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# Standard Operating Procedure: Field Sampling Methods for Invasive Mussel Early Detection

Field Sampling Standard Operating Procedure (SOP)  
Version 6 (Date Revised: 2020)  
Document No. EcoLab-FA981-2020-02

Bureau of Reclamation  
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.

# Standard Operating Procedure: Field Sampling Methods for Invasive Mussel Early Detection

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Version 6 (Date Revised: 2020)  
Document No. EcoLab-FA981-2020-02

*Prepared by:*

**Bureau of Reclamation  
Technical Service Center  
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Ecological Research Laboratory (86-68560)**

## **Previous Versions:**

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

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## **1. ACRONYMS AND DEFINITIONS**

**ANS:** Aquatic Nuisance Species  
**COC:** Chain of Custody  
**Cod-end:** Weighted end of the plankton tow net  
**DI water:** Deionized water  
**DNA:** Deoxyribonucleic acid, the genetic material used to identify organisms  
**Dreissenid:** Genus of freshwater mussel  
**JHA:** Job Hazard Analysis  
**MSDS:** Material Safety Data Sheet  
**Plankton tow net:** Mesh net used to collect plankton samples  
**Quagga mussel:** (*Dreissena rostriformis bugensis*) Invasive mussel species  
**PFD:** Personal Flotation Device (life jacket)  
**Eco-Lab:** Ecological Research Laboratory (Reclamation Technical Service Center)  
**Reclamation:** Bureau of Reclamation  
**Sample Site/s:** Sample sites are the locations (usually at least 3) selected at a waterbody where plankton tow samples are collected  
**Secchi disk:** Circular disk used to measure water transparency  
**SOP:** Standard Operating Procedure  
**Veliger:** The microscopic, free-floating larval stage of dreissenid mussels  
**YSI:** Multi-probe used to collect water quality data  
**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 personnel collecting invasive dreissenid mussel (zebra and quagga mussel) larvae (veliger) early detection samples. This method is applicable to collection of dreissenid mussel plankton samples that are shipped to the Ecological Research Laboratory (Eco-Lab) at the Reclamation Technical Service Center, Denver CO. The goal of this SOP is to standardize the collection, preservation, and shipment of dreissenid mussel early detection samples, and collection of water quality data. This SOP is not a replacement for training.

## **3. INTERFERENCES**

The main interference with accurate early detection of invasive mussels is cross-contamination from other samples and waterbodies. In order to mitigate this interference, new and well-labeled bottles will be used for each sample. It is highly recommended that a separate and dedicated plankton tow net and cod-end be used at each waterbody whether or not veligers have been previously detected. It is important to decontaminate nets after sampling at each site, even within the same waterbody to ensure veligers do not remain on the net or cod-end. Veliger decontamination can be accomplished by soaking the net and cod-end in a vinegar bath for at least 10 minutes between sites. Before the next sample is collected, rinse the vinegar off the net and cod-end by dipping in the water without submerging the net opening. Always soak the net and cod-end in vinegar at the end of the sampling day, and sampling trip, and rinse the vinegar off the net and cod-end with tap, well, deionized (DI), or distilled water and allow to dry before storing. Always store nets and cod-ends separately

from nets used at other waterbodies, especially nets that are used at waters with known mussel populations.

A secondary interference with accurate mussel early detection is improper sample preservation and handling in the field. If samples are not preserved correctly (see preservation description in Section 10), and if samples are not kept cool and shipped promptly, it is possible for sample integrity to become compromised by low pH or presence of bacteria. Improper sample handling could result in degradation of the veliger shell, tissue, and genetic material which may prevent detection.

#### **4. HAZARDS**

A variety of chemicals are used in the collection of mussel detection samples. While the majority of these chemicals may cause minimal injury or irritation, field personnel should refer to the Material Safety Data Sheet (MSDS) for each chemical used. Specific chemical hazards are not listed in this SOP because each sampling agency uses slightly different chemicals for preservation and multi-probe calibration. Additionally, there are non-chemical hazards that can impact sampling efforts. These hazards are often site and situation-specific and can range from weather extremes, to dangerous wildlife, to hazards associated with operating boats and vehicles. The Eco-Lab Job Hazard Analysis (JHA) is included for reference in Appendix A; however, personnel should refer to the JHA, or equivalent document developed by their own office or agency, which should include information about site and situation-specific hazards.

