BUREAU OF RECLAMATION

Standard Operating Procedure: Preparation and Analysis of Water Samples for Dreissenid Mussel Veliger Detection: Microscopy

Laboratory Standard Operating Procedure (SOP)
Version 6 (Date Revised: 2020)
Document No. EcoLab-FA981-2020-04

Bureau of Reclamation
Ecological Research Laboratory

The Ecological Research Laboratory

Mission Statements

The Department of the Interior (DOI) conserves and manages the Nation's natural resources and cultural heritage for the benefit and enjoyment of the American people, provides scientific and other information about natural resources and natural hazards to address societal challenges and create opportunities for the American people, and honors the Nation's trust responsibilities or special commitments to American Indians, Alaska Natives, and affiliated island communities to help them prosper.

The mission of the Bureau of Reclamation is to manage, develop, and protect water and related resources in an environmentally and economically sound manner in the interest of the American public.

Peer Review

Bureau of Reclamation
Technical Service Center

Final Report EcoLab-FA981-2020-04

Standard Operating Procedure: Preparation and Analysis of Water Samples for Dreissenid Mussel Veliger Detection: Microscopy

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Prepared by:

Bureau of Reclamation
Technical Service Center
Hydraulic Investigations and Laboratory Services
Ecological Research Laboratory (86-68560)

Previous Versions:

Lab SOP Version 1	6/2007
Lab SOP Version 2	6/2010
Lab SOP Version 3	2/2011
Lab SOP Version 4	7/2013
Lab SOP Version 5	5/2019

Table of Contents

1. ACRONYMS / DEFINITIONS

COC: Chain of Custody
DI water: Deionized Water
Dreissenid: Genus of freshwater mussel
SDS: Safety Data Sheet
PCR: Polymerase Chain Reaction; technique used to amplify DNA
Quagga mussel: *Dreissena rostriformis bugensis*, invasive mussel species
Eco-Lab: Ecological Research Laboratory (Reclamation Technical Service Center)
Reclamation: Bureau of Reclamation
Settling cone: modified Imhoff cone used to measure the volume of solids in water
SOP: Standard Operating Procedure
Veliger: larval zebra/quagga mussel
Venoset: plastic tubing with roller wheel system to control flow out of settling cone
Zebra mussel: *Dreissena polymorpha*, invasive mussel species

2. SCOPE AND APPLICABILITY

This Standard Operating Procedure (SOP) is used to establish a uniform format for duties performed by laboratory staff. This method is applicable to dreissenid mussel early detection water samples that are prepared and analyzed by laboratory staff 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. INTERFERENCES

The main interference in early mussel detection is cross-contamination from other samples. To mitigate this interference, glassware is decontaminated overnight in 5% acetic acid. A new disposable pipette and decontaminated petri dish is used for every sample. Separate pipette bulbs are used for positive and negative samples. Settling cones are decontaminated overnight with 5% acetic acid, scrubbed with a dedicated cone brush, and thoroughly rinsed with DI water.

4. HAZARDS

A variety of chemicals are used in the preparation, analysis, and disposal of water samples collected for the early detection of dreissenid mussels. While most of these chemicals may cause minimal injury or irritation, lab personnel should refer to the Laboratory Job Hazard Analysis (JHA) (Appendix A) and Safety Data Sheets (SDS) for detailed information regarding hazards, handling, storage, and disposal of specific chemicals. Prior to beginning work, all lab personnel will be familiar with the different chemicals and will be prepared to deal with spills and exposures appropriately. Current SDS are in the microscopy lab documentation binder, located in the lab next to the printer.

5. MATERIALS

5.1. Glassware

Graduated test tubes, 15 mL (Glass: Pyrex, 8101-15; Plastic test tube with screw on lid: Falcon, 352097)
Petri dishes (Pyrex, 3160-60)
Transfer pipettes (Fisherbrand, 13-678-20B)
Watch glasses, 12-cm diameter

5.2. Microscopes

Compound: Spencer AO, 40X – 100X magnification
Inverted: Zeiss Axio Observer A1, 100X – 400X magnification
Research: Olympus SZH10, 10.5X – 210X magnification
Micrometrics 318CU digital camera mounted on research scope
Screening: Meiji EMZ-5D, 7X-45X magnification
Screening: Meiji EMZ-8TR, 7X-45X magnification

5.3. Miscellaneous Items

2-mL Eppendorf tube (USA Scientific, 1615-5510)
Cone brush for washing settling cones (Fisher, 03-562)
Cooler login binder
Designated wash bottles for DI water (Fisher, 03-409-22C)
Fisherbrand labeling tape, rainbow pack (Fisher, 15-901-R)
Kimwipes (Fisher,06-666A)
Labels (Label Value: LV-30330R, LV-3033POLY)
Lens paper (Fisherbrand, 11-996)
Measuring spoons for baking soda (0.1 gram) (Scienceware 367210010)
Micro pipette (Fisherbrand Finnpipette, 20-200 uL)
Micro pipette tips (Fisherbrand SureOne, 02707422)
Modified Imhoff settling cones (Fisher, 15-438) (Appendix B)
Parafilm (PM-999)
pH strips (MColorpHast, 1.08533.0001)
Rubber bulbs (Fisher, 03-448-26)
Safety gloves, nitrile (small, medium, and large) (VersaPro)
Specimen counters (Counting Devices, Inc)
Technician notebooks
Venoset (Jorgenson Laboratories, Inc J400X)
1-gallon plastic jugs for 5% acetic acid solutions

6. SAMPLE RECEIVING / RETURN

All coolers should be opened as soon as possible after receipt and the following tasks completed. Care should be taken that samples are not intermixed when opening multiple coolers at the same time. Chain of Custody (COC) or data sheet forms should remain with samples until login is complete.

