

RECLAMATION

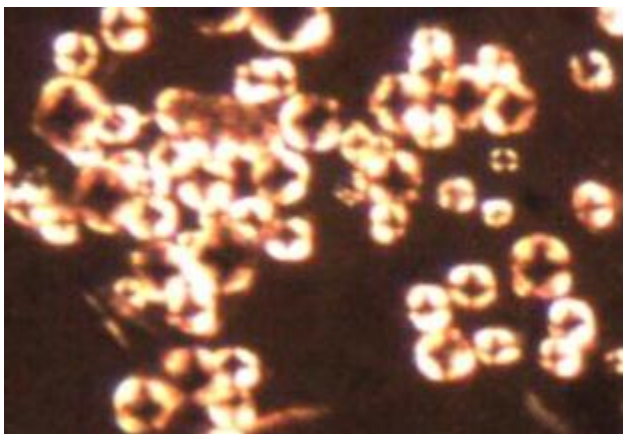
Managing Water in the West

Technical Memorandum No. 86-68220-13-02

Lab Protocol: Preparation and Analysis of Dreissenid Veliger Water Samples

Laboratory Standard Operating Procedure (Lab SOP)
Lab SOP Version 4
Date Revised: 2013

Bureau of Reclamation
Technical Service Center
Reclamation Detection Laboratory for Invasive and Native
Species



Quagga mussel veligers viewed under cross-polarized light microscopy

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Prepared for:

**Bureau of Reclamation
Technical Service Center
Research and Development Office**

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**PEER REVIEW DOCUMENTATION
PROJECT AND DOCUMENT INFORMATION**

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REVIEW REQUIREMENT

Part A: Document Does Not Require Review

Explain _____

Part B: Document Requires Peer Review: SCOPE OF PEER REVIEW

Peer review restricted to the following items/section(s):

Reviewer:

REVIEW CERTIFICATION

Peer Reviewer- I have reviewed the assigned items/section(s) noted for the above document and believe them to be in accordance with the project requirements, standards of the profession, and Reclamation policy.

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Preparer- I have discussed the above document and review requirements with the Peer Reviewer(s) and believe that this review is completed, and that the document will meet the requirements of the project.

Team Member:  Signature Date: 7/1/13

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1. ACRONYMS / DEFINITIONS

Dreissenid: Genus of freshwater mussel

Zebra mussel: (*Dreissena polymorpha*) Invasive mussel species

Quagga mussel: (*Dreissena rostriformis bugensis*) Invasive mussel species

Veliger: For the purpose of this paper, veliger refers to larval zebra/quagga mussel

DI water: Deionized water

mL: Milliliter

L: Liter

PCR: Polymerase chain reaction. Technique used to amplify DNA

SEM: Scanning electron microscope

Settling cone: Modified Imhoff cone used to measure the volume of solids in water

Venuset: Plastic tubing with a vent and roller wheel system to control flow out of the settling cone

2. SCOPE AND APPLICABILITY

This standard operating procedure (SOP) is used to establish a uniform format for duties performed in the zebra/ quagga mussel lab. This method is applicable to dreissenid water samples that are prepped and analyzed in the mussel lab at the Bureau of Reclamation Technical Service Center, Denver, CO. The goal of this SOP is to standardize how each task in the laboratory is performed by every lab technician. This SOP is not a replacement for training.

3. SUMMARY OF METHOD

Preparation, analysis, and disposal of dreissenid water samples.

4. INTERFERENCES

The main interference in early mussel detection is cross contamination from other samples. In order to mitigate this interference, glassware is decontaminated in five percent acetic acid for a minimum of two hours. A new pipette and decontaminated petri dish is used for every sample. Settling cones are triple-rinsed with DI water and decontaminated with five percent acetic acid for a minimum of four hours, rinsed with one percent bleach, and triple-rinsed with DI water. A secondary interference in early mussel detection is improper sample handling in the field resulting in lag times between sample collection and shipping. Improper sample handling also can result in a low pH which will hinder detection.

