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RECLAMATION

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

Field Sampling Standard Operating Procedure (SOP)
Version 8 (Date Revised: 2024)
Document No. EcoLab-F436A-2024-06

Bureau of Reclamation
Ecological Research Laboratory



Mission Statements

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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 8 (Date Revised: 2024)

Document No. EcoLab-F436A-2024-06

Prepared by:

Bureau of Reclamation

Technical Service Center

Hydraulic Investigations and Laboratory Services

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 |
| Field SOP Version 6 | 3/2021 |
| Field SOP Version 7 | 4/2022 |

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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

Plankton tow net: Mesh net used to collect plankton samples

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

PFD: Personal Flotation Device (life jacket)

EcoLab: 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

SDS: Safety Data Sheet

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 (EcoLab) 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 cannot address specific issues related to each and every sample location. We encourage you to expand on the information contained here to tailor this document to the needs and procedures of your agency and location. 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 used at negative waterbodies separately from nets and cod-ends used at positive waterbodies.

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 9), 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. DISCLAIMER

The procedures outlined in this SOP have been developed through collaboration with the Western Regional Panel and industry experts, and in many cases are standard practice. The EcoLab cannot guarantee analysis results of samples collected outside of these procedures.

5. 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 Safety Data Sheet (SDS) 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 EcoLab 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.

6. MATERIALS

Sampling:

Chain of Custody (COC) (Appendix B)

Plankton net (64- μ m mesh) with weighted cod-end (64- μ m mesh, 90cm length) [Simple Plankton Net 30cm Diameter](#)

Rope for plankton net, marked in 1-meter increments up to 50-meters

Labeled sample bottles (new bottles only – do not re-use old bottles)

[Wide Mouth Field Sample Bottles 500mL](#)

[Wide Mouth Field Sample Bottles 250mL](#)

Secchi disk [Secchi Disk](#)

Measuring Tape [50M Tape Measure Reel](#)

Water quality instrument (YSI multiprobe or comparable)

Permanent marker

Distilled water

Decontamination buckets for plankton tow nets

Disposable dropper to add Tris buffer to sample [Fisherbrand Transfer Pipette](#)

Cloth storage bag, one for each net

https://www.amazon.com/gp/product/B0992FXK4M/ref=ox_sc_act_image_3?smid=A3AQP8TDYVYCGL&th=1

Sampling permit (if required)

Ruler

Reagents / Chemicals:

Vinegar (suggest 2-3 gallons from grocery store)

Ethanol (90% or greater ethyl) [Decon Labs 200 Proof Ethanol](#) (this product may require an alcohol user permit to purchase)

Tris buffer, 4 molar [4M], pH 7.5 ([4M Tris-HCl, pH 7.5. 250mL, RNase/DNase Tested. Sterile. | Teknova](#), product number T5575)

Calibration standards (pH and conductivity) for multiprobe [Standard All-in-One pH Buffer Kit](#)

Shipping:

Electrical tape to seal sample bottles

Cooler

Ice packs

Garbage bags

Ziplock bags (for COC)

Packing tape to seal coolers

7. 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.

Mark ropes used for collection in 1-meter increments. Marking methods include Sharpie, sewing, tape, etc. Consider using different colors for 1-meter marks vs. 5-meter marks for easier measurements.

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.

8. 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.

9. 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. Please note that it is not a requirement to use the EcoLab's COC as long as all of the information the EcoLab requires is included.

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 EcoLab (**bor-sha-ecolab@usbr.gov**).

Field-blank Sample Collection Method: Field blanks are used to confirm that nets have been properly cleaned before sampling and will help to validate any positive DNA (genetic) findings. A single field-blank sample should be collected each time a net is used. If you are using the same net to collect samples at multiple waterbodies during a single day or trip, one field blank should be collected AT EACH WATERBODY. Nets should still be decontaminated between waterbodies, and the vinegar should be rinsed off prior to collecting the field blank. Field blanks should be collected before the first sample is collected.