- Open cooler and remove all tape, stickers, and labels related to shipping. **Do not discard shipping label until cooler is prepared for return.**

- Remove all contents of cooler, including packing materials, to ensure nothing is overlooked.
- Enter shipping information into the Cooler Login binder. ALL samples, including those collected by Eco-Lab staff, should be recorded in the Login binder.
- On a self-stick note write date received, where the samples are from, and number of samples received. Attach this **with tape** to one of the sample bottles.
- Store samples and applicable documentation on designated shelf in refrigerator, with self-stick note in front until they're logged in and barcoded.
- Drain and dry cooler, then replace all packing materials, including ice packs, bubble wrap, newspaper, Ziplock bags, unused diapers, etc. Empty cardboard boxes are not returned – only coolers and cooler boxes.
- Prepare FedEx request with return shipping information on the COC. Return the cooler via ground 4-5-day shipping. Tape cooler securely closed and attach FedEx request to the lid.
- Enter return information into Cooler Login binder.
- Coolers can be set aside for the mailroom to collect. If not collected within 2 days, take coolers to the mailroom in building 67.

Coolers should be returned as soon as possible after opening, to reduce confusion related to return shipping and to avoid congestion in the lab. If there is an issue with the cooler, inform the laboratory manager so that the owner of the cooler can be contacted, and the issue can be addressed.

7. SAMPLE LOGIN PROCEDURE

All samples received in the lab will be logged into the Mussels Database and assigned a unique identifying number prior to being set up. This number will be tied into a barcode and printed on a label that will remain with the sample throughout the analysis process. The sample number will also be written on the top of the bottle.

Samples will be logged in following the most current procedures (Appendix C). Samples should be entered in the order received when possible. At least two sets of labels will be printed for each batch: one set on permanent labels which will be affixed to the original sample bottle; one set on removable labels which will be affixed to the corresponding test tube during set up.

Samples from priority water bodies will need an additional removable label set printed and will be identified with red tape on the lid. These labels will be affixed to a sterile 50 mL conical collection tube for eDNA analysis. As samples from priority water bodies are logged in, shake thoroughly and pour 40 mL from the sample bottles into a labeled 50 mL conical tube. The 50 mL tubes should then be placed on the PCR shelf in the refrigerator for analysis.

Field blanks (DI water used to rinse the net and cod-end in the field) should be logged into the database. The "Analysis Type" for these should be *PCR Only*. A permanent label should be printed and affixed to the original sample bottle. These samples are then placed on the Field Blank shelf in the refrigerator. For field blanks from priority water bodies, or where eDNA testing is requested, a subsample will be taken. A second permanent label set should be printed, affixed to a 50-ml conical tube, and 40-mL of the sample poured into the conical tube. The field blank subsample should be placed on the PCR shelf in the refrigerator for analysis and the sample bottle placed on the field blank shelf. Field blanks will not be analyzed by microscopy.

7.1. Table for Types of Labels to Print

Type of Sample	Required Number of Labels to Print	
	Permanent	Removable
Field Blank (Non-Priority waterbody)	1 (for bottle)	0
Field Blank (Priority waterbody)	2 (bottle & 50-mL tube)	0
Priority waterbody sample	2 (bottle & 50-mL tube)	1 (for test tube)
Non-priority waterbody sample	1 (for bottle)	1 (for test tube)

8. SAMPLE PREPARATION PROCEDURE

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

8.1. Sample Setup

- Arrange Imhoff cones on sturdy countertop with enough room to ensure samples do not fall off or get knocked over (Figure 1A). Samples from waterbodies with known mussel populations will be set up in a separate area using dedicated cones.
- Turn cones so that the graduation lines face front.
- Apply roller clamp and back up clamp to each cone (Figure 1B). Ensure roller clamps are completely locked to prevent leaks.
- Samples should be set up in numerical order unless priority processing has been requested. Samples with known veliger presence may be set up out of order as needed.
- Place sample bottles under the cone in which they will be settled, ensuring that they are in the correct numerical order. ALWAYS double-check that samples are in the correct order!
- Place watch glass on top of each cone to prevent cross-contamination by splashing.
- Shake sample bottle to homogenize the sample, remove watch glass, and carefully pour sample into designated cone.
- Rinse sample bottle with DI water, using care so the tip of the wash bottle does not come into contact with the bottle, and pour contents into cone. Place sample bottle under cone.
- Replace watch glass on top of cone.
- Log into database and follow setup procedures (Appendix C, Section 3) for each sample.
- Settle samples overnight.
- Repeat these steps for all samples in the group.

Figure 1: A. Cones are set up with samples. B. Close-up image of the closed roller clamp and the secondary clamp in place on the tubing.

8.2. Sample Takedown

- 15 mL of each sample will be collected into a plastic test tube with a screw top.
- Carefully drain the bottom (heaviest) 15 mL of each sample into a test tube and screw the top on securely.
  - If venoset is plugged with debris, manipulate the roller back and forth to try to dislodge sediment/debris. If needed, use a metal rod (available in lab) to push sediment/debris through the venoset. **Do not lift cones and allow to drop back onto rack** – this will not dislodge debris, and there are numerous risks for injury, sample loss, and sample contamination.
  - Decontaminate metal rod in 5% acetic acid after each use and rinse with DI water. **Do not use metal rods in more than one sample without decontaminating in between.**
- Locate the label that corresponds to the sample just collected and affix to the test tube. DO NOT apply the label prior to collecting the sample – the amount of ethanol in each sample will erase the information on the label if it spills.
- Drain remaining volume of settling cone into the original sample bottle and rinse cone with DI water. Any excess that does not fit in the original sample bottle can be collected in a separate container and discarded down the sink.
- Place sample bottle into refrigerator in numerical order. Test tubes will be stored in numerical order in a Styrofoam holder.
- **ALWAYS CONFIRM THAT SAMPLES ARE BEING STORED IN THE CORRECT ORDER!!**

8.3. Cone Decontamination

- Re-apply secondary clamp to the bottom of the collection tube. Move venoset roller just above secondary clamp and close completely.
- Pour at least 300 mL of 5% acetic acid (Appendix D) into cones to decontaminate and cover each cone with a watch glass.
- After 24 hours, scrub each cone with designated cone brush before draining acetic acid and rinse thoroughly inside and out with DI water, ensuring that ample amounts of acetic acid and water run through the venoset tubing. Safety gear should be worn during the cleaning process to include nitrile gloves, safety glasses, and a chemical resistant smock.

- Check the cone for any debris that might have gotten stuck in the tubing and perform additional washing if there is any debris.
- Set cones aside to air dry.