5. HAZARDS

- 5.1. Acetic acid, glacial: CAS-No 64-19-7 UN-No2789 Hazard Class: 8
- 5.1.1. Handling: Keep away from heat. Keep away from sources of ignition. Do not ingest. Do not breathe gas/fumes/vapor/spray. Never add water to this product; when diluting always add acid to water. Always prepare in fume hood. In case of insufficient ventilation, immediately contact a physician or Poison Control Center and bring the container or the label. Avoid contact with skin and eyes. Keep away from incompatibles such as oxidizing agents, reducing agents, metals, acids, and alkalis.
- 5.1.2. Storage: Store in a segregated and approved area; under fume hood. Keep container in a cool, well-ventilated area. Keep container tightly closed and sealed until ready for use. Avoid all possible sources of ignition (spark or flame).
- 5.1.3. Engineering Controls: Provide exhaust ventilation or other engineering controls to keep the airborne concentrations of vapors below their respective threshold limit value. Ensure that eyewash stations and safety showers are proximal to the work-station location.
- 5.1.4. Personal Protective Equipment: Splash goggles, synthetic apron, vapor respirator, and rubber or latex gloves. Be sure to use an approved/certified respirator or equivalent (MSDS, NoA/0407/PB08, 2010).
- 5.2. Clorox Regular Bleach: EPA Regulation No. 5813-50
- 5.2.1. Health Hazard Data: Corrosive: May cause irritation or damage to eyes and skin. Vapor or mist may irritate. Harmful if swallowed. No special protection or precautions have been identified with using this product under directed consumer use conditions.
- 5.2.2. First Aid: Eyes: Hold eye open and rinse with water for 15 to 20 minutes. Remove contact lenses, after first five minutes. Continue rinsing eye. Call physician. Skin: Wash skin with water for 15 to 20 minutes. If irritation develops, call a physician. Ingestion: Do not induce vomiting. Drink a glassful of water. If irritation develops, call a physician. Do not give anything by mouth to an unconscious person. Inhalation: Remove to fresh air. If breathing is affected, call a physician.
- 5.2.3. Spill Procedures: Control spill; use absorbents to clean spill. Wash area and let dry. Dispose of in accordance with all applicable federal, state and local regulations (MSDS EPA Regulation No. 5813-50, 2009).
- 5.3. Ethanol, Denatured with up to five percent V/V Ether: CAS-No-60-29-7 UN-No1170 Hazard Class: 3
- 5.3.1. Handling: Wash hands thoroughly after handling. Use only in a well-ventilated area. Take precaution to avoid static discharges. Avoid contact with eyes, skin, and clothing. Do not breathe fumes. Empty containers retain product residue (liquid and/or vapor), and can be dangerous. Keep container tightly closed. Avoid contact with heat, sparks and flame. Avoid ingestion and inhalation. Do not pressurize, cut, weld, braze, solder, drill, grind, or expose empty containers to heat, sparks or open flames.
- 5.3.2. Storage: Keep away from heat, sparks, and flame. Keep away from sources of ignition. Store in a tightly closed container. Keep from contact with oxidizing materials. Store in a cool, dry, well-ventilated area away from incompatible substances. Store in a designated area marked flammables. Do not store near perchlorates, peroxides, chromic acid or nitric acid.
- 5.3.3. Engineering Controls: Use explosion-proof ventilation equipment. Facilities storing or utilizing this material should be equipped with an eyewash facility and a safety shower close to workstation location. Use adequate general or local exhaust ventilation to keep airborne concentrations below the permissible exposure limits.

- 5.3.4. Personal Protective Equipment: Eyes: Wear appropriate protective eyeglasses or chemical safety goggles as described by OSHA's eye and face protection regulations in 29 CFR 1910.133 or European Standard EN166. Skin: Wear appropriate protective gloves to prevent skin exposure. Clothing: Wear appropriate protective clothing to prevent skin exposure. Respirators: A respiratory protection program that meets OSHA's 29 CFR 1910.134 and ANSI Z88.2 requirements or European Standard EN 149 must be followed whenever workplace conditions warrant a respirator's use (MSDS, No. 270660000, 2010).
- 5.4. Lemon Fresh Clorox Disinfecting Wipes: EPA Regulation No. 5813-5
- 5.4.1. Health Hazard: Eyes: Can cause moderate eye irritation. Ingestion: Liquid should be non-toxic if ingested. Skin: Prolonged skin contact may produce minor irritation. No special protection or precautions have been identified for using this product under directed consumer use conditions.
- 5.4.2. First Aid: Eyes: Flush with water. Call a physician if irritation. Ingested: Drink a glassful of water. Call a physician or poison control center. Skin: Wash thoroughly with soap and water after handling. Inhalation: If breathing is affected, move to fresh air. Under normal consumer use conditions the likelihood of any adverse health effects are low.
- 5.4.3. Spill Procedures: Absorb and containerize. Wash residual down to sanitary sewer. Contact the sanitary treatment facility in advance to assure ability to process washed down material. Waste disposal: Disposal must be made in accordance with applicable federal, state and local regulations.
- 5.5. Sodium Bicarbonate: CAS-No 144-55-8, UN-No Not Regulated, Hazard Class-Not Regulated
- 5.5.1. Handling: Use with adequate ventilation. Minimize dust generation and accumulation. Do not get in eyes or skin. Do not ingest or inhale.
- 5.5.2. Storage: Store in cool, dry place.
- 5.5.3. Personal Protective Equipment: Eyes: Wear appropriate protective eyeglasses or chemical safety goggles as described by OSHA's eye and face protection regulations in 29 CFR 1910.133 or European Standard EN166. Skin and clothes: Wear appropriate protective clothing to prevent skin exposure. Respirators: A respiratory protection program that meets OSHA's 29 CFR 1910.134 and ANSI Z88.2 requirements or European Standard EN 149 must be followed whenever workplace conditions warrant a respirator's use (MSDS, No 20970, 2008).
- 5.5.4. Engineering Controls: Facilities storing or utilizing this material should be equipped with an eyewash facility and a safety shower close to workstation location. Use adequate ventilation to keep airborne concentration low.
- 5.6. Buffer Solutions: pH 4, pH 7, pH 10, UN-No Not Regulated, Hazard Class-Not Regulated
- 5.6.1. Handling: Wear personal protective equipment. Ensure adequate ventilation. Do not breathe vapors or spray mist. Avoid contact with skin, eyes and clothing.
- 5.6.2. Storage: Keep containers tightly closed in a dry, cool and well-ventilated place.
- 5.6.3. Engineering Controls: Ensure adequate ventilation, especially in confined areas. Ensure that eyewash stations and safety showers are close to the workstation location.
- 5.6.4. Personal Protective Equipment: Eyes: Wear appropriate protective eyeglasses or chemical safety goggles as described by OSHA's eye and face protection regulations in 29 CFR 1910.133 or European Standard EN166. Skin/Clothing: Wear appropriate protective gloves and clothing to prevent skin exposure. Respirators: Follow the OSHA respirator regulations found in 29 CFR 1910.134 or European Standard EN 149. Use a NIOSH/MSHA or European Standard EN 149 approved respirator if exposure limits are exceeded or if irritation or other symptoms are experienced (MSDS, No C2948, 2006).

