WESTERN REGIONAL PANEL

On Aquatic Nuisance Species

Zebra and Quagga Mussel Field Sampling and Monitoring Protocol

Zebra mussels (*Dreissena polymorpha*)

Quagga mussels (*Dreissena bugensis*)

WESTERN REGIONAL PANEL
ON AQUATIC NUISANCE SPECIES

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PURPOSE

The goal of Western Regional Panel is to protect limited western aquatic resources by preventing the introduction and spread of non-native nuisance species into western marine and freshwater systems though the coordinated management and research activities of state, tribal, federal, commercial, environmental, research entities and other regional panels.

The zebra and quagga mussel field sampling and monitoring protocols are used for both early detection and long-term monitoring. The protocol includes sampling techniques aimed at three stages of the mussels' life cycle: veliger, settler, and adult. Mussel veligers (i.e. free-floating microscopic larvae) are sampled via plankton tows. Settlers (i.e. juveniles) and adult mussels are sampled via substrate samplers and other direct observations such as shoreline surveys. Samples can be analyzed using both visual and molecular techniques. Please see WRP's document *Lab Standards for Dreissena Analysis* (2018) for more information on laboratory analysis protocols and standards.

This document provides guidance on the minimum recommended standard operating procedures (SOPs) for utilizing plankton tow, shoreline and substrate surveys to identify the presence of dreissenid mussels (e.g. quagga/zebra mussels) and monitor population growth over time. These SOPs cover when and where to sample, tow types, substrate materials, sampling effort, sampling methods, sample collection and preservation, and equipment decontamination.

The methods described herein are not intended for the sampling or analysis of free-floating DNA in aquatic environments ("eDNA"), nor are the sample handling procedures described adequate to prevent sample contamination from occurring in the field or laboratory. Methods that would be appropriate for eDNA sampling and analysis are outside the scope of this document.

Plankton sampling is conducted for the purpose of detecting the presence of dreissenid mussel veligers. It is one of several methods that can be used for the early detection of dreissenid mussels and can be used alone or in combination with other monitoring methods. Use of this method depends on the goal of monitoring, resources that are available to devote to monitoring, and the expectations for accuracy of monitoring results (i.e., the tolerance for error). Where as substrate and shoreline monitoring in a sampling program can be used to detect incipient populations of dreissenid mussels and is an effective method for evaluating water body status following detection of dreissenid veligers in plankton samples.

This protocol describes the recommended method for sampling using plankton tows, substrates and shoreline surveys, specifically targeting dreissenid mussel (quagga and zebra; taxonomic family *Dreissenidae*) veligers1 for cross-polarized light microscopy (CPLM) followed by a molecular technique such as polymerase chain reaction (PCR) to confirm the identification of a veliger detected by optical methods.

For many authorities responsible in managing dreissenid mussels and/or waterbodies, the presence of dreissenid mussel (veligers and/or adult mussels) triggers significant regulatory response requirements. This sampling and monitoring protocol along with subsequent analytical methods appropriate to identify juveniles and/or adults is intended to provide reliable, science-based information on which to base management decisions.

[1] "Veligers" are the microscopic, free-floating (planktonic) larval life stage of dreissenid mussels.

INTRODUCTION

Monitoring can be prioritized based on perceived levels of risk, and are generally defined by individual states and management agencies. Levels of risk, often described on a qualitative scale of high, medium, and low, are often based on various factors such as intensity of use (described by metrics including the numbers of boats using the reservoir, boat ramps, marinas, out of state boat use, complex boat types, etc.), water chemistry (both CHALK and trophic variables), and proximity to an infested or suspect water. Currently, there is no standardized method for assigning dreissenid mussel risk of introduction or establishment, and monitoring efforts are prioritized based on relative levels of risk and constraints of resources available for monitoring.

Before any sampling is initiated, it is highly recommended that sampling staff contact the laboratory that will be analyzing the samples to discuss specific requirements for sample preservation, transportation, and shipping, as these requirements may vary by laboratory.

To optimize the potential for detecting the presence of mussels, plankton tows as well as substrate (i.e., deployed artificial substrates or opportunistic survey of other artificial substrates) and shoreline monitoring should follow standardized sampling methods; sample a large area or multiple sites and target the seasons and locations where mussel populations are most likely to first occur or to reach densities where detection is likely. Samples must also be preserved and handled properly to ensure bio-security and facilitate expert identification.

Watercraft, equipment, tools and all gear used in sampling and monitoring in waters classified as suspect, positive, or infested for quagga/zebra mussels should ideally be dedicated to that water, and definitely be fully decontaminated before use in any other water body.

When multiple age classes of dreissenid juveniles and/or adults are found during shoreline surveys, this is usually indicative of an established, reproducing population (i.e., 'infested' status). One possible exception to this indication would be sites where the presence of multiple age classes may be indicative of consistent propagule pressure from an upstream source (e.g., in rivers downstream of infested impoundments), without which the population would not persist. When individuals of only one size class are detected, additional sampling will be needed to determine whether an established, reproducing population persists.