#### **5. MATERIALS**

##### **Sampling:**

Chain of Custody (COC) (Appendix B)  
Plankton net (64- $\mu$ m mesh) with weighted cod-end (64- $\mu$ m mesh)  
Rope, marked in 1-meter increments up to 50-meters  
Labeled sample bottles (new bottles only – do not re-use old bottles)  
Secchi disk  
Water quality instrument (YSI multiprobe or comparable)  
Permanent marker  
Distilled water  
Decontamination buckets for plankton tow nets  
Plastic spoons to measure sodium bicarbonate buffer, 0.1 gram  
(OR) Disposable dropper to add Tris buffer to sample  
Personal Flotation Device (PFD)  
Sampling permit (if required)  
Tape measure  
GPS

**Reagents / Chemicals:**

Vinegar

Alcohol (90% or greater ethyl [ethanol] or isopropyl [isopropanol])

Tris buffer (4 molar [4M], pH 7.5) OR Sodium bicarbonate (baking soda) buffer

Calibration standards (pH and conductivity) for multiprobe

**Shipping:**

Electrical tape to seal sample bottles

Cooler

Ice packs

Garbage bags

Ziplock bags (for COC)

Packing tape to seal coolers

**6. FIELD SAMPLE PREPARATION**

Inspect plankton tow net for holes, rips, or tears. Check metal/PVC collar to ensure cod-end is not broken and screws on securely. Check that mesh is attached to the inside of cod-end with no gaps. Check that the weight on the bottom of the cod-end is secure. Make sure all knots are securely tightened. Make sure plankton tow net and cod-end have been decontaminated in vinegar and rinsed.

Calibrate water quality instrument (YSI or comparable) prior to departure using manufacturer's recommended calibration method for the specific model being used.

Assemble all supplies needed to perform sampling (see list in Section 5). Review and sign JHA.

**7. SITE SELECTION**

Veliger distribution can be highly localized; therefore, to increase the potential for detection, sampling should occur at several spatially distinct sites across the waterbody. A minimum of three sampling sites per waterbody is recommended. Sampling sites should include areas of high use and likely sites of mussel introductions such as boat docks, boat launch ramps, floating restrooms, marinas, and waterbody inlets/outlets (e.g. mouth of tributaries and dams). Sampling can be conducted in both open water and near shore depending on ease of access.

**8. SAMPLING**

**Boat Sampling:** When using boats to conduct sampling, it is important that boats traveling from one negative waterbody to another negative waterbody be cleaned, drained, and dried in accordance with the Aquatic Nuisance Species (ANS) plan for the applicable state.

Waterbodies with known mussel populations should have dedicated boats if boat sampling is to be conducted. Boats used on waterbodies with known mussel populations should be decontaminated with hot water treatment performed by local State Authorities at a watercraft inspection and decontamination station before the boat is transported to another waterbody.

State and local regulations regarding boat transport and launching should be checked and observed.

**Chain of Custody (COC):** At each site complete the COC Sampling Log (Appendix B). The COC should be shipped to the lab along with samples.

**Secchi Disk:** Lower Secchi disk with a rope marked at 1/10-meter increments until the black and white markings are indistinguishable from one another. Record this depth as “Secchi Depth” on the COC. Then lower disk to the deepest depth at the sample site and record as “Total Depth” on the COC. All depths should be recorded in meters.

**Water Quality Sampling:** Calibrate water quality multi-probe instrument (YSI or comparable) each morning prior to sampling, using manufacturer’s recommended calibration method for the specific model being used. Collect and record water quality readings at 1-meter increments from the water’s surface to the bottom. After each sampling trip, email water quality data to the Eco-Lab ([bor-sha-ecolab@usbr.gov](mailto:bor-sha-ecolab@usbr.gov)).