A field-blank sample is collected by first ensuring the cod-end is securely attached to the net, then pouring approximately 1L of distilled water around the inside of the opening of the net and over the entire interior surface of the net. While pouring the water through the net be sure water passes over the majority of the inside of the net before it flows into 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). It is not necessary to collect the entire volume of water used to rinse the net as long as the net is thoroughly rinsed in this process.

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 the 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. 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 pull up the net (~1 meter per second), hand-over-hand, to the surface. If the cod-end is filled with sediment due to a bottom-drag, 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. 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 settled sediment due to a bottom-drag, discard that tow, rinse cod-end, and collect another tow.

Sample Collection: 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 sample bottle. Remove any large debris such as sticks,

leaves, fish, clumps of algae or plant material, etc., prior to pouring into the 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 – this must be done prior to adding Tris and ethanol. The final volume of the sample and alcohol preservative should not exceed the shoulder of the bottle (refer to Appendix C).

Sample Preservation: Buffer each sample with at least 1 mL of Tris buffer to each 250 mL sample. It is important to add the Tris before the alcohol. Buffer 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 has better buffering capacity than baking soda and will not precipitate out of solution, which can interfere with microscopy. Tris buffer solution can be provided by the EcoLab (requests can be sent to any lab staff or to the EcoLab shared email (bor-sha-ecolab@usbr.gov)). If Tris buffer is not available, baking soda can be used (0.2 grams per every 100 mL of sample). **DO NOT USE BOTH.**

Alcohol must be added to the sample to prevent degradation. The EcoLab prefers that samples are preserved with Ethanol (90% or greater ethyl). Isopropyl alcohol can be used if necessary and can be purchased at most grocery and drug stores. However, lower concentration alcohol solutions, such as 70% isopropyl alcohol, should be avoided as they may not be sufficient to achieve the final required concentration of 70% alcohol by volume in the final sample. The following instructions apply to the use of 90% or higher alcohol.

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.**

10. 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.

11. 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. When nets and cod-ends are completely dry, place in dedicated storage bag and return to the correct trip tote. 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. It is not necessary to use a dedicated net for each sample site on the same waterbody. Even dedicated nets and cod-ends must be decontaminated using vinegar, as described above, regardless of mussel presence/absence.

12. SHIPMENT

If your cooler has a drain valve, make sure it is closed and sealed with tape. Make sure cooler is clean and dry. **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. If you are using gel ice packs, please enclose them in a Ziploc bag as they can tear open and create a slimy mess inside the cooler and on the sample bottles. 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 EcoLab main email (**bor-sha-ecolab@usbr.gov**) or any of the EcoLab 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 may require special handling and shipping labels, depending upon the carrier being used. **PLEASE CHECK WITH YOUR LOCAL CARRIER FOR THEIR RECOMMENDED SHIPPING PROCEDURES**. It is important to disclose that the samples you are shipping contain alcohol. You may be required to report the total volume of alcohol in each cooler or the amount of alcohol in each sample. Please note that failure to disclose the presence of alcohol in a cooler, inaccurate reporting of the volume of alcohol contained in a cooler, or improper labeling of a cooler may result in the carrier imposing fines against you. The EcoLab will not be responsible for these fines.

Ship samples immediately after collection to prevent degradation, ideally within 2 days of collection. Please ensure that samples will not **ARRIVE** at the EcoLab later than noon on Friday. The mailroom does not always process/deliver packages that arrive later on Friday afternoons and the lab is not staffed on weekends. If necessary, it is acceptable to hold samples over the weekend, however, please keep them refrigerated. **DO NOT HOLD SAMPLES FOR MULTIPLE WEEKS OR FOR THE ENTIRE SAMPLE SEASON**. The EcoLab 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.

If you would like email confirmation of sample arrival at the lab, email the tracking number to the EcoLab at bor-sha-ecolab@usbr.gov.

Ship samples to the following address:

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

13. 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 EcoLab 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 the important details described in this SOP. Additionally, Appendix D of this SOP addresses Frequently Asked Questions (FAQ) received by the Eco Lab.