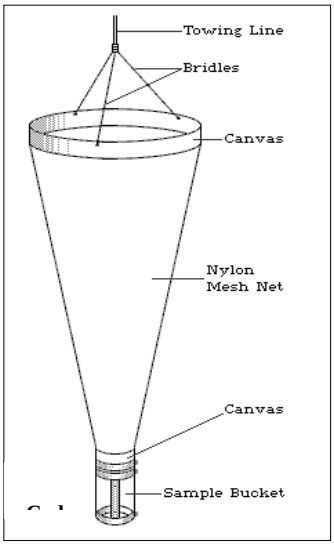

Figure 1: Simple Conical

Plankton sampling filters out organisms, and any other matter in the water, that is too large to fit through the mesh of the net. Organisms may include algae, protozoa, arthropods, including dreissenid mussel veligers, that enter the net. Plankton sampling can be used for early-detection monitoring for dreissenid mussel veligers, as well as for monitoring veliger density in infested water bodies.

Plankton samples are collected by pulling a fine-mesh net through the water column henceforth referred to as a "tow." To effectively sample for dreissenid mussel veligers the plankton net must have a mesh size of not less than 64 micrometer (µm) and not more than 65 µm. Figure 1 provides an illustration of a typical plankton-net. In addition, Appendix A provides a list of generally accepted equipment and supplies necessary for field sampling utilizing plankton nets. Ideally each water body sampled should have a dedicated set of equipment. This will insure samples and water bodies are not cross-contaminated, and avoid the added time and wear-and-tear on equipment from decontamination after each use. If dedicated

gear is not possible, minimally water bodies classified as suspect, positive, or infested with dreissenid mussels should have dedicated equipment that is kept isolated from nets and gear used for early-detection monitoring at water bodies believed to be un-infested. Decontamination procedures for sampling gear can be found in Appendix B.

WATER BODY CLASSIFICATION

In 2013, the WRP's Building Consensus Committee determined guidelines for classifying water bodies in which zebra or quagga mussels have been detected. The classification structure is as follows:

- **Unsampled** – Water body is not being sampled or monitored for AIS.
- **Undetected/Negative** - Sampling/testing is ongoing and nothing has been detected, or nothing has been detected within the time frames for de-listing.
- **Inconclusive** (temporary status) - Water body has not met the minimum criteria for detection.
- **Suspect** – Water body that has met the minimum criteria for detection.
- **Positive** – Multiple (2 or more) subsequent sampling events that meet the minimum criteria for detection.
- **Infested** – A water body that has an established population of AIS.

The **minimum criteria for detection** are 2 independent lab results from the same sample using scientifically accepted techniques (e.g. microscopy, PCR, gene sequencing, taxonomic identification).

In addition to classification of detected waters, the WRP also determined timelines and requirements for "de-listing" waters.

- **Inconclusive** – 1 year of negative testing including at least one sample taken in the same month of subsequent year as the positive sample (accounting for seasonal environment variability) to get to undetected/negative.
- **Suspect** – 3 years of negative testing to get to undetected/negative.
- **Positive** – 5 years of negative testing to get to undetected/negative.
- **Infested** – Following a successful eradication or extirpation event including a minimum of 5 years post-event testing/monitoring with negative results to get to undetected/negative.

WHEN AND WHERE TO SAMPLE

Plankton Water Temperature

Plankton monitoring targeting dreissenid mussel veligers is typically conducted when water temperatures are above 9 °C (48 °F), when spawning may be occurring. In warmer regions, where water temperatures remain above 9 °C throughout the year, it is recommended plankton sampling be conducted at least monthly, especially in medium to high risk water bodies. Water body risk assessment varies from agency to agency and state to state consequently sampling frequency needs to be addressed by individual basis for every water body.

Plankton Location

Veliger distribution can be highly localized; therefore, to increase the potential for detection, sampling should occur throughout the water body at multiple sites. Sampling sites should include areas of high use and likely sites of mussel introductions such as around boat docks, boat launch ramps, floating restrooms,

marinas, at inlets and outlets of the water body (e.g. mouth of tributaries; dams), and in the downwind areas and eddies which can be identified by the accumulation of leaves, pollen, and debris on the surface of the water. Sampling should also be conducted in both open water and near shore, if both habitats are present. The goal of the sample should be that it be representative, in size and diversity of areas, of the entire water body.

A minimum of three sampling sites is recommended per water body. The number of tows at each site should be based on the net diameter and the depth of each tow, with the goal of a minimum total volume of 1000 liters (L) per site filtered through the net. Based on the diameter of the net and depth of each tow, the number of tows per site to filter 1000 L can be calculated using Appendix C.

Table 1: Summary of Plankton Sampling Recommendations

Parameter	***Recommendation***
Water Temperature	≥9 °C (≥48 °F)
Waterbody Locations	Around floating structures, marinas, inlets and outlets, coves, downwind areas and eddies
Depth to Sample	15 m (50 ft) but will depend on depth of waterbody and the depth of the thermocline
# of sampling sites per waterbody	Variable; based on the size of waterbody and diversity of area; minimum of 3 sites
# of tows per sampling site	Variable; based on depth and net size
Total volume sampled	Minimum 1000 L per site

Substrate and Shoreline Water Temperature and Monitoring Season
Dreissenid veligers begin to settle out of the water column 2-3 weeks after spawning and develop a mussel-shaped shell 3-5 weeks after spawning. Spawning occurs between 9°-18°C (48°-64° F). Therefore, it is recommended that substrate checks begin as early as 3-5 weeks after water bodies have reached spawning temperatures (i.e., warmed to ≥9C or cooled to ≤18C) and veligers begin to settle out of the water column and continue for at least two months, possibly longer, after temperatures are no longer conducive to spawning. In regions where water temperatures remain within this range throughout the year, mussels can spawn year round. Volunteers may be used to increase the frequency of sampling on low risk water bodies but, depending on state laws, may not be able to possess and transport specimens for identification.