**Field-blank Sample Collection Method:** A single field-blank sample should be collected at each waterbody after the net and cod-end are decontaminated and rinsed, and before the first sample is collected at the first site. After soaking the net and cod-end in vinegar for at least 10 minutes, first rinse off the vinegar with distilled water, then collect the field-blank sample by pouring a gallon of distilled water through the opening of the net. While pouring the water through the net be sure that water passes over the majority of the inside of the net before it passes through the cod-end. Pour the water that is retained in the cod-end into a labeled sample bottle and buffer and preserve in the same manner as all other samples (see **Sample Preservation** section below for details). The field blanks will be used to confirm that nets have been properly cleaned before sampling and will help to validate any positive DNA (genetic) findings.

**Plankton Tow Net Sampling:** Label bottles with date collected, waterbody, sample site (or field-blank), tow type (vertical or horizontal), number of tows in the bottle, and length of tows. Please use meters for all length and depth measurements (refer to Appendix C). Be sure to also include all of this information on the COC along with the sample site coordinates (in decimal degrees if possible). If using pre-labeled bottles, make sure correct sample bottle is used for the site being sampled. A separate bottle should be used for each sample site at a waterbody (i.e. if you are sampling three sites at a waterbody you should use three sample bottles).

Keep the plankton tow net and cod-end submerged in a bucket with vinegar for decontamination between sample sites. Rinse the vinegar off the net and cod-end before collecting the first tow. This is accomplished by dipping the net into the water without submerging the net opening. Do not collect samples from the exact same spot where the net was rinsed as there may be residual vinegar that could compromise the sample.

**Vertical Plankton Tow:** Collect a vertical plankton tow when water is deeper than 4 meters. After rinsing vinegar from the net and cod-end and collecting the field blank sample, lower

the plankton tow net vertically from dock or boat to 1 meter above the “Total Depth”. The “Total Depth” at the site should be pre-determined by using the Secchi disk, water quality instrument, or the boat’s depth finder. If the “Total Depth” is deeper than the length of the plankton tow net rope, lower the plankton tow net to the deepest depth possible. Slowly (~1 meter per second) pull up the net, hand-over-hand, to the surface. If the cod-end is filled with sediment, discard sample, rinse cod-end, and re-sample by raising the sample depth by half a meter. Record the length of the tow, in meters, on the sample bottle and on the COC.

**Horizontal Plankton Tow:** Collect a horizontal plankton tow when water is less than 4 meters deep or in flowing water. After rinsing vinegar from the net and cod-end and collecting the field blank sample, throw the net as far as possible and estimate the distance thrown. Record this distance, in meters, on the sample bottle and on the COC. Slowly reel in the net, making sure the entire opening of the net is submerged and that the net is not dragging along the bottom. If the cod-end contains sediment, discard sample, rinse cod-end, and re-sample.

**Sample Completion:** After each tow is complete, rinse the contents of the net into the cod-end by dunking the net into the water three-times without submerging the opening of the net. Unscrew the cod-end, gently swirl the sample around to drain excess water, and pour the remaining sample into the appropriate pre-labeled sample bottle. Using a wash-bottle containing distilled or DI water, rinse the cod-end and pour the rinse water into the sample bottle, repeating this step three times.

Collect 5 tows per site and combine all 5 tows into a single sample bottle. Be sure to empty and rinse the cod-end into the bottle after each tow. Leave enough space for the addition of the alcohol preservative. If there is too much water in the bottle to accommodate the preservative, pour the unpreserved sample back into the cod-end and swirl gently to drain excess water. The final volume of the sample and alcohol preservative should not exceed the shoulder of the bottle (refer to Appendix C).

**Sample Preservation:** Either 4M Tris pH 7.5 OR baking soda may be used to buffer the pH of the sample. 4M Tris pH 7.5 can be purchased from [teknova.com](https://www.teknova.com); product number T5575 (<https://www.teknova.com/tris-hcl-4-m-ph-75-rnase-dnase-tested.html>). Buffer (Tris or baking soda) helps maintain the pH of the sample, which is important because a low pH can degrade the veliger shell and prevent identification during analysis. Tris buffer is preferred because it has better buffering capacity than baking soda. Also, unlike baking soda, it will not precipitate out of solution, which can interfere with microscopy. Tris buffer solution can be provided by the Eco-Lab (requests can be sent to any lab staff or to the Eco-Lab shared email ([bor-sha-ecolab@usbr.gov](mailto:bor-sha-ecolab@usbr.gov))). If Tris buffer is not available, baking soda should be used.  
**DO NOT USE BOTH.**