Contacts

| | | |
|---------------------|--------------|--|
| EcoLab | 303-445-2498 | bor-sha-ecolab@usbr.gov |
| ECOLAB STAFF | | |
| Sherri Pucherelli | 303-445-2015 | spucherelli@usbr.gov |
| Diane Mench | 303-445-2050 | dmench@usbr.gov |
| Jacque Keele | 303-445-2187 | jkeele@usbr.gov |
| Yale Passamaneck | 303-445-2480 | ypassamaneck@usbr.gov |
| Annie Quattlebaum | 303-445-2798 | rquattlebaum@usbr.gov |

APPENDIX A: JOB HAZARD ANALYSIS (JHA)

Project Title: Dreissenid Mussel Field Sampling

Purpose: To conduct Dreissenid mussel larvae sampling and collect water quality data within the 17 western states.

Start Date: May 2024

End Date: May 2025

Personnel Requirements: Work will be performed by Bureau of Reclamation (Reclamation) employees and other contract personnel as required.

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. TRIS, ethanol, isopropyl alcohol, and vinegar) are relatively harmless and pose little risk of illness or injury. However, all personnel should review the SDS' 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 8.0: 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.

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.

Ethanol: Wash thoroughly after handling. Highly flammable liquid and vapor. 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. May cause drowsiness or dizziness.

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.

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.

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.

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.

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.

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: Connie Svoboda, 303-524-0285

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 _____



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APPENDIX B - CHAIN OF CUSTODY
ECOLOGICAL RESEARCH LABORATORY
INVASIVE MUSSELS FIELD SAMPLING LOG

Sample Collector and Agency:
Phone Number:
Email:
Net Diameter:
Type and % Alcohol Added:
Baking Soda Added (circle): Y / N | Tris Buffer Added (circle): Y / N

Table with 10 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)

Special Instructions:
Address to Return Cooler:
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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 15 drops of Tris buffer per 250-500 mL sample OR 0.2 grams of baking soda per 100 mL sample. **Do not add both!**
- 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: ECOLAB (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**



APPENDIX D: FREQUENTLY ASKED QUESTIONS

1. We have individual nets for each of our priority waterbodies, and we clean them every time we are done in a tub of vinegar. Is there a need for field blanks when we have individual nets for each waterbody?
 - A. It is very important that you collect field blanks, as they are for validating eDNA results, and it is a separate issue from using dedicated nets.
2. When during the collection process should Tris and EtOH be added?
 - A. For best results, Tris and EtOH should be added to the complete sample within 15 minutes of collection.
3. The EcoLab's Chain of Custody does not have enough space for all the data we collect. Can I create one of my own, or am I required to use the one provided?
 - A. The Chain of Custody developed by the EcoLab contains the information WE need in order to track and process samples. Any additional data collected is extraneous to our processes but can also be useful and informative in other areas. Samplers can absolutely create a document that works for them, as long as the information the EcoLab needs is included in a clear, understandable, legible manner.
4. I received notification that my samples were rejected for analysis – why?
 - A. The EcoLab strives to process every sample we receive regardless of offensive odors or physical interferences. Occasionally, however, differences in organic material at each waterbody, even each location, as well as the timing of preservation, and temperature fluctuations during shipping can create a pungent, foul-smelling sample that is intolerable for lab staff and leads to health concerns. Samples that contain a high amount of sediment, either from a bottom drag or simple turbidity may also create such heavy interference that analysis is simply not possible.
5. I need sample results quickly to meet a deadline, is it possible to expedite sample processing?
 - A. Yes! Please let us know either on the CoC or via email that you require expedited processing. Please understand that we are not able to prioritize every sample this way.
6. Why are “tow depth”, “total water depth”, and “secchi depth” recorded for horizontal tows?
 - A. It is not absolutely necessary to record these depths for horizontal tows. However, if it is possible to record a depth, please do as it would be informative if mussels are found.
7. We use leak-proof bottles, and they work. Can we skip the electrical tape?
 - A. In our experience, there is no such thing as a “leak-proof bottle”. Coolers are not handled gently in transit and things can shift inside. Lids can loosen, crack, or break on the way to our lab and once the sample leaks or spills, we cannot guarantee that

