Standard Operating Procedure for Dreissenid Veliger Sampling with Plankton Tows for qPCR Analysis

Vers. 1.1

Last Updated: 20 August 2015

Scope and Application

1. This standard operation procedure (SOP) outlines the format for sampling Amistad Lake to detect *Dreissenid* species veliger using a plankton tow net.

Sampling Supplies

1. Plankton tow net (63 or 64 micron mesh size), cod end assembly
2. Line for deploying net marked at 2-m intervals
3. Labeled sample bottles – 250mL, 500mL and 1-L with leak-proof screw lid
4. Preservative – non-denatured ethanol (190 PROOF)
5. Spray bottle containing DI
6. Spray bottle containing white vinegar
7. Field data sheets, pen/pencil, sharpie
8. Multiprobe water quality instrument
9. GPS instrument
10. Secchi disk
11. Zip lock bags -1 gallon
12. Disposable Diapers
13. Plastic Garbage Bags
14. Shipping boxes

Sample Collection/Vertical Plankton net tow

1. Lower the net to the desired, measured depth and slowly raise the net to the surface. The volume of water that is sampled can be determined based on the diameter of the net opening and how many times the net was lifted. A minimum sample volume of 1,000 L is recommended (see Table 1). Record depth of tow.

2. Pay attention to tow angle, if line angle goes over ~30 degrees due to wind or current, discard tow and repeat until 30 degree angle or less is achieved.

3. Using a spray bottle with DI water, wash down the net from the outside to concentrate plankton into the collection cup. Carefully unscrew the collection cup and pour the sample into a Nalgene leak proof poly bottle. Rinse the collection cup with spray.
bottle with minimal volume of water and transfer the rinses into the same sample bottle. Take care to keep the wash and/or rinse water away from the opening of the plankton net and wash only along the outside of the plankton net and cup, so that the filtered volume remains unchanged. Unless you will be pooling samples, MARK THE WATER LEVEL ON THE SAMPLE BOTTLE WITH PERMANENT INK (Draw a line on the bottle and label “Level 1”). If you are pooling samples, place first sample into empty Nalgene bottle. As samples are collected, continue to add to the Nalgene bottle. Once all samples are collected, shake to mix, pour off necessary amount into a final sample bottle, and then label the water level with permanent ink.

4. Add an appropriate volume of ethanol to get 70% final concentration in the sample bottle (visual estimate, does not have to be exact). Use 1 lake water and 3 part ethanol.

5. Replace bottle cap snugly. (Note: The volume of ethanol will be needed in the calculation of number of veligers per unit volume; therefore be sure that the sample bottle is marked with a second line to indicate total volume (sample + ethanol) so that the lab can also determine the volume of ethanol that was added.) Draw a line on the bottle and label “Level after ETOH”. Wrap the bottle in a disposable diaper and place in a Ziploc bag (push all air out of bag before closing).

6. Label the bottle with waterbody, site name, date/time and name and contact information of collector. Be careful to avoid spillage of ethanol. For backup, record sample bottle information with a mechanical pencil on a piece of waterproof paper and insert paper into the Ziploc bag along with the sample bottle.

7. Complete datasheet and laboratory submission form.

8. Wrap sample bottle using a disposable diaper and place it into a zip lock bag and then place this bag and a datasheet copy and submission form into a garbage bag.

9. Place sealed bags into a cardboard box and add cushioning material. Label the cardboard box with “this side up” label. Seal box with clear packing tape.

10. Because the samples are preserved in alcohol, ice packs are not required; shipping at ambient temperatures is sufficient.

11. Ship samples (for q-PCR analysis) using FedEx ground to:
Table 1: Net tow Calculator

<table>
<thead>
<tr>
<th>Depth (feet)</th>
<th>Depth (meters)</th>
<th>#Net Tow for a minimum of 1,000L</th>
<th>Liters (L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.6</td>
<td>2</td>
<td>3</td>
<td>1,200</td>
</tr>
<tr>
<td>13.2</td>
<td>4</td>
<td>2</td>
<td>1,600</td>
</tr>
<tr>
<td>19.8</td>
<td>6</td>
<td>1</td>
<td>1,200</td>
</tr>
<tr>
<td>26.4</td>
<td>8</td>
<td>1</td>
<td>1,600</td>
</tr>
<tr>
<td>33</td>
<td>10</td>
<td>1</td>
<td>2,000</td>
</tr>
</tbody>
</table>

Water Quality Data

1. Describe Secchi lower, take depth, then raise, take depth, average the 2

2. Lower calibrated YSI to same depth as tow was taken from; allow 30 secs to stabilize readings before recording on datasheet.