9. SAMPLE ANALYSIS

- Remove original sample bottle and associated test tube containing the settled 15-mL sample from refrigerator. Ensure the sample ID on bottle's label matches sample ID on the test tube. Do not use the handwritten number on the lid to confirm matching ID.
- Remove label from test tube and place in technician notebook.
- Use a new glass pipette and a clean glass Petri dish for every sample.
- Use the appropriate rubber bulb for the sample being analyzed. Bulbs marked with an "X" on the top should only be used for known positive samples. Bulbs with no "X" should only be used for non-positive samples.
- If needed, draw a grid on the bottom of the Petri dish with a sharpie.
- Invert test tube a few times to mix contents.
- Pipette a small amount of sample from test tube into the Petri dish, diluting with DI water if necessary.
- Analyze contents of the Petri dish thoroughly, using a cross-polarized filter on a stereo/ dissecting microscope. Reference the identification book (prepared by the Eco-Lab) to differentiate organisms.
- Pour contents of Petri dish into original sample bottle and rinse with DI water. Repeat these steps until all 15 mL from test tube has been analyzed. Then, rinse the pipet inside and out and analyze the contents.
- When analysis is complete, record findings in technician notebook.
- Place pipette into glass recycling container and other glassware into the dishpan for decontamination.
- Technician performing analysis should write their initials on the lid of the sample bottle and place in the appropriate place in the storage refrigerator.
    - Priority samples – those marked with red tape – should be placed on PCR shelf.
    - Non-priority samples should be placed on non-priority shelf.
- All efforts should be made to complete analysis of a sample in one sitting. In the event this is not possible, technician should use a sharpie to write the sample number, date started, and their initials on the test tube. Reseal test tube with screw top lid and place with sample bottle on shelf in refrigerator. The same technician should complete analysis on the next business day.
- Soak glassware overnight in 5% acetic acid, then use scrub pad and bottle brush to thoroughly clean, ensuring all sharpie markings are removed. Rinse thoroughly with DI water and allow to air dry.
- At the end of the day, clean work surfaces and microscope with bleach disinfecting wipes. Clean lenses of microscope with Kimwipes. Right after the analysis of a positive sample, clean work surfaces and microscope with disinfecting wipes, and wash hands.

9.1. SUSPECT ORGANISMS AND POSITIVE WATERBODIES

- If a suspect organism is found, request assistance from other lab personnel in confirming identification.
- Remove suspect organism(s) from primary sample with a micro pipette, using a new micro pipette tip for every sample. Place each suspect into a clean Petri dish, preserve with

alcohol, and examine under higher magnification microscope, taking digital photographs for further analysis.
- Inform the lab manager of the finding so the reporting process can begin.
- Once pictures of suspect organism(s) have been taken, pipet the organism into an Eppendorf tube with minimum liquid. Label the top of the tube with the sample number. Place the suspect in the rack on the PCR shelf. Inform the molecular biologist that the sample needs to be analyzed as soon as possible.
- If counting veligers in a known positive sample, use a counter to keep an accurate tally. Subsampling may be used for samples with more than 2000 veligers (Appendix E). Record final veliger count in technician notebook.

10. SAMPLE SPILLS

- When sample spills occur, salvage whatever remains in the test tube to complete analysis. To prevent cross-contamination DO NOT return spilled sample material to tube.
- Clean work surface with paper towels, then disinfecting wipes.
- If sample was from a known positive waterbody, first decontaminate work surface by soaking for at least 10 minutes with 5% acetic acid. Then wipe away the acid, rinse the area with DI water, and disinfect with bleach wipes.
- Note spill in technician notebook next to appropriate label.
- Analyze remaining sample.

11. TECHNICIAN NOTEBOOK

- Remove label from test tube of sample being analyzed and place in notebook.
- Complete analysis of entire 15 mL of each test tube.
  - Make note of organisms, other than dreissenid mussels, that are present (snails, glochidia, ostracods, seeds, etc.), estimating the number of each in the sample.
    - Less than 10 = Low
    - 10 – 30 = Medium
    - More than 30 = High
  - Record the counted number of suspect/un-confirmed dreissenid mussels – do not estimate.
  - For known positive samples record the number of counted dreissenid veligers, or the subsample total - do not estimate.
- When analysis is complete, technician should initial and date next to the appropriate label in the notebook.
- At the end of the workday, technician should log results into the database (Appendix C) and place a check mark on the label to indicate this has been done.

12. DISPOSAL OF SAMPLES

- Samples that are non-priority may be purged 1 month after analysis. Priority samples (those marked with red tape) and field blanks will be purged at the discretion of the molecular biologist.
- Sample purging procedure:
  - Pour sample into the sink
  - Rinse sample bottle thoroughly
  - Recycle empty sample bottles

- It is not necessary to remove labels, tape, sharpie markings, etc.
- Keep track of which samples have been purged and the date
- Make a note in the database if field blanks were not used for analysis
- Enter the purged samples into the database

13. CONTACT INFORMATION

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Appendix A – Laboratory Job Hazard Analysis (JHA)

ECOLOGICAL RESEARCH LABORATORY
JOB HAZARD ANALYSIS (JHA)

1. **Project Title:** Invasive Dreissenid Mussel Early Detection and Monitoring Program
2. **Purpose:** To prepare and conduct analysis of water samples for the presence of dreissenid mussels within the 17 western states.
3. **Start Date:** May 2019
4. **End Date:** May 2021
5. **Personnel Requirements:** Work will be performed by Bureau of Reclamation (Reclamation) employees and seasonal interns assigned to the lab.
6. **Hazards**