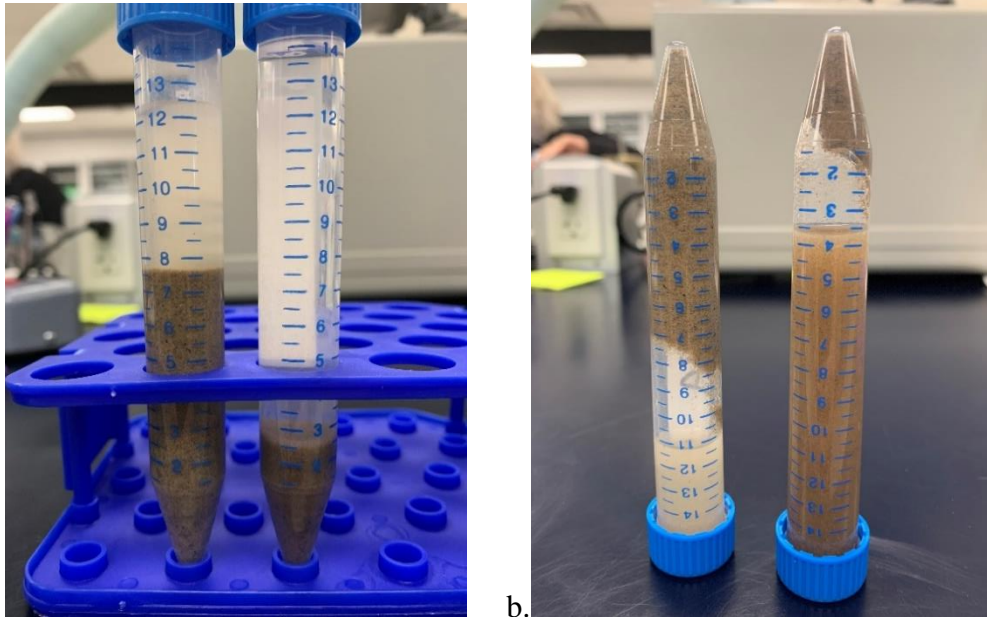
cross-contamination has not occurred. EtOH in the sample degrades any labeling on the outside of the bottle, making sample identification inaccurate. It is a quick, inexpensive step to add electrical tape to each bottle to eliminate this potential.

8. The SOP says to do 5 separate tows for each sample. For horizontal tows, wouldn't it make more sense to ask for a minimum tow length, since one person may not be able to throw the net as far as another person?
 - A. We did not request a specific depth for vertical tows, so we did not want to request a specific length for horizontal tows. + or - 1 meter in horizontal tow length does not have a huge impact on our calculations, so one person's ability to throw farther than another person is not of great concern – the important part is to record the total distance sampled.
9. Collecting 5 tows results in too much volume for the sample bottle. What should I do?
 - A. First, try condensing the sample by pouring what you have collected so far back into the cod-end and swirling some of the water out. **THIS MUST BE DONE PRIOR TO ADDING TRIS OR ETHANOL!** You can use your hand or a small rubber spatula on the inside of the cod-end to make this a little easier (please remember to clean and dry your hands and any implements you use in between samples).

In some places, there is simply too much algae and/or zooplankton to condense the sample enough. In this case, it is acceptable to reduce the number of tows you collect – just be sure to record the correct number on the Chain of Custody (COC).

As a last resort, you can divide the sample into multiple bottles. However, it is imperative that you correctly label each bottle, and that you note on the CoC if a sample has been divided. All bottles must be correctly labeled, buffered, and preserved.