Substrate and Shoreline Water Level
Artificial substrates should be placed in locations where water level variability does not affect probability of settlement and where substrates remain immersed year-round (see location section). Shoreline surveys should consider variation in water level and should occur when water levels are declining, rather than rising, such as after events such as drawdowns or seasonal fluctuations.

Substrate and Shoreline Location
Distribution of early mussel populations can be highly localized; therefore sampling should occur throughout the water body at multiple sites to increase the potential for detection. These include likely sites of mussel introductions such as around marinas, boat docks, boat launch ramps, floating restrooms,

inlets and outlets of the water body (e.g. mouth of tributaries; dam tailraces), and in the downwind and downstream areas (e.g., dams) and eddies or slackwaters. Such areas are especially important in riverine sites where dreissenids may only form reproducing populations in lentic areas (Churchill and Quigley 2017) which can be identified by the accumulation of leaves, pollen, and debris on the surface of the water or the presence of lowhead dams or other barriers to flow. Research suggests that the probability of veliger detection at marina sites is ~72%, compared to ~52% at non-marina sites (Zehfuss 2010) and marina substrates (e.g., encapsulated floats) rise and fall with water levels and survey of these substrates or sampling substrates attached to marinas are not subject to the same limitations as shoreline surveys. Artificial substrate deployment should occur in both open water and near shore, with the number and location of sites selected in such a way to represent the entire water body. Sampling sites may be co-located at or near sites where plankton tows are conducted for efficiency and likelihood of detection, as priority sites for plankton sampling are often conducive to settlement and detection of settled mussels.

Artificial Substrate Deployment Depth

Depth of placement for artificial substrates should be based on individual characteristics of the water body as settlement of juvenile and adult mussels as well as density of attached mussels at different depths depends upon factors such as temperature, oxygen and food supply (Navarro et al., 2009). It should also be noted that multiple artificial substrates can be deployed at multiple depths on a single line**.** During times when water body stratification occurs, the greatest dreissenid mussel attachment—or survival of attached mussels (i.e., due to low oxygen in the hypolimnion)—occurs in the epilimnion and the mesolimnion (highly variable, but approximately 6-32 m; Muetang et al. 2010). During the warmest months, surface water temperatures in some areas may exceed the physiological tolerances of dreissenid mussels, and dissolved oxygen levels in the hypolimnion may be too low for survival (particularly for zebra mussels). Artificial substrates should therefore be placed in the epilimnion or mesolimnion at depths of 6 meters or greater when possible, especially in water bodies with high summer surface water temperatures.

PLANKTON TOW METHODS

Plankton tows can be made in one of two ways, based on the type of the waterbody.

- **Vertical Tows** - For waterbodies with little flow and depth greater than 4m, tows are made by lowering the net to the desired depth and pulling it back vertically. Tow depths of 15m (49.2 feet) are recommended for vertical tows.
- **Horizontal Tows** - For waterbodies less than 4m, or that are flowing (drainage ditches, pipes, rivers, streams, etc.), oblique or horizontal tows are recommended. Horizontal tows are made by releasing the net in the flow and either holding it stationary, or by pulling it back at an oblique angle or horizontal to the surface of the water.

Note: Guidance for calculating tow volumes for each tow method is provided in Appendix C.

When taking tows be sure to do so safely. If you are conducting the tow from a boat, anchor/secure the boat at the sampling site and make sure the boat is not drifting. If conducting the tow from a stationary position (e.g. dock, shore), make certain that you have stable footing. Before deploying the net examines both the net and line to be sure the cod end is securely attached and the tow line is free of tangles.

Vertical Plankton Tow Procedure (From Boat or Dock)

This method is preferred because it samples all (or up to 15m of deeper waterbodies) of the water column, maximizing the potential that dreissenid mussel veligers will be captured if present.

1. Calculate the appropriate tow length for water bodies less than 15m deep.
2. Determine the water depth (in meters) using your watercraft depth finder or a marked rope.