5.7. Conductivity Standard: UN-No Not Regulated, Hazard Class-Not Regulated

5.7.1. Handling: Ensure adequate ventilation. Do not breathe vapors or spray mist. Avoid contact with skin, eyes and clothing.

5.7.2. Storage: Keep containers tightly closed in a dry, cool and well-ventilated place.

5.7.3. Engineering Controls: Ensure adequate ventilation, especially in confined area. Ensure that eyewash station and safety showers are close to the workstation location.

5.7.4. Personal Protective Equipment: Wear appropriate protective eyeglasses or chemical safety goggles as described by OSHA's eye and face protection regulations in 29 CFR 1910.133 or European Standard EN166. Skin/Clothing: Wear appropriate protective gloves and clothing to prevent skin exposure. Respirators: Follow the OSHA respirator regulations found in 29 CFR 1910.134 or European Standard EN 149. Use a NIOSH/MSHA or European Standard EN 149 approved respirator if exposure limits are exceeded or if irritation or other symptoms are experienced (MSDS. No 09-328-2.2010).

5.8. Glassware

5.8.1. In the case of broken glassware, obtain a dustpan and broom and sweep up the pieces. Discard them in the container marked 'Recycled Glass' in the lab. Do not handle broken glass by hand, if it can be avoided. Broken glassware with chemical residue should either be cleaned (if there is a way to do so safely), or placed into the trash container. Only place clean broken glass in the 'Recycled Glass' container. Glass pipettes are rinsed and disposed of in the 'Recycled Glass' container after each sample.

5.9. Material Safety Data Sheets (MSDS)

5.9.1. MSDS are located in the microscopy lab documentation binder.

6. MATERIALS

- 6.1. Glassware
 - 6.1.1. Glass transfer pipettes
 - 6.1.2. Glass Petri dishes
 - 6.1.3. Glass graduated cylinders (up to 1000 mL)
 - 6.1.4. Glass watch glasses
 - 6.1.5. Glass slides and Sedgwick-Rafter counting cells, cover slips optional
 - 6.1.6. 15 mL graduated test tubes
 - 6.1.7. >80 mL beakers
- 6.2. Microscopes (appendix A)
 - 6.2.1. Screening scopes – Meiji EMZ-5D, 7X-45X magnification
 - 6.2.2. Research scope – Olympus SZH10, 10.5X-210X magnification
 - 6.2.3. Compound scopes – Spencer AO, 40X-100X magnification
 - 6.2.4. Inverted scope – Axio Observer A1, 100-400X magnification
 - 6.2.5. Micrometrics 318CU digital camera mounted on research scope
- 6.3. Kimwipes
- 6.4. Settling cones (appendix B)
- 6.5. Cone brush for washing settling cones
- 6.6. pH meter, or pH strips
- 6.7. Latex gloves
- 6.8. Measuring spoons for baking soda (0.1 gram)
- 6.9. Designated squirt bottles for DI water and five percent acetic acid
- 6.10. Colored Fischer tape
 - 6.10.1. Red tape used for ‘priority’ water samples only
- 6.11. Parafilm
- 6.12. Rubber bulbs
 - 6.12.1. Separate bulbs for positive waters
- 6.13. 2 mL Eppendorf tube
- 6.14. Dissecting needles
- 6.15. Slide case for SEM slides
- 6.16. Micro pipette
- 6.17. Micro pipette tips
- 6.18. Lab counters
- 6.19. Clorox wipes
- 6.20. Log notebooks
 - 6.20.1. Cooler login notebook
 - 6.20.2. Sample prep notebook
 - 6.20.3. Technician sample notebook
 - 6.20.4. Enumeration notebook
 - 6.20.5. Purge notebook
- 6.21. Computer access to shared H:/ drive
 - 6.21.1. Master login file
 - 6.21.2. Master enumeration file
 - 6.21.3. SEM log file

7. REAGENTS AND CHEMICALS

- 7.1. Glacial acetic acid
 - 7.1.1. Five percent acetic acid (appendix C)
- 7.2. Regular Clorox bleach
 - 7.2.1. One percent bleach solution to decontaminate the cones
- 7.3. Lake water preserved with ethanol
 - 7.3.1. Twenty percent ethanol added in field
- 7.4. Dish soap
- 7.5. Buffers
 - 7.5.1. Sodium bicarbonate (baking soda)
- 7.6. Conductivity standard and pH buffers for calibrating the YSI