10. The SOP says to discard any sample with sediment in it. This is not always practical, as sediment is suspended in lake water. Suggest rewording to “avoid dragging the net on the bottom” and leave it at that.
 - A. Rocks, weeds, sticks, leaves, mud, fish, sand, and other solid material in a sample is a huge interference in our settling process at the lab. Particles suspended in turbid water are unavoidable and are generally manageable. However, there is an ENORMOUS difference between sediment from turbidity and sediment from a bottom-drag (Figures 1a and 1b). It is not necessary to discard the entire sample if the net drags the bottom – just that tow. Additionally, it is completely acceptable for samplers to remove sticks, weeds, leaves, rocks, fish (especially fish!!), etc., collected during the tow without discarding the remainder. The EcoLab may elect not to analyze samples containing excessive amounts of sediment.



Figures 1 a & 1 b. a.) A bottom-drag sample (left) vs. a turbid sample (right). B.) The same samples, inverted, showing how much sediment and how dense it is when the net drags the bottom.

11. The SOP says to add 70% alcohol by volume but describes procedures for adding 75% by volume. Is this a mistake?
 - A. Our preservation requirement is to add 70% alcohol by volume. But for those of us who are not great at math/fractions, it is easier to determine a 3:1 alcohol to sample ratio.

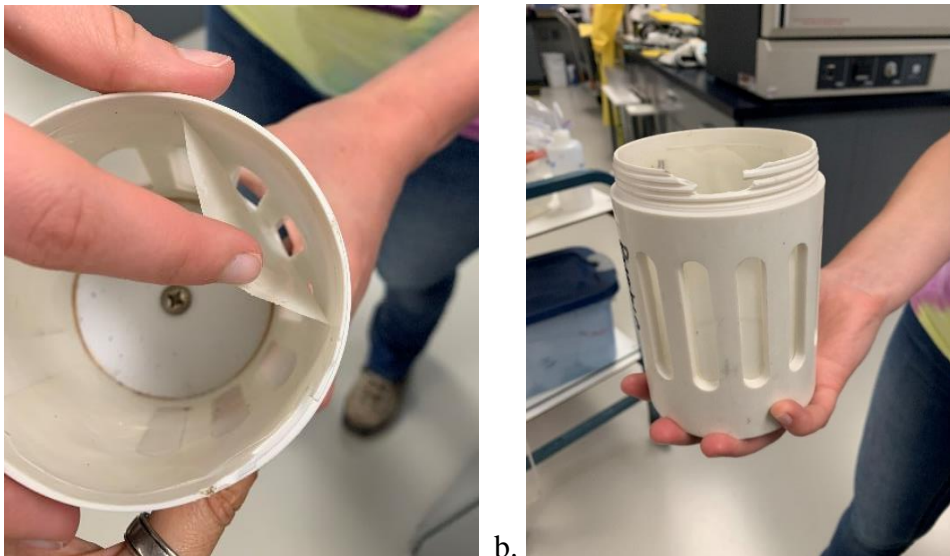
12. When collecting horizontal tows, multiple reservoirs may be sampled on the same day. Do I need to change or decontaminate my clothes/shoes between reservoirs?
 - A. This isn't generally a concern, especially if you are not physically in the water. Try to arrange your sampling trip so that you sample negative waterbodies before sampling positive waterbodies. Also, follow sample collection hygiene: don't spill sample water on your clothes and shoes; let your clothes/shoes dry between waterbodies where you have physical contact with the water;; etc.

13. The SOP says to use DI water to collect the Field Blank. Why can't I use tap water?
 - A. Using DI water to collect a Field Blank is a standard practice adopted by the eDNA community. Tap water can contain contaminants, including potential DNA. If our data is to be shared more broadly, we need our processes to adhere to standard practices. For all other rinses (after the vinegar soak between sites, end-of-day cleanup, etc.), tap water is just fine.