- **Minor Injuries:** Cuts, scrapes, bruises. A first-aid kit is maintained in the lab for small cuts, scrapes, and other minor injuries that might occur.
- **Serious Injuries:** Serious injuries are those that cannot be treated in the lab with a first-aid kit. Contact local authorities and/or medical personnel immediately for serious injuries.
- **Heavy Lifting:** Items such as field equipment, outreach items, racks of settling cones containing 5% acetic acid, and client coolers can be quite heavy. Use caution when lifting heavy items. Two people should lift items weighing more than 50lbs.
- **Ergonomics:** Prolonged periods of sitting. Prolonged periods of work on microscopes. Personnel should plan to take several short breaks throughout the day to rest eyes and stretch.
- **Chemicals:** Glacial acetic acid, which is used to make dilutions, is highly corrosive and flammable and every precaution should be taken when handling this chemical. Most other chemicals used in the lab are relatively harmless and pose little risk of illness or injury. However, all personnel should review the SDS for the specific chemicals used in the lab and understand the risks posed by those chemicals. All chemicals, regardless of associated risk, should be handled, used, stored, and disposed of in accordance with the manufacturer recommendations.
    - **100% Acetic Acid, Glacial** (Fisher, A38C-212)
      Handling: Never add water to this product; when diluting, always add acid to water. Always prepare in a fume hood. Keep away from heat. Keep away from sources of ignition. Do not ingest. Do not breathe gas/fumes/vapor/spray. 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. **Storage:** Store separate from other chemicals and in an approved area, under a fume hood or in a fire or corrosives cabinet. 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). Room temperatures below 63° F could cause acid to freeze. If a glass bottle of acetic acid freezes, wearing protective gloves, gently place bottle inside a plastic bucket and place in fume hood to allow to thaw. Freezing and thawing does not affect product quality.
Personal Protective Equipment: When handling, wear splash goggles, synthetic apron, and rubber or latex gloves. Ensure that eyewash stations and safety showers are proximal to the workstation.
Spills/Leaks: Absorb spill with inert material (e.g. vermiculite, sand or earth), then place in suitable container. Wash area with water. Remove all sources of ignition. Provide ventilation. Use water spray to cool and disperse vapors, protect personnel, and dilute spills to form nonflammable mixtures. Control runoff and isolate discharged material for proper disposal. Spill may be carefully neutralized with soda ash (sodium carbonate). Due to its high flammability and fumes, if a full bottle of glacial acetic acid breaks and spills, do not attempt to clean. Evacuate the laboratory and pull the fire alarm to evacuate the building. Call 911 and notify them of the amount of spill (2.5 liters) and the location (Denver Federal Center, building 56, room 1330).

- **Acetic Acid, 5% (Distilled White Vinegar)**
  Handling: Use with adequate ventilation. Do not get in eyes, on skin, or on clothing. Do not ingest or inhale. May cause eye and skin irritation. May be harmful if absorbed through the skin. Harmful if swallowed, causes gastrointestinal tract irritation. May cause respiratory tract irritation if inhaled.
  Storage: Store in a cool location. Provide ventilation for containers. Avoid storage near extreme heat, ignition sources or open flame. Store away from oxidizing agents. Store in cool, dry conditions in well-sealed containers. Keep containers tightly sealed. Protect from freezing.
  Personal Protective Equipment: Splash goggles, synthetic apron, vapor and rubber or latex gloves. Ensure that eyewash stations and safety showers are proximal to the workstation.
  Spills: Collect liquids using vacuum or by use of absorbents. Place into properly labeled containers for disposal. If necessary, use trained response staff/contractor. Remove from all sources of ignition. Soak with inert material. Use spark-proof tools and explosion-proof equipment.

- **Bleach Disinfecting Wipes**
  Handling: Avoid contact with eyes, skin, and clothing. Do not eat or drink when using this product. Exposure to vapor or mist may irritate respiratory tract. Liquid may cause irritation to eyes and skin. Ingestion of liquid may cause slight irritation to mucous membranes and gastrointestinal tract. Ensure adequate ventilation. Ensure that eyewash stations and safety showers are proximal to the workstation. Wash hands after use.
  Storage: Keep containers tightly closed in a dry, cool, and well-ventilated place.
  Personal Protective Equipment: No special protective equipment is required under normal use conditions.
  Spills: Liquid may be absorbed with paper towels and discarded in the trash.

- **Sodium Bicarbonate** (Baking Soda)
  Handling: Minimize dust generation and accumulation. Do not get in eyes, on skin, or on clothing. Do not ingest or inhale. May cause eye and skin irritation. May be harmful if absorbed through the skin. Causes gastrointestinal tract irritation if swallowed. May cause respiratory tract irritation if inhaled. Ensure adequate ventilation. Ensure that eyewash stations and safety showers are proximal to the workstation.
  Storage: Keep containers tightly closed in a cool, dry place.
  Personal Protective Equipment: Wear appropriate protective eyewear, gloves, and clothing to prevent exposure.
  Spills/Leaks: Vacuum or sweep up material and place into a suitable disposal container. Avoid generating dusty conditions. Provide ventilation. Do not let this chemical enter the environment.

- **Tris-HCl (4M, pH 7.5)** (Teknova 5575)
  Handling: Wash hands after use. Do not eat or drink in work areas.
  Storage: Keep away from food, drink, drains, surface and ground water. Use in well-ventilated areas.
  Personal Protective Equipment: No special protective equipment is required under normal use conditions.
  Spills/Leaks: Wipe up with absorbent material (e.g. cloth, paper towel).

- **Alcohol (Isopropyl or Ethyl)**
  Handling: Avoid contact with eyes, skin, and clothing. Empty containers retain product residue (liquid and/or vapor) and can be dangerous. They should be left with the lid off to off-gas in the fume hood for at least 24 hours before being placed in mixed recycling container. Take precautionary measures against static discharges. Avoid breathing vapor or mist. Ensure adequate ventilation. Wash hands thoroughly after handling. Ensure that eyewash stations and safety showers are proximal to the workstation.
  Storage: 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.
  Personal Protective Equipment: Wear appropriate protective eyewear, gloves, and clothing to prevent exposure.
  Spills/Leaks: If spilled on clothing, remove clothing and wash before reuse. Absorb spill with inert material (e.g. vermiculite, sand or earth), then place in suitable container. Use water spray to dilute spill to a non-flammable mixture. Clean up spills immediately, observing precautions in the Personal Protective Equipment section (Section 7). Remove all sources of ignition. Provide ventilation.

- **Glassware**
  Clean, unbroken glass can be placed in the mixed recycle container. In the case of broken glassware, use a dustpan and broom or a paper towel to sweep up the pieces. Discard in the container marked “Glass Disposal,” located by the main door to the lab. Do not handle broken glass by hand if it can be avoided. Glass pipettes are rinsed and disposed of in the “Glass Disposal” container after each sample. Contact Lise Pedersen (lpederson@usbr.gov) to empty Glass Disposal container.