For 4M Tris pH 7.5 buffer, use a 1 mL plastic transfer pipette (available from [Amazon.com](https://www.amazon.com)) to add 6 drops of Tris buffer per every 100 mL of sample. For baking soda, add 0.2 grams (0.2 mL or 2 level scoops with a 0.1-gram measuring spoon) per every 100 mL of sample in order to buffer the pH. Gently shake the bottle to mix. It is important to add the buffer (either Tris or baking soda) before the alcohol. **Be careful not to add excess baking soda to**

**the sample. Additional baking soda above 0.2 gram/100 mL of sample will not dissolve and can cause significant interference with sample analysis.**

Alcohol must be added to the sample to prevent degradation. Isopropyl alcohol is commonly used because it can be purchased as a 91% solution at most grocery stores and pharmacies. Each sample should contain a final 70% alcohol by volume. The following instructions apply to the use of 90% or higher alcohol. Lower concentration alcohol solutions, such as 70% isopropyl alcohol, may not be sufficient to achieve a final concentration of 70% alcohol by volume in the final sample.

To calculate the amount of alcohol to add, measure the height of the collected sample (in centimeters or inches) and multiply by 3.0. The result of this calculation is the amount of alcohol, in centimeters or inches, that should be added to the sample. Add alcohol to the sample until the height of liquid increases by the calculated number. Total volume of the sample and alcohol should not exceed the shoulder of the sample bottle (refer to the image in Appendix C). Gently shake the sample to mix. Release built up pressure in the bottle by opening the lid of the bottle, then close the lid tightly and seal the bottle with electrical tape around the base of the lid and the top of the bottle to prevent leaking. Place sample in cooler with ice. **Ensure any writing or labeling on the bottle was not inadvertently rinsed off while adding the alcohol and re-label if necessary.**

Decontaminate the plankton tow net and the cod-end by submerging them in a bucket with vinegar for at least 10 minutes between sites.

## **9. END OF DAY / TRIP PROCEDURES**

Remove plankton tow net and cod-end from the vinegar bath and rinse with tap, well, DI, or distilled water. Also rinse the Secchi disk and all water quality instrument components, following manufacturer's recommendations for storage. Do not use lake water for rinsing or storage. Store samples on ice in the cooler or keep samples refrigerated until they are shipped.

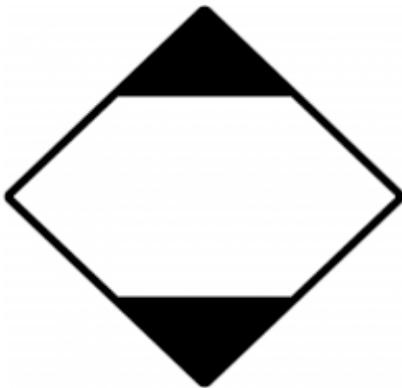
## **10. NET DECONTAMINATION**

Between all sample sites, even on the same waterbody, soak net and cod-end in a vinegar bath for at least 10 minutes to remove veligers and prevent contamination. Rinse with lake water to remove vinegar before collecting sample. Do not collect samples from the same spot net is rinsed. At the end of each sampling trip, rinse nets and cod-ends thoroughly with tap, well, DI, or distilled water and hang to dry. Do not use lake water to rinse nets and cod-ends at the end of the day. Use dedicated nets and cod-ends for positive waterbodies. It is highly recommended to use a dedicated net and cod-end for **every** waterbody, regardless of mussel presence/absence. Even dedicated nets and cod-ends must be decontaminated using vinegar, as described above, regardless of mussel presence/absence.

## 11. SHIPMENT

If your cooler has a drain valve, make sure it is closed and sealed with tape. Make sure cooler is clean and dry. Open a trash bag and lay it in the cooler to contain any leaks that may occur. **DO NOT USE ICE.** Coolers that leak, sweat, or are wet in any way will be rejected by all shipping companies and may end up lost. Instead, use frozen ice packs to keep samples cool during shipping. Once all samples and ice packs are inside, close garbage bag by tying a tight knot to prevent spills during shipping. Confirm that all information has been added to the COC and if you have any special instructions or would like the sample analysis to be prioritized due to special circumstances please provide instructions on the COC and send an email the Eco-Lab main email ([bor-sha-ecolab@usbr.gov](mailto:bor-sha-ecolab@usbr.gov)) or any of the Eco-Lab staff members (see contact information in Section 12). Put the COC into a plastic bag and place on top of the samples. Tape lid and sides of cooler securely closed.