14. Please suggest both minimum and maximum times for the vinegar soak. A maximum time is needed to avoid damage to net hardware.
 - A. A minimum soak time is mentioned in the SOP, section 10 (at least 10 minutes). The EcoLab does not have a maximum soak time, but it is recommended that cod-ends

specifically are thoroughly rinsed at the end of the sample day. Allowing them to sit with vinegar on them, even overnight, seems to degrade the adhesive used to attach the mesh to the inside of the cod-end (Figures 2a and 2b), especially in the increasingly hot weather.

Thorough inspection of your nets and cod-ends is recommended prior to every sampling trip. You should look for weak attachments to the ring and lines at the top of the net, small holes and tears in the net, detached mesh inside the cod-end, broken threads on the cod-end, etc. You should also check that the metal clamp securing the cod-end to the net is tight, and that the screw securing the weight on the cod-end is tight. Small holes (less than 1 mm) in the net can be repaired with super glue.



a. *Detached mesh in a cod-end.* b. *Broken threads on a cod end.*

15. Can vinegar be poured down the drain? If not, what are the disposal procedures?
 - A. It is best to refer to the SDS and your local area chemical disposal procedures for guidance. However, the vinegar you are using in the field is no different than the vinegar you use in your home and is generally considered safe to discard down the sink. It is recommended that you run water while discarding in this manner, but this isn't a requirement. DO NOT dump vinegar on the ground, even in a paved parking lot, as most areas of the US treat storm water differently than municipal sewer water. Again, your best bet is to refer to the SDS and your local community guidance.

16. At reservoirs where dedicated nets are used, is the vinegar soak really needed? It's all the same water, and if a mussel is found, it seems that follow-up sampling would be needed even if the mussel was captured during a prior sampling event.
 - A. First-time-findings require a lot of information to be provided to our partners during the notification process. The vinegar soak between sampling sites is a standard community practice and is necessary even at negative waterbodies. If a mussel were to be found, this practice allows for an immediate, localized response if appropriate.

Additionally, we look for mussel tissue as well as the shell, and this may not be present if the mussel was captured during a prior sampling event.

17. Do I need to have a dedicated net for each site I sample, even if they are on the same waterbody?
- A. This is ultimately your agency's decision. The EcoLab has dedicated nets for each waterbody, but not for each sample location on that waterbody.
18. Can I wait until the end of the day and decontaminate all my nets at the same time?
- A. No. Due to the risk of contamination, particularly in the close confines of a vehicle, the best practice is to decontaminate all equipment as you move through your sampling trip.
19. It is basically impossible to collect a Field Blank with 1 liter of water – it pours through the net and onto the floor. Is there another procedure that can be used? For example: running copious tap water through the net with a hose, capturing water with the cod, and proceeding as for a sample. Or soaking the net in a tub of water, then pulling sample from that, rinsing 3 times with fresh water between nets.
- A. The goal of collecting the Field Blank is not so much about collecting the full 1 L of water – it is about collecting any DNA that might be remaining on the net from the previous collection event. The water is meant to wash this DNA into the cod-end for collection, so it is fine if you don't collect the full volume used in the rinse. Additionally, Field Blanks should be collected in the field prior to the first sample tow. It is important that you do not use tap water to collect Field Blanks as there may be contaminants in the water, including DNA (see question 13 above).

The EcoLab suspends the net, so the cod-end is off the ground, then pours DI water around the inside top of the net, getting as much of the inside of then net wet as possible (Figure 3). Whatever collects in the cod-end is our Field Blank.



Figure 3. Collecting a Field Blank

20. The SOP says that when drying clean nets, don't let them touch each other. Is this a best management practice or a requirement? Separate drying and storage is logistically challenging. If nets are clean, it shouldn't matter if they touch.
 - A. The EcoLab's recommendation is to keep positive and negative equipment, including nets, wet or dry, as separate as possible. Our end-of-day procedure is to rinse all nets thoroughly with tap water, then hang them side-by-side to dry. Once dry, each net is neatly folded, placed in a dedicated cloth storage bag, and packed side-by-side in a trip box. However, we do not have ANY positive equipment in our facility, so your situation may be different.