8. SAMPLE RECEIVING

- 8.1. Unpack and dry the cooler
 - 8.1.1. Save or record shipping label for return shipping
 - 8.1.2. Enter shipping label information into the cooler login notebook (appendix D)
- 8.2. Open master login file
 - 8.2.1. H:\EnvRes Share\MUSSEL SAMPLES\DATA and LOGIN
- 8.3. Enter login data per sample labels and/or chain of custody forms (appendix E)
 - 8.3.1. Make sure samples are on ice
 - 8.3.2. If the samples are warm, note in the computer under comments in the master login file
 - 8.3.3. Write the assigned sample ID numbers from master login file on both the sample bottle and the lid
- 8.4. Test pH and record in master login file
 - 8.4.1. Add baking soda if sample is less than 7.0 pH
 - 8.4.1.1. Add small amounts of baking soda using 0.1 g spoons, and re-test until pH is 7.0 or higher
- 8.5. The priority water list is posted in the lab
 - 8.5.1. Flag priority sample bottles with a small piece of red tape
- 8.6. Samples with a COC form
 - 8.6.1. Write assigned sample ID numbers and the date received on the COC
 - 8.6.2. Place COC in ZQM sampling metadata sheets binder
- 8.7. Store samples in refrigerator on designated shelf, until ready for prep
- 8.8. Save master login file as a new spreadsheet with current date
 - 8.8.1. Move old spreadsheet to 'Old Log-in Files' folder
- 8.9. Ship cooler back to sender along with all packing material, including ice packs and diapers and tape closed
 - 8.9.1. Enter the return information for the cooler into the cooler login notebook (appendix D)
 - 8.9.2. Use standard shipping forms available in lab, also located on the H:/ drive
 - 8.9.2.1. H:\EnvRes Share\MUSSEL SAMPLES\FEDEX Request
 - 8.9.3. Take packaged cooler to the mail room in building 67

9. SAMPLE PREPARATION PROCEDURE

The sample preparation method was developed from Hosler (2011), which was adapted from the Army Corps of Engineers (ZMIS 2002), the Standard Method (Standard Methods 2001), and the US Environmental Protection Agency (USEPA 2003). Appendix B shows instructions on construction of a modified Imhoff cone with a passive venoset system.

- 9.1. Check that the settling cone is decontaminated and dry
 - 9.1.1. Roller clamp is locked to prevent leaks
 - 9.1.2. Place watch glasses on all of the cones to prevent accidental contamination
- 9.2. Remove watch glass for the sample
- 9.3. Pour contents of sample bottle into settling cone
- 9.4. Rinse the sample bottle with DI water
 - 9.4.1. Use a minimal amount of water
 - 9.4.2. Rinse sample bottle three times
 - 9.4.3. Place rinse water into the cone
- 9.5. Rinse top edges of cone with DI water to clean any debris from sides
- 9.6. Replace watch glass
- 9.7. Repeat 9.1 to 9.6 for all samples
- 9.8. Fill out sample prep notebook (appendix F)
 - 9.8.1. Low debris: To middle of drip chamber on the venoset (appendix B)
 - 9.8.2. Medium debris: To top of drip chamber on the venoset
 - 9.8.3. High debris: Anything above the drip chamber on the venoset
- 9.9. Let the sample settle 18-24 hours
- 9.10. Use tape to label a small (~100 mL) beaker and 15 mL test tube with sample information (appendix G)
 - 9.10.1. Beaker label
 - 9.10.1.1. Sample ID number, location, sample date
 - 9.10.2. Test tube label
 - 9.10.2.1. Sample ID number
 - 9.10.3. Priority water label
 - 9.10.3.1. Use red tape only on 'priority' water samples
 - 9.10.3.2. Priority list posted in lab
- 9.11. Drain bottom 15 mL of sample from the cone into test tube and seal with parafilm
 - 9.11.1. When plug occurs, roll the roller clamp back and forth to try and release particulates/sediment
 - 9.11.2. If needed, use thin wire, provided in lab, to push particulates/sediment through the venoset
 - 9.11.3. Decontaminate wire with five percent acetic acid, rinse with DI water
- 9.12. Empty the remaining volume of the settling cone into the original sample bottle
 - 9.12.1. If needed, label another clean sample bottle for additional volume and tape to original sample bottle
- 9.13. Put sample bottle and test tube/ beaker into refrigerator in numerical sample ID order

- 9.14. Triple rinse and scrub the settling cone with DI water
- 9.15. Decontaminate settling cone with 200-250 mL five percent acetic acid for a minimum of four hours (appendix B and C)
 - 9.15.1. After four hours triple-rinse cone with DI water
 - 9.15.2. Scrub cone with designated cone brush
 - 9.15.3. Rinse cone in one percent bleach
 - 9.15.4. Triple-rinse cone with DI water
 - 9.15.5. Set cone aside to dry