Due to the increased volume of ethanol required to preserve samples, coolers must be shipped via Ground transport only, and must be labeled with a “Limited Quantity” label, shown below. This label indicates that each individual sample bottle contains less than 1L/1000 mL of ethanol. The label can be printed from the internet and taped to the cooler. Adhesive labels are also available for purchase from a variety of online vendors. Note that the center of this label is blank – a similar label with a “Y” in the center is for air transport. Coolers shipped via UPS may contain no more than 11 – 500 mL sample bottles and must be labeled with the statement “Contains Ethyl Alcohol”. Coolers shipped via FedEx must weigh less than 66 lbs when loaded with samples and icepacks. Additional questions regarding shipping requirements should be directed to your chosen shipping company.



Ship samples as soon as possible after collection, ideally within 2 days of collection. Confirm that samples will not arrive in the Denver mailroom on Friday afternoon or over a weekend, even if that means holding samples for an additional day or two.

Ship samples to the following address:

**US Bureau of Reclamation  
Attn: ECO-LAB (86-68560)  
1 Denver Federal Center  
Denver, CO 80225**

Ship samples immediately after collection to prevent degradation, ideally within 2 days of collection. The Eco-Lab may elect not to analyze samples that are held for an extended period prior to shipment, as sample integrity can be compromised, and analysis results may not be reliable. It is more important that samples do not arrive in the Denver mailroom later than noon on Fridays. The mailroom does not always process/deliver packages that arrive later on Friday afternoons and the lab is not staffed on weekends. If you would like email confirmation of sample arrival at the lab, email the tracking number to the Eco-Lab at: [bor-sha-ecolab@usbr.gov](mailto:bor-sha-ecolab@usbr.gov).

## **12. RESOURCES AND CONTACT INFORMATION**

This SOP, the COC, and other supporting documents can be found on the Reclamation Invasive Mussels Webpage, <https://www.usbr.gov/mussels>, along with additional information and resources. The Eco-Lab has prepared an instructional video, which can also be found on the website, that describes and demonstrates many aspects of the sampling process. The video is not a replacement for training or for reading this SOP. The video does not provide all of the important details described in this SOP.

### **Contacts**

Eco-Lab	303-445-2498	<a href="mailto:bor-sha-ecolab@usbr.gov">bor-sha-ecolab@usbr.gov</a>
<b>ECO-LAB STAFF</b>		
Sherri Pucherelli	303-445-2015	<a href="mailto:spucherelli@usbr.gov">spucherelli@usbr.gov</a>
Diane Mench	303-445-2050	<a href="mailto:dmench@usbr.gov">dmench@usbr.gov</a>
Jacque Keele	303-445-2187	<a href="mailto:jkeele@usbr.gov">jkeele@usbr.gov</a>
Yale Passamaneck	303-445-2480	<a href="mailto:ypassamaneck@usbr.gov">ypassamaneck@usbr.gov</a>
Annie Quattlebaum	303-445-2798	<a href="mailto:rquattlebaum@usbr.gov">rquattlebaum@usbr.gov</a>
Aaron Murphy	303-445-2157	<a href="mailto:amurphy@usbr.gov">amurphy@usbr.gov</a>

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### **APPENDIX A - INVASIVE MUSSEL FIELD SAMPLING JOB HAZARD ANALYSIS (JHA)**