3. Subtract (at least) the length of your plankton net plus 0.5m from the depth. This length will prevent your net from hitting the bottom of the waterbody, disturbing the sediment, and fouling your sample. Large amounts of sediment interfere with sample analysis.
4. Lower the net to the appropriate depth. (Tip: If air is trapped in the net, lower the net slowly with the opening tilted to the side.) Wait approximately 10 seconds for the cod end of the net to settle to the prescribed depth before proceeding to step 4. As previously mentioned, be careful not to hit bottom with the net. If you hit bottom, raise the net, thoroughly clean out the net and cod end, and redo the tow.
5. Slowly pull up the net using a hand-over-hand motion, no faster than 0.5m per second. Raise the net until the opening is out of the water but the cod end is still submerged. Keeping the opening out of the water, pulse the net up and down several times as you finish pulling it up – this flushes the plankton off the sides of the net and into the cod end.
6. Remove the net from the water and carefully unscrew the cod end from the net without spilling any of the collected sample. You can concentrate the plankton sample by gently swirling the cod end and allowing some of the water to drain out through the mesh. Pour the plankton sample into a leak-proof polyethylene bottle (e.g. Nalgene®). Thoroughly rinse the cod end several times using a spray or squirt bottle filled with tap water. Pour the rinse water from the cod end into the sample bottle. Be sure to rinse out the cod end after each tow.
7. Repeat the vertical tow procedure in order to collect replicate samples at the same location. All dreissenid veliger tows from the same sampling site or waterbody may be composited into a single bottle, if needed to reach a minimum volume sampled or to save sample processing expenses. Be sure to allow adequate space in your sample bottle for EtOH and baking soda solution if the sample will be preserved (70% empty for preserving at 70% EtOH).
8. Clearly label the sample bottle as appropriate and store in an ice chest.

Oblique or Horizontal Plankton Tow Procedure (From a Moving Boat or in Flowing Water)

If sampling in moving water that is not deep enough or wide enough to deploy the plankton net via rope, hold the net beneath the surface and allow the water to flow through it. Record the amount of time that you held the net in the water, the percentage of the net opening that was under water, and the rate of flow of the water.

1. If appropriate, attach a weight (1-2 kg [2-4 lbs]) to the line immediately in front of the net opening to help keep the net below the water surface.
2. Hold the net by the mouth. Make large loops with the line and hold it loosely in the same hand that is holding the net. Firmly grasp the other end of the line with your free hand.
3. Throw the net using a sidearm-style, opening your hand upon release to allow the line to feed out with the net.
4. Allow the net to sink into the water to within 0.5-1m of the bottom. Your goal is to pull the net on a diagonal path from bottom to top, sampling as much of the water column as possible without letting the net or cod end hit the bottom. If an air bubble gets trapped in the net, retrieve the net and start again.
5. Slowly pull in the net using a hand-over-hand motion, no faster than 0.5 meter per second. Keep the net and cod end off the bottom to avoid both collecting debris and snagging the net. If you hit bottom, gently pull in the net, thoroughly clean out the net and cod end, and redo the tow.
6. Raise the net until the opening is out of the water but the cod end is still submerged. Keeping the opening out of the water, pulse the net up and down several times as you finish pulling it up – this flushes the plankton off the sides of the net and into the cod end.
7. Remove the net from the water and carefully unscrew the cod end from the net without spilling any of the collected sample. You can concentrate the plankton sample by gently swirling the cod end and allowing some of the water to drain out through the mesh. Pour the plankton sample into a leak-proof

polyethylene bottle (e.g. Nalgene®). Thoroughly rinse the cod end several times using a spray or squirt bottle filled with tap water. Pour the rinse water from the cod end into the sample bottle.

8. Repeat the oblique/horizontal tow procedure in order to collect replicate samples at the same location. All dreissenid veliger tows from the same sampling site or waterbody may be composited into a single bottle, if needed to reach a minimum volume sampled or to save sample processing expenses. Be sure to allow adequate space in your sample bottle for EtOH and baking soda solution if the sample will be preserved (70% empty for preserving at 70% EtOH).
9. Clearly label the sample bottle as appropriate and store in an ice chest.

SUBSTRATE MATERIALS AND METHODS

Artificial substrates which may be surveyed opportunistically include encapsulated and un-encapsulated marina floats, buoys, bridge pilings, docks, and other manmade substrates. Floating, manmade substrates are not subject to periodic desiccation and so may provide enhanced detection probability, although high summer temperatures may reduce mussel attachment and survival in surface waters.

There are a variety of artificial substrate materials and designs that are appropriate for sampling. Plate-style artificial sampling substrates (i.e., Hester-Dendy samplers) are commercially available and offer the added benefit of facilitating quantification. The simplest artificial sampling substrates may consist of a brick or a concrete block suspended from a rope. There are a variety of other designs for artificial substrate sampling devices that vary from PVC tube style (e.g., Portland samplers) to scouring pads framed/fortified with Plexiglas. Research has shown no significant preference for mussel attachment by artificial substrate material or shape, with the exceptions that mussels prefer to attach to dark surfaces and do not attach well to galvanized steel. See Appendix D for substrate design and information.

Biofilm accumulation on newly deployed sampling devices takes approximately two months to occur and is important for zebra mussel attachment but may not be necessary for quagga mussel attachment (Marsden 1992, Wainman et al, 1996, Kavouras and Maki 2003, Mueting et al. 2011).

Table 2: Summary of Artificial Substrate Sampling Recommendations

Parameter	***Recommendation***
Timing	beginning 3-5 weeks after water bodies have warmed to ≥9C or cooled to ≤18C and continuing for ~2 months after temperatures are no longer conducive to spawning
Waterbody Locations	Around floating structures, marinas, inlets and outlets, boat ramps, docks, coves, downwind areas and eddies
Artificial Substrate Depth	Epilimnion or mesolimnion; ≥6 meters

Artificial Substrate Check Protocol

Retrieve deployed artificial substrate slowly, place substrate into a plastic tub and closely examine substrate for settler and/or adult mussels. First visually inspect the substrate and then attempt to gently

push any attached organisms that might be a mussel. If no mussels are observed, then redeploy the substrate without removing any algal growth or biofilm from the substrate.