10. SAMPLE ANALYSIS

- 10.1. Remove sample from refrigerator. Check that sample information on test tube/beaker matches information on sample bottle
- 10.2. Obtain a clean glass pipette and a clean glass Petri dish for every sample
 - 10.2.1. Invert the test tube a few times to mix the contents
 - 10.2.2. Pipette a small amount of sample from the test tube into the Petri dish
 - 10.2.3. If needed, dilute with water from sample or DI water
- 10.3. Use the identification book (posted in the lab) to help distinguish between organisms
 - 10.3.1. If suspect is found (section 14: Identification of a suspect)
- 10.4. Pour the contents of the Petri dish back into the sample bottle and rinse with sample water, using the same pipette, or with a small amount of DI water from a designated DI water bottle
- 10.5. Repeat this process until all 15 mL are analyzed
- 10.6. Instructions for counting known positive water samples
 - 10.6.1. Use designated positive water rubber bulbs
 - 10.6.2. Place a small amount of sample in a petri dish with DI water
 - 10.6.3. Obtain a counter
 - 10.6.4. Add a drop of dish soap, if necessary, to still the Brownian motion of the water pushing the sample around the dish
- 10.7. When analysis is finished
 - 10.7.1. Rinse petri dish into the sample bottle with DI water or water from the sample
 - 10.7.2. Rinse test tube with DI water and place rinse water into sample bottle
 - 10.7.3. Rinse glass pipette with DI water and dispose in designated glass recycling container
 - 10.7.4. Put initials on top of sample bottle and place in back storage refrigerator
 - 10.7.4.1. If sample is from a priority reservoir, place on PCR shelf
- 10.8. Soak the glass petri dish and 15 mL test tube in five percent acetic acid for a minimum of two hours
- 10.9. At end of day, clean work area and surface of microscope with Clorox Wipes. Clean lenses of scope with kimwipes

11. SPILLS

- 11.1. When spills occur
 - 11.1.1. Try to save as much of the sample as possible
 - 11.1.2. Clean rest with paper towels
 - 11.1.3. Clean counter with Clorox Wipes
 - 11.1.4. Decontaminate counter with five percent acetic acid (appendix C)
 - 11.1.5. Re-settle sample as per direction in sample preparation procedure (section 9: Sample preparation procedure)
 - 11.1.6. Note spill in technician sample notebook and enumeration notebook (appendix G and H)
 - 11.1.7. Analyze the rest of the sample

12. TECHNICIAN SAMPLE NOTEBOOK

- 12.1. Remove tape from sample and place in technician sample notebook
 - 12.1.1. Make note of debris and sediment type (i.e. Algae, mud, etc.)
 - 12.1.2. Make note of organisms presents, ie snails, glochidia
 - 12.1.3. Note the number of suspects in the technician sample log (section 14: Identification of a suspect)
- 12.2. Estimate the number of ostracods and / or corbicula and record as low, med or high
 - 12.2.1. Less than 10: Low organisms
 - 12.2.2. Between 10 and 30: Medium organisms
 - 12.2.3. Over 30: High organisms
- 12.3. Transfer data from technician sample notebook to enumeration notebook (appendix G and H)

13. DATA LOGGING PROCEDURES

- 13.1. Input results from the enumeration notebook into the master enumeration file (appendix H and I)
 - 13.1.1. Located in H:\EnvRes Share\MUSSEL SAMPLES\DATA and LOGIN
 - 13.1.2. Save master enumeration file as a new spreadsheet with current date
 - 13.1.3. Move old spreadsheet into 'Old Data Files' folder
 - 13.1.4. Enter the date microscopy was completed in the master login file, under the 'Date Microscopy Completed' column

14. IDENTIFICATION OF A SUSPECT

- 14.1. If sample is positive by PCR but negative by microscopy
 - 14.1.1. Re-settle per sample preparation procedure (section 9)
 - 14.1.2. Re-scan the sample with light and cross polarized light microscopy
- 14.2. If a suspect organism is found by microscopy
 - 14.2.1. Use the identification notebook (posted in the lab) to classify the organism(s)
 - 14.2.2. Notify other personnel and lab manager
 - 14.2.3. Examine suspect(s) under higher-magnification microscope
 - 14.2.4. Take a digital photograph of organism
 - 14.2.5. Remove suspect(s) with micro pipette using a new micro pipette tip and place each suspect into a separate 2-mL Eppendorf tube preserved with alcohol
 - 14.2.5.1. Record sample ID number on Eppendorf tube
 - 14.2.5.2. Mark Eppendorf tube with either PCR or SEM as determined by lab manager
 - 14.2.6. Place suspect(s) in the sample refrigerator
 - 14.2.7. Place sample bottle on PCR shelf in sample refrigerator
 - 14.2.7.1. Place red tape on the sample bottle to mark it as a priority sample
- 14.3. Prepare suspect(s) for SEM analysis
 - 14.3.1. Only to be done when SEM technician is ready to analyze samples
 - 14.3.2. Wear latex gloves
 - 14.3.3. Locate SEM prep box in the lab
 - 14.3.3.1. Obtain a glass slide
 - 14.3.3.1.1. Record sample number, location and sample date on the slide
 - 14.3.4. Place double sided tape in the middle of the slide
 - 14.3.4.1. Store double sided tape in sanitary glass jar
 - 14.3.5. Capture the suspect with minimal water using a micro pipette and a new micro pipette tip
 - 14.3.6. Place a small drop of water containing the suspect on the prepared slide
 - 14.3.7. If multiple suspects are present, repeat 14.3.5 to 14.3.6, and place suspects in a line on the same slide
 - 14.3.8. Cover and allow the slide to dry in a dust free area
 - 14.3.9. Circle the location of the suspect(s) on the back of the slide so that it is easier to find during SEM examination
 - 14.3.10. Place slide in a sterile glass slide container
 - 14.3.11. Fill out the SEM log file (appendix J)
 - 14.3.11.1. H:/ Env Res Share/ MUSSEL SAMPLES/DATA and LOGIN/SEM LOG Date
 - 14.3.11.2. Write down sample ID, location, sample date, slide prep date, and initials of preparer
 - 14.3.11.3. Indicate how many suspicious organisms were placed on the prepared slide