1. **Project Title:** Dreissenid Mussel Field Sampling
2. **Purpose:** To conduct Dreissenid mussel larvae sampling and collect water quality data within the 17 western states.
3. **Start Date:** May 2020
4. **End date:** May 2021
5. **Personnel Requirements:** Work will be performed by a Bureau of Reclamation (Reclamation) employee and other contract personnel as required.
6. **Hazards**
  - **Vehicle Operation:** Reclamation vehicles will only be operated by personnel who have current Defensive Driver Training. Trucks with heavy loads, boats, and towable vehicles such as trailers filled with supplies may be used. Be aware of shifting loads in vehicles, boats, and trailers. Make sure loads are tied down when in transport.
  - **Boat Operation:** Reclamation boats will only be towed and operated by personnel who have completed the Motorboat Operator Certification Course (MOCC). All personnel are required to wear a Personal Flotation Device (PFD) while onboard a boat. Personnel should be aware of the risk of falling overboard, drowning, and hypothermia due to cold water temperatures.
  - **Chemicals:** In general, chemicals used in the field (i.e. sodium bicarbonate, distilled vinegar, isopropyl alcohol) are relatively harmless and pose little risk of illness or injury. However, all personnel should review the MSDS' for the specific chemicals used by their agency 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.
    - **Tris hydrochloride solution pH 7.5:** Use with adequate ventilation. Wear personal protective equipment. Avoid contact with skin, eyes, and clothing. Avoid ingestion and inhalation. Keep away from food, drink, and animal feeding stuffs. Do not eat, drink, or smoke when using this product. Remove and wash contaminated clothing before re-use. Wash hands before breaks and at the end of workday.
    - **Sodium Bicarbonate:** Use with adequate ventilation. 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

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harmful if absorbed through the skin. May cause gastrointestinal tract irritation if swallowed. May cause respiratory tract irritation if inhaled.

- **Distilled White Vinegar:** 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.
- **Isopropyl Alcohol:** Wash thoroughly after handling. Avoid contact with eyes, skin, and clothing. Use only with adequate ventilation. 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.
- **Weather:** Dress appropriately for weather conditions. Wear sunscreen, sunglasses, a hat, long sleeves, and/or rain gear as appropriate. Protective weather clothing and equipment is the responsibility of each individual.
- **Minor Injuries:** Reclamation vehicles are equipped with a first-aid kit to treat small cuts, scrapes, and other minor injuries that might occur.
- **Serious Injuries:** Serious injuries are those that cannot be treated in the field with a first-aid kit. Contact local authorities and/or medical personnel immediately for serious injuries.
- **Heavy Equipment:** Equipment such as coolers and toolboxes may need to be loaded and unloaded from the vehicle. Items weighing over 50lbs should be lifted by two people. Secure or stow items properly in the vehicle to prevent shifting loads that may cause injury.
- **Dehydration:** Personnel should be aware of the possibility of and risks associated with dehydration and are expected to monitor their water intake in order to prevent it. Drinking a minimum of one quart every two hours is suggested, more in cases of high heat, extended sun exposure, or increased physical activity. Drinking water will be provided by the crew leader for all field personnel.
- **Exhaustion:** Personnel should be aware of the possibility of and risks associated with exhaustion. Long driving distances, extended sun exposure, and increased physical activity are among the factors that can contribute to exhaustion. Personnel should plan to be well-rested, take necessary breaks, and use other precautions (i.e. drinking plenty of water, limiting or countering sun exposure, sharing driving responsibilities, etc.) to limit the risk of exhaustion.

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- **Wildlife Encounters:** Personnel should be aware of wildlife that may possibly be in the sampling area to include poisonous animals, and insect bites. Particular attention should be paid in areas where venomous snakes, scorpions, spiders, and other arthropods may be encountered.
7. **Personal Protective Equipment (PPE):** Personnel are responsible for maintaining their own PPE to ensure adequate protection.
- **Personal Flotation Devices (PFDs):** PFDs are required for each individual working on or near the water and will be provided by the field crew leader.
  - **Footwear:** Shoes offering protection from heavy objects and weather should be worn at all times. All footwear should be closed toe and have nonskid soles.
  - **Clothing:** Weather-appropriate clothing should be worn. Long-sleeved shirt, a hat with a brim, sunglasses, and SPF or greater sunscreen are recommended year-round. Dress in layers in order to maintain sufficient comfort level.
8. **Training Requirements**
- Reclamation vehicles will only be operated by personnel with current Defensive Driver Training. Reclamation boats will only be towed and/or operated by personnel with current MOCC training. Boat orientation will be provided to orient all passengers to the details of the boat and its operation, location of fire extinguishers, radio operations, first aid kits, and other emergency equipment.
  - Training on sample collection and preservation, water quality collection, net hygiene and other field protocols will be conducted by experienced personnel.
9. **Security Requirements**
- Due to increased pressure from the general public in reference to the US Government and its associated property and personnel, extra caution should be taken when in the field to safeguard yourself, your vehicle, and your possessions. Ensure vehicles are locked whenever not in use. To the extent possible, do not leave valuables such as cell phones, laptops, wallets, etc., in plain sight in an unattended vehicle. All vehicle incidents including break-in, theft, and accidents should be reported to your supervisor immediately. A low profile is advised for all US Government employees and their interactions with the public.
10. **Emergency Contact Numbers**
- Personnel should be aware of local emergency contact numbers such as law enforcement, medical response, Poison Control, and the closest Reclamation facility. It may also be useful to have on hand phone numbers for local towing and locksmith businesses.