7. **Personal Protective Equipment (PPE):** Personnel are responsible for maintaining their own PPE to ensure adequate protection. Safety glasses, gloves and lab coats are provided by the lab.
   - **Footwear:** All footwear should be closed toe and have nonskid soles. Due to the high volume of water used in the lab and the associated risk of slipping, high-heeled shoes are not permitted in the lab.

   - **Clothing:** Flowing or loose long-sleeved shirts are not permitted in the lab due to the high volume of chemicals and glassware used, as well as the risk of sample cross-contamination. When working with acetic acid, both glacial and 5%, nitrile gloves and safety glasses will be worn. When washing dishes, a lab coat or apron will be worn to protect clothing and skin. Lab coats will not be worn at any time other than washing dishes to minimize the risk of sample cross-contamination.

   - **Rubber or Nitrile Gloves:** Disposable gloves should be worn when unpacking coolers and while handling chemicals and cleaning glassware and cones.

8. **Training Requirements**
   Training on safety, sample handling, and lab hygiene will be conducted by experienced personnel.

9. **Security Requirements**
   Due to the sensitive nature of the work being conducted in the lab all personnel, including Reclamation employees and seasonal interns, will have a DOI Access Card prior to beginning work. All visitors/tours will be escorted by Eco-Lab employee.

10. **Emergency Contact Numbers**
    Personnel should be aware of local emergency contact numbers such as law enforcement, fire/medical response, and Poison Control.

    Emergency number: 911 or the GSA Denver MegaCenter: 877-437-7411.

    Non-emergency number: Bob Einhellig, 303-579-3536, Diane Mench, 303-445-2050

    Emergency room services: St. Anthony's Hospital, 11600 W 2nd Pl, Lakewood, CO 80228
    (720) 321-0000

11. **Acknowledgement Signatures**

All personnel assigned to the lab will read and acknowledge the above information prior to beginning work.

I have been briefed on the details of this JHA, and what my role and responsibilities will be during the project. My signature below indicates that I have read and understand the requirements.

Signature_____ Date_____

Appendix B - How to Make a Modified Imhoff Cone

1. Materials

- Imhoff Cone
- Large animal venoset
- 3/16" 1D flexible tubing
- Plumber's tape
- Clamp

2. Tools

- Drill with 3/16" drill bit
- Pliers
- Flathead screwdriver
- Silicone gel lubricant
- Scissors
- Razor blade
- Ruler

3. Instructions

1) Pull out the small tube at the opening of the venoset using pliers (Figure 1).
2) Enlarge the opening of the venoset using a 3/16" drill bit and remove any plastic pieces still inside (Figure 1).
3) Apply silicone gel to the outside of the venoset opening, coating the entire bottom portion to the shoulder (Figure 1).
4) Attach the tubing by sliding over the outside of the venoset. Push the tubing as far as it will go; with some pressure it should slide all the way up (Figure 2).
5) Cut off the hard-plastic parts of the venoset using razor blade (Figure 1).
6) Trim the tubing so it is long but not touching the stand when attached to the cone (Figure 1).
7) Wrap the collar of the cone with a liberal amount of plumber's tape and check for leaks using water (Figure 2).
8) Slide a clamp onto venoset and over the collar of the cone. The collar of the cone should sit on the line on the venoset with the clamp sitting just above the collar (Figures 1 and 3).
9) Tighten clamp firmly using screwdriver. It should not wiggle or slide in any direction.
10) Test for leakage using water. Apply more tape and tighten clamp if there are any leaks and test again.
11) Place the fully assembled cone in the stand (Figure 4).