15. SAMPLE ARCHIVING

- 15.1. All samples will be kept for at least one month after analysis
- 15.2. Samples from water bodies designated as 'priority' will be kept past the end of the sample season

16. DISPOSAL OF SAMPLES

- 16.1. Purge old samples
 - 16.1.1. Only purge samples that are non-priority and are over one month old
 - 16.1.2. Mark sample with a checkmark as 'purged' in purge notebook (appendix K)
 - 16.1.3. Remove label if possible
 - 16.1.4. Pour the sample into the sink
 - 16.1.5. Rinse bottle several times with water
 - 16.1.6. Place sample bottle in dishwasher rack; place lid in a basket
- 16.2. Consolidate priority samples (section 17: Compositing priority samples)
- 16.3. Run dishwasher
 - 16.3.1. Use Alcojet detergent
 - 16.3.2. Press start button twice to begin cycle
- 16.4. Remove clean bottles and let dry
- 16.5. Replace lids on bottles and rub off labels
- 16.6. Box and store clean bottles by volume (125, 250, 500, 1000 mL)

17. COMPOSITING PRIORITY SAMPLES

- 17.1. Only to be done after sampling season and by request of the lab manager
- 17.2. Lab manager will decide which water bodies will be composited by location and which will be composited by sample site
- 17.3. Label each composite bottle and lid with a unique composite number (appendix L)
- 17.4. Begin the composite process by shaking the sample and pouring approximately 50 mL of each sample into a 1000-mL (1-L) bottle
- 17.5. Each priority sample will be preserved in 70 percent alcohol
 - 17.5.1. 25 mL of ethanol per 50 mL of sample will allow for 11 samples to be stored in a 1-L bottle (including room for headspace)
 - 17.5.2. $X = [(0.7*V)] - (V*P)$
 - 17.5.2.1. V= Volume of Sample
 - 17.5.2.2. P=Percent Ethanol in Sample
 - 17.5.2.3. X=Volume of Ethanol to add
 - 17.5.3. Example: 50 mL sample in 20 percent ethanol
 - 17.5.3.1. $X = [(0.7*50) - (50*0.2)]$
 - 17.5.3.2. X=25 mL
- 17.6. Place sample in refrigerator. Keep bottles in numerical composite ID order

18. ONLINE DATABASE

This is currently in the Beta testing phase

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20. CONTACT INFORMATION

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Appendix A - Microscopes / Digital Camera

1. DEFINITIONS

Dimmer switch: Switch on the microscope used to turn on illuminator
Illuminator: Light source for the microscope
Pillar collar: Post located on the back of the microscope which holds the ocular and objective
Objective: Lens closest to the specimen
Zoom control knob: Controls the amount of magnification of the subject
Focusing knob: Moves the objective up and down to bring specimen into focus
Fine focusing knob: Used to gain sharper focus
Stage: Platform which holds specimen
Brightfield light: Produces a dark image on a brighter background
Cross-polarized light: Polarizing filters in the light path creating birefringence for certain organisms
Transmitted light: Light which is projected from an illuminator

2. CALIBRATE MICROSCOPE

- 2.1. **Refer to instruction manual of particular microscope for calibration instructions**
- 2.2. Set magnification to highest magnification by using zoom control knob
- 2.3. Use focusing knob to focus sample until clear
- 2.4. Set magnification to lowest magnification using zoom control knob
- 2.5. Using right eye, look through the right eyepiece and turn the eyepiece diopter until sample is clear
 - 2.5.1. Repeat with left eyepiece
- 2.6. Sample should now be clear for all magnifications. Repeat if necessary

3. REPLACE LIGHT BULB

- 3.1. Turn off selector switch and dimmer switch
- 3.2. Unplug microscope from electrical outlet
- 3.3. Locate where light bulb is housed and open housing
 - 3.3.1. Some microscopes need a screwdriver to access light bulb housing
- 3.4. Using Kimwipe, carefully remove old light bulb from illuminator
- 3.5. Dispose of bulb in the trash receptacle
- 3.6. Using Kimwipe, take new light bulb from box and place the two wires coming out from it in the holes in which the old bulb came from
- 3.7. Once the new bulb has been installed, replace cover to light bulb housing
- 3.8. Turn microscope back to its original position, reconnect plug to electrical socket and test by turning on the microscope

4. SCREENING SCOPE – Meiji EMZ-5D

- 4.1. Remove cover from microscope and place Petri dish containing sample in the center of the microscope staging area
- 4.2. Turn selector switch, located in the back of the microscope, to transmitted light (T)
- 4.3. Turn dimmer switch, located next to selector switch, to desired light intensity
- 4.4. Turn zoom control knob, located on the zoom body, to desired magnification
- 4.5. Use both the focusing and fine focusing knobs until sample is in focus
- 4.6. To change between cross-polarized and non-cross polarized light, turn the cross polarizer on the bottom of the stage

5. RESEARCH SCOPE – Olympus SZH10

- 5.1. Remove cover from microscope and place Petri dish containing sample in the center of the microscope staging area
- 5.2. Turn on power switch to desired light intensity
- 5.3. Turn zoom knob to desired magnification of sample
- 5.4. Use both the focusing and fine focusing knobs until sample is in focus
- 5.5. If non-cross polarized light is needed to view sample, turn the cross polarizer on the bottom of the stage