SHORELINE SURVEYS

Shoreline surveys can be conducted using natural substrates including rocks, wood, vegetation and other debris. Similar to an artificial substrate, sampling staff examines the shoreline materials searching for invasive mussels.

Table 3: Summary of Shoreline Sampling Recommendations

Parameter	***Recommendation***
Timing	beginning 3-5 weeks after water bodies have warmed to ≥9C or cooled to ≤18C and continuing for ~2 months after temperatures are no longer conducive to spawning
Waterbody Locations	Identify locations representative of waterbody and with physical access and appropriate substrates for settlement.
Water level	Variable: Ideally at low water levels or soon after water levels decline

Shoreline Survey (& Opportunistic Artificial Substrate Survey) Protocol
Examine rocks, wood, vegetation and other debris or other existing substrates for settlers and/or adult mussels. When necessary, retrieve substrates (e.g., rocks) slowly for closer examination. Examine marina or pier/dock floats, buoys, or other artificial substrates for juveniles and/or adults.

PONAR GRAB SAMPLING

Petite Ponar Sediment Grab Sampler
Deploy the sediment dredge in areas of gravel, small cobble, sand and mud in water depths up to 6m (20ft). Then engage the spring-pin into dredge and carefully lower the dredge keeping tension on the rope. Lower until the dredge settles in or on the bottom, and then quickly jerk the rope up to trigger the dredge. You can feel the dredge deploy. Quickly retrieve dredge and dump contents into a metal sieve and rinse in the lake. Inspect sieve for bivalves and snails. Record GPS location and sampling activities at each site on a datasheet provided. Retain suspect specimens in a 250mL sample container or zip lock bag with lake water. Record the sample container numbers in the datasheet and place sample in cooler on ice.

Sample Quality Assurance/Quality Control (QA/QC)

Proper training and evaluation of field staff can help reduce the risk of data error or improper preservation. Data sheet checks can also be performed to reduce error.

Equipment Decontamination

Generally, equipment decontaminations between sites on the same waterbody occurring on the same day are not necessary, unless the waterbody is suspect or positive for invasive mussels. In the event the waterbody is suspect for mussels, decontamination of sampling equipment can assist in isolating the location within the waterbody where veligers could be occurring.

Watercraft and trailers should be decontaminated in between each water body following the WRP's *Student Training Curriculum for Watercraft Inspectors and Decontaminators to Prevent and Contain the Spread of Aquatic Invasive Species in the U.S.A.* available at https://www.westernais.org/level-2-inspection-and-decon.

SAMPLE PRESERVATION

Plankton Preservation Techniques

Plankton samples that are not immediately analyzed must be preserved, buffered, and then kept refrigerated to prevent the decomposition of biological material and calcium carbonate. This method is suitable for dreissenid mussel veliger microscopic and PCR analysis, shipment via air carriers, and does not require special storage due to flammability concerns. The following preservation method is recommended, however should be preapproved and compatible with the procedures of the laboratory that will be performing the analysis. Check with your laboratory prior to sampling.

When preserving field samples, alcohol and a buffer of either baking soda or liquid Trisaminomethane-Ethylenediaminetetraacetic Acid (Tris) should be used. Either 4M Tris pH7.5 to 8.0 OR baking soda may be used to buffer the pH of the sample. Buffering the sample will prevent drops in pH that can result in degradation of veligers' calcium carbonate shells (Meara 2013). Controlling the pH is important as a low pH will begin to degrade any veliger shells and may make identification of veliger larvae in samples impossible. Tris buffer is a preferred buffer as it is better at buffering the solution and does not precipitate out of solution. However, if Tris buffer is not available, baking soda should be used. Only one of these buffers should be used in your samples. Do not use both Tris and baking soda.

- For 4M Tris pH7.5 buffer, use a 1 mL plastic transfer pipette to add 2 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

  Source: Standard Operating Procedure: Field Sampling Methods for Invasive Mussel Early Detection, Bureau of Reclamation Ecological Research Laboratory, May 2020.

Next, alcohol (preferably absolute ethanol or Ethyl Alcohol 200 Proof, or EtOH 200 Proof) should be added to the sample for preservation. Avoid using denatured EtOH or isopropyl alcohol (rubbing alcohol) as these may interfere with analysis or pose a health risk to laboratory staff.

The finished, preserved sample needs to be at a 70% alcohol content. Lower alcohol percentages (below 90%) may not allow you to reach the end goal of 70% alcohol in the sample. 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 (*Standard Operating Procedure: Field Sampling Methods for Invasive Mussel Early Detection*, Bureau of Reclamation Ecological Research Laboratory, May 2020).

Do not freeze plankton samples. Freezing damages shells and reduces detection sensitivity.