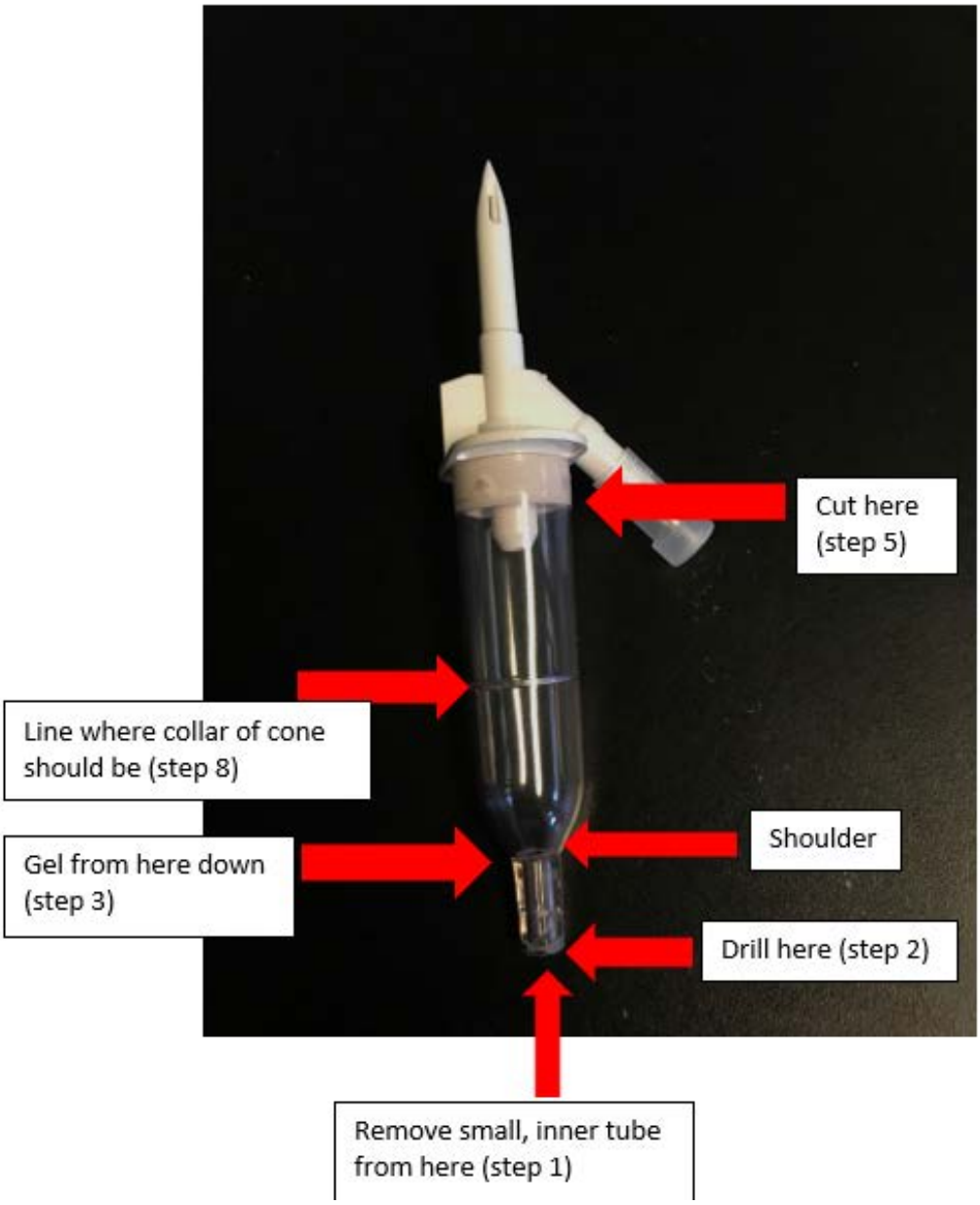


Figure 1: Venoset displaying modifications that need to be made

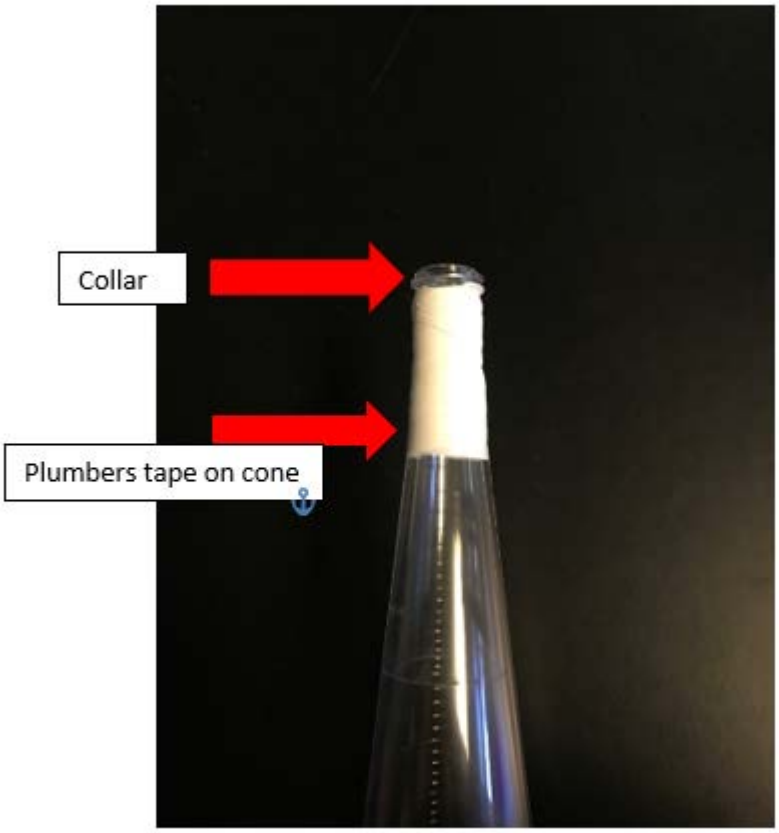
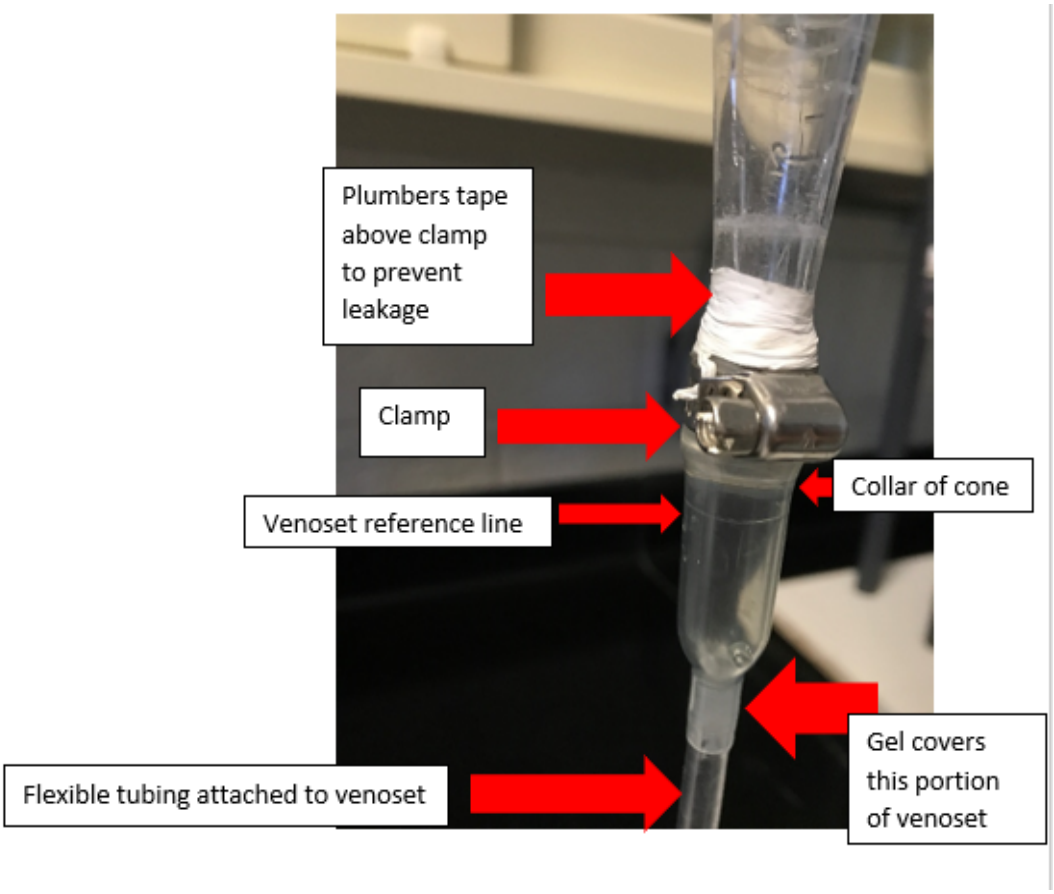