6. COMPOUND SCOPE – Spencer AO

- 6.1. These scopes use a Sedgwick-Rafter counting cell
- 6.2. Remove cover from microscope and place Sedgwick-Rafter cell containing the sample in the center of the microscope staging area between the two clips by pulling them apart
- 6.3. Turn dimmer switch to desired light intensity
- 6.4. Depending on the microscope the dimmer switch may be located on illuminator power box or located on the front of the microscope
- 6.5. Use both the focusing and fine focusing knobs until sample is in focus
- 6.6. If non-cross polarized light is needed to view sample, turn the cross polarizer on the bottom of the stage
- 6.7. Different objectives can be used with these microscopes to zoom in or zoom out of the image
- 6.8. These can be changed by twisting the objective piece to the desired objective
- 6.9. To move the sample, use stage dials located on the right side underneath the stage to move the stage while viewing sample
- 6.10. The easiest way to view is to start in one corner of the Sedgwick-Rafter cell and move horizontally to the next corner, move down and return to the previous side. This may also be done vertically

7. INVERTED SCOPE – AXIO Observer. A1

- 7.1. This scope uses a Sedgwick-Rafter counting cell
- 7.2. Remove cover from microscope and place Sedgwick-Rafter cell containing the sample in the center of the microscope staging area
- 7.3. Open Luminera Program on computer
- 7.4. Turn dimmer switch to desired light intensity
- 7.5. Microscope is equipped with different objectives (10X, 20X, 40X)
- 7.6. Use both the focusing and fine focusing knobs until sample is in focus
- 7.7. If cross polarized light is needed to view sample, turn the cross polarizer filter
- 7.8. These can be changed by twisting the objective piece to the desired objective
- 7.9. To move the sample, use stage dials
- 7.10. The easiest way to view is to start in one corner of the Sedgwick-Rafter cell and move horizontally to the next corner, move down and return to the previous side. This may also be done vertically
- 7.11. Choose the correct objective in the Luminera program
- 7.12. Press capture on Luminera program
- 7.13. Obtain measurements by using the point to point measurement option

8. DIGITAL CAMERA

- 8.1. Log onto the computer
- 8.2. Open Micrometrics LE program from desktop or start menu
 - 8.2.1. Make sure camera is plugged into computer
- 8.3. Turn on research scope (appendix A section 5)
 - 8.3.1. Switch the microscope from Bi to Bi/Photo
- 8.4. In Micrometrics LE program, switch to 'Camera View'
 - 8.4.1. Choose the 'Acquire' tab
- 8.5. Focus microscope with the camera until you capture the organism
 - 8.5.1. Keep the microscope in transmitted light mode, not cross-polarized
- 8.6. Under the 'Camera Control' tab choose automatic focusing
 - 8.6.1. After organism is in focus, press the 'Capture' button to take the picture
- 8.7. Save in Micrometrics LE folder
- 8.8. Transfer picture to H:/ drive for public lab use

Appendix B – Imhoff Cone Directions

1. EQUIPMENT

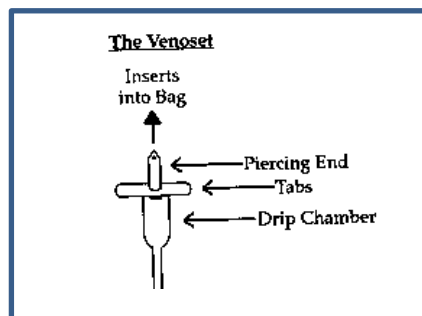
- 1.1. Nalgene Imhoff Cones 1000 mL (Nalgene 1000-0010)
- 1.2. Large animal venoset IV tube with valve and wheel
- 1.3. Inert wide bore medical tubing
- 1.4. Hose clamps
- 1.5. Silicone lubricant
- 1.6. Plumbers tape

2. MODIFY THE VENOSET

- 2.1. With scissors, cut below the plastic piercing end and tabs (Picture 1)
 - 2.1.1. Remove the plastic piercing end and tabs from the very top of the venoset
- 2.2. Widen the end of the drip chamber with a drill (drill bit 5/32")
- 2.3. Add silicone lubricant to the end of the drip chamber
- 2.4. Place the medical tubing, of the desired length, on the end of the drip chamber with the silicone lubricant
- 2.5. Place the valve and wheel on the venoset tubing and cut to desired length

3. MODIFIED VENOSET TO IMHOFF CONE

- 3.1. Wrap plumbers tape around the bottom of the Imhoff cone
- 3.2. Push the modified venoset onto the Imhoff cone as far as it will go
- 3.3. Fix with a hose clamp
- 3.4. If leaking occurs, apply more plumbers tape



Picture 1: Modified venoset for Imhoff cone
(Clock Tower Animal Hospital 2013)

Appendix C - Five Percent Acetic Acid Preparation

1. PREPARATION PROCEDURE

- 1.1. Obtain the pre-labeled five percent acetic acid, 4-L container from underneath the fume hood
- 1.2. Use a 1000-mL graduated cylinder to pour 3800 mL of DI water into the 4-L sealable container
- 1.3. In the fume hood
 - 1.3.1. Add 200 mL of glacial acetic acid to the 4-L container
 - 1.3.2. Replace lid and mix the contents in the 4-L container
- 1.4. Fill up pre-labeled five percent acetic acid squirt bottles as needed
- 1.5. Store the 4-L container under the fume hood