**Emergency number:** 9-1-1

**Non-emergency number:** Bob Einhellig, 303-579-3536

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**11. Acknowledgement Signatures**

All personnel conducting field sampling 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\_\_\_\_\_



ECOLOGICAL RESEARCH LABORATORY
INVASIVE MUSSELS FIELD SAMPLING LOG

Sample Collector and Agency: \_\_\_\_\_

Net Diameter: \_\_\_\_\_

Phone Number: \_\_\_\_\_

Type and % Alcohol Added: \_\_\_\_\_

Email: \_\_\_\_\_

Baking Soda Added (circle): Y / N | Tris Buffer Added (circle): Y / N

Table with 9 columns: Date Collected, Water Body, Sample Location, Tow Type (V or H), Number of Tows, Length of Tows (M), Total Water Depth (M), Secchi Depth (M), Coordinates (decimal degrees preferred). The table contains 8 empty rows for data entry.

Special Instructions: \_\_\_\_\_

Address to Return Cooler: \_\_\_\_\_

## APPENDIX C - INVASIVE MUSSEL FIELD SAMPLING METHODS

### Secchi Depth Reading & Total Water Depth

- Lower Secchi disk until black and white marking is indistinguishable and record Secchi Depth.
- Lower disk to bottom of sample site and record Total Depth.

### Sampling

- Record all sample information on Chain of Custody sheet.
- Collect a field-blank at each new waterbody. See full SOP for details.
- Vertical Tow: Use in water deeper than 4 m. Lower plankton tow net vertically from dock or boat to 1 m above maximum depth, and slowly (~1 m/s) pull up.
- Horizontal Tow: Use for shore sampling or flowing water. Throw net as far as possible and estimate distance. Slowly reel in keeping entire opening of net submerged but not dragging along the bottom.
- Collect 5 tows per Sample Site and put in one bottle. (See “Collected Sample” in figure below).  
    **Note:** Leave room for alcohol. Sample can be swirled around in cod end to condense.

### Preservation

- Add 6 drops of Tris buffer per 100 mL sample OR 0.2 grams of baking soda per 100 mL sample. Add volume of alcohol equal to 3x volume of Collected Sample (See Figure).
  - **Note:** The final sample that is mailed should have an alcohol concentration of ~70%.
  - **Do not fill bottle past shoulder!** (See “Max Fill Line” in figure below)

### Bottle Labeling

- Label bottle with: date collected, water body and sample site, tow type (vertical or horizontal), number of tows, and length of tows (use meters for all measurements).

### Net Hygiene

- Use dedicated nets for any positive waterbody.
- Ideally every waterbody (positive or not) should have its own net.
- Soak net in vinegar between each site and rinse before next sample.

### Water Quality Data

- Collect as much water quality data as possible using a multiprobe.
- Email water quality data to: **bor-sha-ecolab@usbr.gov**.

### Shipping

- Ship samples as soon as possible after collection, ideally within two days of collection.
- Seal bottles with electrical tape and place in a plastic bag.
- Pack sample bottles in a cooler with ice packs, **NO WET ICE**.

Address:       **US Bureau of Reclamation**  
                  **Attn: ECO-LAB (86-68560)**  
                  **1 Denver Federal Center**  
                  **Denver, CO 80225**

### Contact Information

- If you have questions or would like confirmation of cooler arrival email tracking number to: **bor-sha-ecolab@usbr.gov**