Figure 2: Plumbers tape added to base of cone (step 7)

Figure 3: Assembled bottom portion of cone



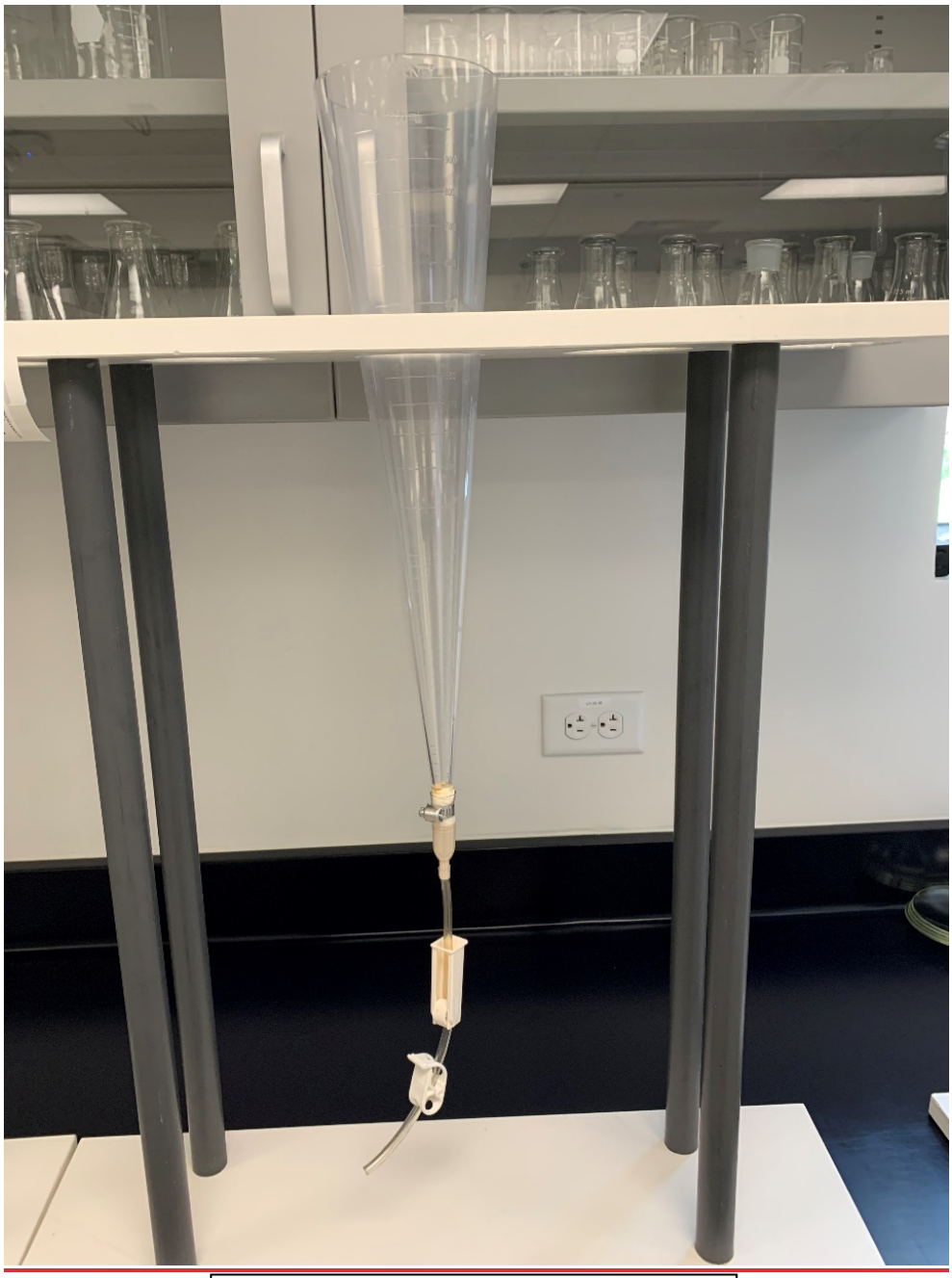


Figure 4: Fully assembled cone on stand

Appendix C – Mussel Database and Label Instructions

1. **Checking pH**
   - As samples arrive, check their pH with pH strips. If pH is less than 6.5, add either baking soda or Tris (depending on what was used when samples were collected), to raise the pH to 6.5 or higher. Record initial pH when logging samples into database and add note if pH/Tris was used to raise pH.

2. **Accessing the Database**
   - Enter URL into Google Chrome browser
   - When given a security warning screen, click "details" and "go onto webpage," then enter login information (Windows name & password) when prompted. If the username/password form reappears, it is likely because the individual does not have access to database yet.

3. **Sample Login**
   - Samples can be uploaded one at a time by selecting "Quick Add: Sample" on the main page of the database, or in batches (no more than 12 in a batch) by selecting "Sample" from the Submission Type drop-down menu on the Batch Upload portion of the site and uploading a CSV file formatted with the required fields as noted on the website.
     - All necessary files can be found at: Z:\DO\TSC\Programs\Ecological Research Laboratory\DATA\MUSSEL SAMPLES\DATA & LOGIN
       - Excel template for Batch Upload (for Samples)
       - Waterbody and Site ID numbers
       - List of WOID's by site
     - The Excel template must be saved as CSV file prior to being uploaded.
     - If upload is successful, a green bar will appear at the top of the screen with the assigned samples numbers, in order of the lines in the CSV file.
     - If an error message is received, review data on CSV file to ensure formats, waterbody, and site ID's are correct, and necessary data is present in correct formats in all required columns
   - For single sample entry: On the database main page, select "Quick Add: Sample." Select waterbody and location, being sure to use the ID name in the database. If the name on the bottle does not appear, check the list of alternate names (i.e. "Lake Elwell" for Tiber Reservoir).
     - If it has been verified with a Laboratory Technician or Manager that the waterbody does not have an alternate name and is indeed a new reservoir, scroll to the bottom of the "Quick Add: Sample" field and select "Create Waterbody."
     - For "Sampler Location," use the location given on the bottle. Often this is the same as "Location." (i.e. If "Dam" is written on the bottle, but *Folsom Dam* is the option for "Location," use "Dam" here). If the location is not listed select *Unknown.* If the location is not listed scroll to the bottom of the "Quick Add: Sample" field and select "Create Location". Verify with a Laboratory Technician or Laboratory Manager prior to doing this.
   - Format all dates as YYYY-MM-DD.
   - Sampler should be entered as: Agency Last Name. (i.e. USBR Keele)
   - Fill out as much information on the location (use decimal degrees format for latitude and longitude), tow, and net as is given, either on the Chain of Custody form or on the bottle.