70% EtOH

1. After tows have been poured into the collection bottle, mark the level with a permanent marker and measure the height (H) of the liquid using a ruler with millimeter (mm) graduations.
2. Divide H by 0.95. This measurement (mm) is the level to which the baking soda solution is added. This will be a relatively small quantity. A small cup should be used to pour the baking soda solution into the sample to avoid adding too much.
3. Divide H by 0.76. This measurement (mm) is the level to which absolute ethanol is added.
4. Pack all sample bottles in an ice chest with ice packs and keep cool until they can be stored in a refrigerator.
5. Deliver or ship the samples in an ice chest with ice or blue ice packs to the laboratory as soon as possible after collection. If possible, same day or overnight delivery is recommended.

Substrate Specimen Collection and Preservation

If suspect or confirmed mussels are found, collect voucher photographs of each specimen. If quantification is desired, remove and preserve entire substrate if possible. Place specimen in a container and preserve in 70% ethanol or refrigerate (optional) immediately after collection to prevent degradation or decomposition. Do not add a buffer to plant or animal samples that are not plankton tows.

All samples should be clearly labeled with water body name, sample location name and coordinates, date, collector's name/initials, affiliation and the % absolute ethanol. If ethanol is not available, place in a container without water, label, and refrigerate.

Contact your State or Province AIS Coordinator to report the detection immediately.

REFERENCES

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An evaluation of artificial substrates for monitoring the quagga mussel (*Dreissena bugensis*) in Lake Mead, Nevada-Arizona (Mueting et al. 2011)

APPENDIX A. PLANKTON EQUIPMENT LISTS

Plankton Sampling[2]

1. Boat, if needed
2. Plankton net: simple, conical plankton tow net, 63-64 μm mesh size, 0.3 m (1 ft) diameter net opening, removable, weighted cod-end piece. See Figure 1.
3. Line for deploying the net: 30 m (100 ft), marked at one-meter inter`vals.
4. Measuring tape or ruler (with mm marks)
5. Field data sheets that includes at a minimum date, time, sampler/agency, volume, water temperature, pH, GPS location, net diameter, and number of tows.
6. Water quality instrument multi-probe (optional)
7. Water thermometer and pH strips (minimum)
8. Global positioning satellite unit (recommended)
9. Waterproof marker and appropriate writing instrument for data sheets
10. Sample bottles: polyethylene material with sufficient volume for sample with screw lid. 500mL or 1L volumes are generally appropriate and available.
11. Preservative: absolute ethanol (EtOH), also called Ethyl Alcohol 200 Proof or EtOH 200 Proof
12. Baking soda solution or Tris-EDTA
13. Sealable plastic bags (Ziploc®), if needed
14. Ice chest with ice or blue ice packs

Equipment Decontamination

1. Personal protective gear
2. Large tubs (~18 g) (1-3 required)
3. White vinegar or 5% acetic acid solution
4. Bleach
5. Water (tap and/or deionized)
6. Spray bottles

[2] Prior to sampling, check with laboratory for preservation requirements.

APPENDIX B. WATERCRAFT AND PLANKTON EQUIPMENT DECONTAMINATION

All gear and equipment that comes into contact with a waterbody must be decontaminated prior to re-use, even if used in the same waterbody. If possible, dedicated plankton tows nets and equipment should be used for each waterbody. For waterbodies that are suspect or know to have dreissenid mussels, separate sampling equipment is strongly recommended. As with sample preservation methods, decontamination protocols should be recommended and/or reviewed by the laboratory that will be analyzing your samples prior to sampling.

Use of chemicals (bleach and vinegar) and hot water present a safety concern. Adequate and appropriate personal protective gear should be worn at all times when working with chemicals and hot water. Consult Material Safety Data Sheets (MSDS) for appropriate safety procedures. Bleach also rapidly degrades gear and equipment, and inadequate rinsing of bleach from the equipment can lead to false-negative PCR results. Therefore, thoroughly rinse gear after decontamination.

Location
Decontaminations (using either hot water or chemicals) must be conducted such that runoff does not reach any waterbody. Decontamination solutions must be properly discarded away from any waterbody, into a drain that goes to a wastewater treatment facility or is appropriately stored for reuse. If possible, decontaminate equipment after returning from the field.

Watercraft Decontamination
Watercraft, trailers, fenders, lines, anchors, and any other equipment that contacts the water should be fully decontaminated between each waterbody utilizing 60°C/140°F water as recommended in *Uniform Minimum Protocols and Standards for Watercraft Inspection and Decontamination Programs for Dreissenid Mussels in the Western United States* (Elwell and Phillips 2016), or check with your appropriate state agency for other recommended method for watercraft decontamination. Also, check with your state water pollution control regulators to ensure decontamination does not violate state water quality regulations.

Equipment Decontamination
Note: Water quality meters should only be cleaned by methods recommended by the manufacturer. Chemical or hot water decontamination may damage the sondes commonly used in water quality meters.

Method #1 below provides a generally acceptable and common equipment decontamination method and is based on guidance developed by California Department of Fish and Wildlife (CDFW 2017). Method #2 is based on guidance developed by U.S. Geological Survey (Sepulveda 2017; Wilcox et al. 2016).

