a safe way to dissolve the dreissenid shells, as remnant shells may lead to false positives. Reusable supplies include but are not limited to:
 - a. Plankton tow net
 - b. Dolphin bucket
 - c. Boots/Waders, etc.
2. Rinse net and dolphin bucket with copious amounts of water to free of excess vinegar.
3. Let all supplies air dry.

4. Assemble sample kits with decontaminated gear that are specific to each site (Plankton tow nets can be stored in garbage bags).

C. Sample Collection

Horizontal Plankton Tow

1. From an appropriate surface (boat dock, boat, shoreline), measure out a length of 50 - 100 meters to be sampled. Doubling back to achieve a longer transect is acceptable.
2. At the start of the transect, proceed to dip the net until the net is completely submersed under water with the net's rim being vertical. At a slow and steady pace continue on for the 50 - 100 meters. The pace should be fast enough that the water filters through the net but slow enough as to not let water back flush out of the net. Prevent large air bubbles from getting into the net and bucket, as this prevents proper collection.
3. At the end of transect, swiftly lift the net straight up out of the water and hold until most of the water drains from the net through the dolphin bucket.
4. Quickly sweep the net opening through the water 2 more times to flush all organic material on the net sides down through the dolphin bucket.
5. Remove the dolphin bucket, and remove excess water by tapping water out through the screen.
6. Using the ethanol squirt bottle, dislodge as much organic material as possible with a small amount of ethanol from the screens to the insides of the dolphin bucket.
7. Swish the contents to mix and transfer to the 60 mL sample bottle. Minimize sediment as much as possible.
8. Add enough 95% ethanol to create a preservative ratio of about 1:3 (sample to ethanol). Higher concentrations of alcohol may be required for samples with high amounts of biological material.
9. Add about a teaspoon of 5% baking soda solution. Seal the bottle with electrical tape around the lid to prevent leakage.
10. Keep samples out of sunlight but do not freeze samples.
11. Record sample information.
12. Rinse all field and personal gear well before leaving the site.
13. Fill out the Montana Fish, Wildlife & Parks submission form and include with sample shipment. Please do not ship samples in coolers.
14. After shipping, email SSchmidt@mt.gov with the number of samples shipped and the shipping date.
15. Samples should be shipped to:
Montana Fish, Wildlife & Parks

Attn: Stacy Schmidt
1420 E 6th Ave
Helena, MT 59620

16. Email Durae Belcer (durae@whitefishlake.org) the Survey 123 data form with lake name, water temperature, Secchi depth, GPS coordinates, date and sampler name.

D. Sample Preservation

Ethanol (EtOH) works as a preservative by dehydrating cells. We use 200 proof (absolute) EtOH to end up with an overall ~%90 EtOH concentration. Avoid using denatured EtOH or isopropyl alcohol with acetone as these may interfere with analysis and may cause health concerns. If isopropyl alcohol without acetone is used, ensure your final alcohol concentration is at least 70%.

Developing dreissenid shells are degraded naturally by lower pH levels (more acidic) and thus it is recommended that all samples be buffered with 5 ml of 5% baking soda to keep the pH of the sample around 7 (to prevent shells from dissolving). To make a 5% baking soda solution, simply add 50 grams baking soda to 1-liter distilled water and mix well.

1. Sample vials should be pre-labeled whenever possible to avoid cross-contamination, and carefully wrapped with clear packing tape. Ethanol will cause any label ink to bleed, and the packing tape protects the ink.
2. For each site, label a 60mL bottle with the collection date, site ID, waterbody, and sample ID.
3. When done, transfer a well-drained dolphin bucket and net to its garbage bag and keep separated from the other clean kits. All material in the bag must be decontaminated (see **Decontamination**, above) before being reused.
4. Store tubes upright, covered, and cool - preferably refrigerated).

Appendix 1: List of Supplies

1. 95% EtOH – approx. 0.5 liter per site
2. 5% baking soda solution
3. Wilco plankton net, dolphin bucket– 1 kit per site, decontaminated
4. Squirt bottles for 95-100% ethanol and DI water
5. 60 mL sample bottle
6. Label and pencil
7. MT FWP Submission form and Survey 123 data form
8. Clear packing tape
9. Electrical tape
10. Garbage bags

11. Field notebook, writing utensil
12. GPS
13. Camera

References Cited

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