2. DISPOSAL OF CHEMICALS

- 2.1. Glacial acetic acid container
 - 2.1.1. Rinse 3–5 times with DI water
 - 2.1.2. With a sharpie write ‘Empty’ across the label in large visible lettering
 - 2.1.3. Place in “Recycled Glass” container located in the lab

3. SPILLS

- 3.1. Glacial acetic acid spill (section 5.1: Acetic acid, glacial)
 - 3.1.1. Remove sources of ignition
 - 3.1.2. Soak up spill with inert absorbent material
- 3.2. Five percent acetic acid spill
 - 3.2.1 Clean with paper towel

Appendix D - Cooler Login Notebook

Cooler Login

Return

Sender	Date Received	# of Samples Received	Chain of Custody (Y/N)	Initials	Date Shipped	Shipped To	Initials
Example	12/6/10	9	Y	AB	12/10/10	Parker Dam	YZ

Appendix E - Master Login File

Location:

H:/EnvRes Share/MUSSEL SAMPLES/DATA and LOGIN/Master Login File Date

Password:

Always reference previous samples from the same water body to ensure consistent and accurate data entry and copy the information into the appropriate cells

Lab SampleID	WOID	Water Facilities Owner	USBR AreaOffice	State	WaterName
Example	ZQAR1	USBR	PRAO	UT	Reservoir

SampleLocation	SampleDate (mm/dd/yyyy)	Sampler / Client	TowType (V or H)	Num of Tows
Marina	Date	USBR	Vertical or Horizontal	5

Tow Length (meters)	DateReceived (mm/dd/yyyy)	AnalysisType	LogIn Name/Initials	LabProjectMger
X meters	Date	Microscopy, PCR	AB	Hosler

SampleCmmt (LogIn Condition)	Microscopy: DateAnalyzed	PCR: DateAnalyzed	SEM: DateAnalyzed	FlowCam: DateAnalyzed
Cold, pH 7.7	Date	Date	Date	Date

Appendix F - Sample Prep Notebook

Sample ID	Location	Sample Date	Prep Date	Volume (mL)	Color	Clarity	Debris (org, inorg, algae)
Example	Shadow Mt -034	12/6/10	12/6/10	325	Clear w/ yellow	Cloudy	Low, Med, High (See below)

Low debris: To middle of venoset

Medium debris: To top of venoset

High debris: Anything above venoset

Appendix G - Technician Sample Notebook

Take tape from sample beaker and place in notebook. Save notebook till end of season. This is a double check to ensure that all samples are recorded into the Enumeration Notebook

Sample ID Location of Sample Sample Date	Count	Comments
Sample ID Location of Sample Sample Date		

Appendix H - Enumeration Notebook

Sample ID	Location	Sample Date	Count Date	Count	Comments	Initials
Example	Mtn. View	12/6/10	12/7/10	0	Low ostracods (See below)	AB

Low organisms: Less than 10

Medium organisms: Between 10 and 30

High organisms: Over 30

Appendix I - Master Enumeration File

Location:

H:/EnvRes Share/ MUSSEL SAMPLES/ DATA and LOGIN/ Veliger Enumeration Date

Password:

Always reference previous samples from the same water body to ensure consistent and accurate data entry and copy the information into the appropriate cells

Lab SampleID	FacilitiesOwner	USBR AreaOffice	State	WaterName
Example	USBR	PRAO	UT	Reservoir

SampleLocation	SampleDate (mm/dd/yyyy)	SettleStart (mm/dd/yyyy)	SettleEnd (mm/dd/yyyy)	DateAnalyzed (mm/dd/yyyy)
Marina	Date	Date	18-24 hours later	Date

Analyst Name / Initials	Count	Micro Comments	PCR Result
XY	Total 15 mls	*Low Ostracods (See below)	Filled out by PRC analyst

Low organisms: Less than 10

Medium organisms: Between 10 and 30

High organisms: Over 30

Appendix J - SEM Log File

Location:

H:/EnvRes Share/ MUSSEL SAMPLES/ DATA and LOGIN/ SEM Log Date

Password:

Always reference previous samples from the same water body to ensure consistent and accurate data entry and copy the information into the appropriate cells

Lab SampleID	FacilitiesOwner	USBR AreaOffice	State	WaterName	SampleLocation
Example	USBR	PRAO	UT	Reservoir	Marina

LocDescription	CDOW UniqueID	SampleDate (mm/dd/yyyy)	SEM: DateAnalyzed (mm/dd/yyyy)	Analyst Name/Initials
		Date	Date	Initials

Results	SEM Cmnts	Photo Location	Report	Report Figure
Leave for SEM analyst	Leave for SEM analyst	Leave for SEM analyst	Leave for SEM analyst	Leave for SEM analyst

Appendix K - Purge Notebook

Sample ID	Location	Sample Date	Analysis Type
S1	Reservoir/ Site location	Mm/dd/yyyy	Microscopy / PCR

Appendix L - Composite Label

<p>Sample ID Composite- Year</p> <p>ID Numbers in Composite: Sample 1, S2, S3, S4, S5, S6, S7</p> <p>70% EtOH</p> <p>pH: 7.0</p>
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