Equipment Decontamination Method #1 – For Decontamination of Calcium Carbonate and DNA
This method uses a two-step process of vinegar to dissolve calcium carbonate (such as veliger shells), and bleach to denature DNA. The vinegar must be used before the bleach to ensure DNA is exposed to the bleach.

1. Place items to be decontaminated into an 18 gallon rubber tote.
2. Fill the tote with enough household vinegar to completely cover all of the items.
3. Soak the items in vinegar for a minimum of 1 to 2 hours (2 hours is preferred).
4. Note: The vinegar can be reused multiple times. It is recommended that vinegar be poured back into the original container for storage. The vinegar should be periodically checked with pH test strips to make sure the pH level remains at approximately 2-3.
5. After soaking in vinegar thoroughly rinse the items in tap water.
6. Decontaminate the items with a 10% bleach solution (Appendix C).

7. Spray the items with a 10% bleach solution and allow the items to sit for 10 minutes, or
8. Soak the items in a 10% bleach solution prepared in a rubber rote or similar type container and allow the items to sit for 10 minutes.
9. After the bleach treatment, thoroughly rinse the items in tap water.
10. Allow the items to air dry completely between uses.

APPENDIX C. PLANKTON SAMPLING CALCULATIONS

Vertical Tow Volume
Tow volume (L) = Area of the net mouth (m2) x tow depth (m) x 1000 liters

Plankton net diameter and the corresponding area (m2) of the net mouth, used to determine the minimum tow depth required to achieve a 1000 liter tow volume.

Net Diameter	Area of Plankton Net Mouth	Minimum Tow Depth to get 1000 Liters Total Volume
13 cm (5 in)	0.01 m2	100 m (328.0 ft)
20 cm (8 in)	0.03 m2	33.4 m (109.5 ft)
30 cm (12 in)	0.07 m2	14.3 m (46.9 ft)
50 cm (20 in)	0.20 m2	5.3 m (17.3 ft)

Example: A 30 cm net is used to collect 3 x 20 meter tows. All 3 of the tows are dispensed into the sample collection bottle.
0.07 m2 x 60 m x 1000 L/m3 = 4200 liters of source water represented in the bottle

Horizontal Tow Volume
It can be difficult to determine horizontal volume. An estimate can be made in the same way vertical tow volume is calculated: use the length of the tow in meters multiplied by the hoop area in square meters then multiplied by 1000 L/m3.
Record the length of time the net was held in the water, the percentage of the net opening that was under water, and the rate of flow of the water.

Preparation of a 10% bleach (sodium hypochlorite) solution:
Use the following formula to prepare a 10% bleach solution
Total volume of solution desired x 0.1 = volume of bleach to add
Example: Add 50 milliliters of bleach to 450 milliliters to prepare a 10% bleach solution (V/V). A measuring cup can be used to measure the bleach and water at a 1:10 proportion. It is recommended that the bleach solution be prepared in an opaque approximately 32 oz spray bottle. The opaque bottle will help protect the bleach from degradation.

Preparation of a 4% baking soda (sodium bicarbonate) solution:
Use the following formula to prepare a 4% by weight (W/V) solution:
Desired volume in ml x 0.04 g baking soda = grams of baking soda to add
Example: to make a 1 liter solution of 4% baking soda solution, add 40 grams of baking soda to 1000 milliliters (1L) of deionized water. A standard 28 mm soda bottle caps holds about 5 grams of baking soda and ½ teaspoon of baking soda is about 3 grams. These values can be used to prepare a solution that is approximately 4% baking soda. For example, adding a level soda bottle capful of baking soda to a 250 ml Nalgene container that is approximately ½ full with water would provide a solution of baking soda close enough to 4% that it could be used to adjust the pH of plankton tow samples.

Common Conversions
To convert feet to meters multiply by 0.3048
To convert inches to centimeters multiply by 2.54
To convert cubic meters to liters multiply by 1000

APPENDIX D. SUBSTRATE MATERIALS AND DESIGN

Plate Style Samplers

- Hester-Dendy Sampler
https://streamsidescience.usu.edu/ou-files/ezplug/uploads/Who_lives_in_the_water/How_to_make_a_Hester-Dendy_-Who_Lives__in_the_Water.pdf

PVC/ABC Samplers

- Portland Sampler
- https://www.pdx.edu/oregon-lake-watch/sites/www.pdx.edu.oregon-lake-watch/files/OLW%20AIS%20Protocols_6.25.13.pdf

APPENDIX E. EQUIPEMENT FOR SUBSTRATE AND SHORLINE SURVEYS

Equipment List for Artificial Substrate Survey

1. Substrates: PVC, rope (nylon, paracord?), weights, buoys, etc.
2. Large plastic tub
3. Sample bottles: polyethylene material, 500 mL or 1.1 pt volume with screw lid
4. Decontamination materials: Large bucket (>5 gal), white vinegar or 5% acetic acid solution; 1% beta-iodine solution, tap water, spray bottles containing 5-7% solution of household bleach, towels and scrub brush.
5. Gloves for handling substrates to prevent cuts from sharp shells; should also be easy to use for decontamination (e.g., thick rubber)
6. Preservation: absolute ethanol (EtOH) or ≥151 proof Everclear. Do not use denatured ethanol or isopropyl alcohol.
7. Waterproof marker and pencils
8. Cooler with cubed, crushed ice or ice packets (optional)
9. Electrical tape (optional)
10. Field data sheets that includes at a minimum date, time, sampler, affiliation, volume, water temperature, pH, and GPS location
11. Global positioning satellite unit: (recommended
12. Boat (optional)
13. Sealable plastic zipper bags
14. Tweezers or small spatula
15. Magnifying glass
16. Measuring tape or ruler (optional)
17. Camera