- Record data from database onto Chain of Custody form (date received, WOID, assigned sample numbers)
- "Analysis type" should autofill based on the reservoir. However, make sure to verify status of *Microscopy & PCR* analysis with priority samples list posted in the lab.
- pH recorded in the database should be the initial value rather than after adjustment is made using Tris or baking soda, depending on what was used initially to buffer the sample.
- After submitting, a green bar should appear at the top of the screen confirming that login information was successfully processed.
- To view a list of all recent samples added, type a recent sample number in the Edit Sample field at the bottom right of the page, select "Go". Once that sample information pulls up, scroll to the bottom and click "List" to see a list of recent samples. Do this after adding each batch of samples to ensure information is accurate.
- Refer to "cheat sheet" in lab for logging in samples.
- Write sample number of top of bottle with Sharpie.

4. <u>Making Labels</u>

- Once all samples in a batch have been logged into the database, return to the database home screen, and select "Reports" in top left corner.
    - Leave the database's autofill of "sample report" in report type and "sample date year" in by date, just fill out the year.
- Open the data.csv file, and delete columns D, F, and G (State, Analysis Type, Do Not Purge Before). Sort all data by sample code, smallest to largest.
- Access data file at Z:\DO\TSC\Programs\Ecological Research Laboratory\DATA\MUSSEL SAMPLES\Barcode Labels
- Copy and paste new sample information from data.csv file to working file S03_data_auto20xx.
- Open DYMO Label v.8 program, select "file" from top left, then select "import data & print."
- If labels have previously been printed on that computer from that account, select the correct "MyData" file.
- Select only the desired labels, and print two copies: one copy on permanent labels to affix to the bottles, and one on removable labels to be attached to test tubes during sample takedown. If there are any samples from Priority water bodies, print a 3rd (permanent) set for only those samples to affix to 50mL conical tubes.

5. <u>Sample Setup</u>

- On database, select "Quick Add: Sample Prep." Click in "Sample Code" box type in code. "Settle start" and "end" will autofill with current date and following day respectively.
- Enter volume (mL), color (using standard colors), select clarity (cloudy/clear), and debris type (low/medium/high, organic/sediment).
- Record sample setup information on sheet in manila folder in drawer labeled "Setup Supplies."

6. <u>Recording Analysis Results</u>

- Once analysis is complete, results must be logged in database; uploading daily is preferred, but at least weekly is expected.
- On database, select "Quick Add: Microscopy"

    - "Sample Code" is manually entered.
    - "Date analyzed" will autofill with current date. Be sure to enter the correct date if logging in samples analyzed on a different date.
    - "Analyst Name" should be given in First Last but no agency is necessary.
    - Indicate levels of Asian clams, glochidia, ostracods, pollen, and seeds in accordance to scale given in Section 12.
    - "SEM Result Type" must be changed from default "Not Tested" to fourth option in drop-down list, "Not Tested."
    - "Notes" is used to notate priority processing or other significant irregularities.
- When results are recorded and submitted, a green bar at the top of the screen will read "Microscopy Added". If this does not appear, check that the information was recorded by looking up the sample number in the "Edit Sample" portion of the screen and hitting "Go". The site will display all information about the sample including data for the Microscopy, PCR and Sequencing, and Sample Prep.

Appendix D – 5% Acetic Acid Preparation

PREPARATION PROCEDURE

- Obtain the pre-labeled 5% acetic acid, 4-L plastic container from under the fume hood.
- **Never add water to this product; when diluting, always add acid to water.**
- At the sink, fill container with DI water to colored tape on container (3800-mL).
- In the fume hood, use a glass, graduated beaker to add 200-mL of glacial acetic acid to the container.
- Replace lid and mix the contents of the container by gently shaking.
- Store prepared containers under the fume hood.

DISPOSAL OF ACETIC ACID BOTTLES

- Empty glacial acetic acid glass bottles should be rinsed several times with DI water and then allowed to off-gas and dry under the fume hood.
- With a sharpie, write “Empty” and “RC” (to indicate recyclable) across the label in large, visible lettering.
- Place in mixed recycling container in northwest corner of lab

SPILLS

- Soak up 5% acetic acid and DI water spills with paper towels.
- For spills of glacial acetic acid, use spill kit to clean and dispose appropriately following instructions on kit located near the fume hood.

Appendix E - Sub-Sampling Instructions

1. Prepare a glass petri dish with a grid pattern (using a Sharpie) for counting veligers.
2. Set a micropipette to 75 µL and add a yellow pipette tip.
3. Cut approximately 5mm off the pipette tip with a clean razor.
4. With cap still on, homogenize the 15-mL sample by inverting multiple times..
5. Quickly take two pulls of the sample with the pipette (before settling has a chance to recur) and dilute with DI water in the petri dish.
6. Count and record the number of veligers. If less than 20 veligers are observed in 150 µL, the full 15 mL sample must be counted using standard methods.
7. If 20 or more veligers are counted, place the cap on the sample and repeat steps 4-6 for a total of six counts.
8. Calculate the **average** and **sample standard deviation** for the counts. Sample standard deviation can be calculated using the "=STDEV.S( )" function in Excel. Average can be calculated using the "=AVERAGE( )" function.
9. Template formulas can be found at: Z:\DO\TSC\Programs\Ecological Research Laboratory\DATA\SOPs\Lab SOP\Lab SOP 2019\SOP 2019\Appendices\Sub Sampling Instructions
10. Standard deviation should not exceed 10-15% of the average (standard deviation/average). If it does, perform six additional counts and recalculate.
11. Once an acceptable standard deviation has been obtained, use the average number of veligers and multiply that by 100 to obtain the average number of veligers for the 15mL sample. (150µL x 100 = 15,000µL = 15mL)
12. Record this number as the total count for the sample in the technician notebook and include the note "sub-sampled". This information should also be entered into the database.