Equipment List for Shoreline Surveys

1. Sample bottles: polyethylene material, 500 mL or 1.1 pt volume with screw lid
2. Decontamination materials: see above and thick rubber gloves
3. Preservation: absolute ethanol (EtOH) or ≥151 proof Everclear. Do not use denatured ethanol or isopropyl alcohol.
4. Field data sheets that includes at a minimum date, time, sampler, volume, water temperature, pH, GPS location
5. Sealable plastic bags (Ziploc)
6. Petite Ponar sediment dredge
7. Cooler with cubed, crushed ice or ice packets (optional)
8. Tweezers or small spatula
9. Metal Sieve
10. Measuring tape or ruler: (optional)
11. Global positioning satellite unit: (recommended)
12. Waterproof marker and pencils
13. Large tubs (~18 gallon)

APPENDIX F. WATERCRAFT AND SUBSTRATE EQUIPMENT DECONTAMINATION

Watercraft and Equipment Decontamination

The following are various options that can be utilized to decontaminate monitoring equipment and watercraft. All equipment must be decontaminated prior to re-use even if on the same water body.

Watercraft Decontamination

Sampling boats and trailers should be fully decontaminated between each water body. Protocols can be found in Chapter 5 of the *Student Training Curriculum for Watercraft Inspectors and Decontaminators to Prevent and Contain the Spread of Aquatic Invasive Species in the U.S.A.* available here or at www.westernais.org.

Equipment Decontamination

All sampling equipment should be fully decontaminated between each water body.

Decontamination Supplies

- Decontamination unit for hot water decontaminations.
- Decontamination solution for chemical decontaminations.
- Clean tap water (do not use lake or stream water) to make the decontamination solution and for rinsing equipment.
- Large tub or bin for submerging equipment.
- Stiff bristle scrub brush.
- Rubber gloves – It is recommended that you wear rubber gloves when handling quaternary ammonia.

Equipment Decontamination Locations

Conduct hot water decontaminations in a "high and dry" location, where the decontamination water will not run off into any water body. Chemical decontaminations must be conducted at least 60 meters (200 feet) away from a water body. Decontamination solutions must be properly discarded away from any water body, into a drain that goes to a wastewater treatment facility.
Note: Water quality meters should only be decontaminated with water. Chemical decontamination may damage the Sonde.

Equipment Decontamination Options

Option 1: Hot water
Note: Hot water is the preferred method of decontamination. It is preferred that equipment be submerged in hot water first, so that they may remain submerged while the boat and trailer are being decontaminated.

- Equipment - Secchi Disk, Ropes, Waders, Boots, Dip Nets, Crayfish Traps, Anchor, etc.
    1. Thoroughly rinse equipment with clean water. Use a stiff bristle scrub brush to remove all debris, mud, and plant material.
    2. Place the equipment in a large tub or bin.
    3. Fill the bin with 140°F water at low pressure until all equipment is completely submerged.

- Quaternary Ammonia (*CPW Quaternary Ammonia Compound Disinfection Protocols*, 2015)
    - Bath Disinfection Recommendations for Submersion of Small Gear and Waders:
        1. Prior to disinfection, clean debris, mud, and vegetation off of equipment and waders.
        2. Muddy disinfectant solution can lose its effectiveness and capacity to kill invasive organisms.
        3. Visually inspect waders and equipment for New Zealand mudsnails and other invasive aquatic organisms prior to cleaning.
        4. The recommended minimum active QAC concentration for effective disinfection is 0.4% or 4.0 ml of QAC per L of water; amount of disinfectant per gallon varies, and is dependent upon the percent active QAC in the disinfectant being used (Table 1).
        5. Equipment and waders should be submerged in disinfectant solution for a minimum of 10 minutes.
        6. Follow all handling instructions on disinfectant label or Material Safety Data Sheet (MSDS).Vinegar solution
    - Spray Disinfection Recommendations for Cleaning off Small Gear and Waders using Disinfectant Spray:
        1. Prior to disinfection, clean debris, mud, and vegetation off of equipment and waders.
        2. Visually inspect waders and equipment for New Zealand mudsnails and other invasive aquatic organisms prior to cleaning.
        3. The recommended minimum active QAC concentration for effective spray-application disinfection is twice that for submersion disinfection, 0.8% or 8.0 ml of QAC per L of water (Table 2).
        4. Equipment and waders should be fully covered in disinfectant solution for a minimum of 10 minutes. Reapplication may be necessary if hot (evaporative) or wet conditions dilute spray solution on equipment.
        5. Follow all handling instructions on disinfectant label or MSDS.
- Drying
    - Dry equipment for a minimum of 10 days in between each use.
- Freezing
    - Place equipment in a freezer for 48 hours or a minimum overnight between each use


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