

**Sample Collection Protocols
for
Dreissenid Mussel Monitoring:
Planktonic Larvae, Juveniles, and Adults**



Prepared for:
Oregon Department of Fish and Wildlife

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1. SAMPLING PROCESS DESIGN

1.a. Sample Locations

1.a.1. Veliger Plankton Tows

Veliger samples should be collected from a boat, if possible, at approximately ten sites per sampling date. A boat allows the sampling to be independent of land-accessible structures, e.g. docks. Collect one plankton tow at each site. A site represents a discrete location, e.g. every 100 linear feet along a transect. More than ten sites can be sampled to increase the likelihood of collecting veligers, but this may require additional sample containers due to an increased density of algae and zooplankton. Samples collected from different sites can be composited together into a single sample container. A typical sampling event, involving ten 16 m (53 ft) plankton tows taken from the Columbia River, will yield approximately three 500 mL (1.1 pt) plankton samples that are preserved in a 70% solution of ethanol. The density of algae and zooplankton will vary within and between water bodies. Spread out the sites to further increase the likelihood of collecting veligers. In lakes and reservoirs, focus sampling near dams, intakes, outflows, inflows, marinas, boat launches, and in areas that are downwind, downstream, open water and near shore. In large streams and rivers, focus sampling in the main stem, downwind and downstream areas and in near shore areas around boat launches, marinas and other structures that create eddies.

1.a.2. Adults and Juvenile Artificial Settlement Substrates

Settlement substrates should be deployed in continuous or intermittent water flows less than 6 ft/ s (1.8 m/ s) in areas that have a means to secure the substrate rope (e.g. eyebolt, hand railing), and provide safe access during inspections. The efficacy of settlement substrates is proportional to the surface area available for colonization, and multiple substrates may be deployed at each project to increase the likelihood of detecting mussels. Deploy multiple substrates in different areas to further increase the likelihood of collection. Settlement substrates should be continuously submerged, at depths ranging from 5 to 25 ft (1.5 to 7.6 m), preferably at depths greater than 20 ft (6.1 m). Mussel settlement is highest at water flows between 1.6 to 2.3 ft/ s (0.5 to 0.7 m/ s), but dreissenid mussels tolerate flows ranging from 0.02 in/ s to 6.0 ft/ s (0.05 cm/ s to 1.8 m/ s). Dreissenid mussels colonize water depths ranging from the surface to several hundred feet, and although there is variation within and between species as well as between water bodies, the highest density of settled mussels is typically at a depth of approximately 25 ft (7.6 m). Suggested deployment locations for settlement substrates include docks, piers, buoys, booms, and breakwaters.

1.a.3. Existing Surfaces

Existing submerged surfaces such as concrete walls, pilings, rocks, etc. offer a readily available large surface area for mussel colonization and should be examined for adult and

juvenile settlement. Existing surfaces can be actively sampled using a surface scraper, and/ or tactile and visual inspections. Existing surfaces can be inspected during maintenance and other work activities.

1.b. Sampling Frequency

1.b.1. Veliger Plankton Tows

Veligers can exhibit spatial and temporal patchiness in the water column and high sampling frequency (weekly or biweekly) increases the likelihood of collecting veligers. Additionally, repeated detection reduces the likelihood of a false positive. The optimal time to sample veligers in North America is when peak spawning is occurring, which varies with adult population density and location. On the average, optimal time to sample veligers is when water temperatures are between 61° and 66°F (16° and 19°C), the June to September period, especially July and August. Veliger sampling can be performed anytime during the day but preferably not immediately following a storm event. Storm events can increase water turbidity and hence the time required to process the sample.

1.b.2. Adult and Juvenile Settlement Substrates

The frequency of inspecting settlement substrates and existing substrates is either monthly, as dictated by existing maintenance schedules, or as time allows. Temporal patterns of veliger settlement usually parallel temporal spawning patterns, and the time period between spawning and settlement is typically 15 to 20 days. Juveniles are generally found in the Midwest of North America between August and September. Substrates should be inspected year round, however, as year round reproduction is possible, and adult and juveniles translocate throughout the year.

1.c. Sample Collection

1.c.1. Veliger Plankton Tows

Vertical and/ or oblique plankton tows are recommended instead of horizontal plankton tows and pumped samples. Veligers have been found throughout the water column, ranging from near the surface to depths greater than 400 feet (122 m). The depth where peak veliger density occurs can vary within and between water bodies as well as between the different Dreissenid species. Pumped samples are more difficult to collect, requiring more equipment and time. The efficacy of pumped samples, however, is not affected by net clogging as it is with plankton tows (i.e. a pressure wave in front of net). Most studies have reported greater veliger densities with vertical and/ or oblique tows compared to horizontal tows. For these reasons, vertical plankton tows are recommended to collect a depth-integrated sample. Horizontal tows, however, have yielded greater densities in some cases (e.g. San Justo Reservoir, CA, *Dreissena polymorpha*). Horizontal tows may be required when lacking access to a boat. In these cases, horizontal tows can be done from a dock, pier, or the water body shoreline.

Do not keep plankton tows that contain large amounts of sediment. If the net is dragged across the water body bottom, large amounts of sediment can be captured. Large amounts

of sediment interfere with sample analysis, bind up preservative, and may damage sampling equipment. If your sample contains large amounts of sediment, dump the contents of net back into the forebay, thoroughly rinse net and cod-end piece in water body, and then repeat the tow. Some sediment (i.e. suspended solids) will be captured in plankton tows, and this small amount of inorganic debris is acceptable.

Individual plankton tows are composited into a single sample container because the objective of sampling is early detection and this reduces costs. The location of planktonic veligers is not related to the location of adult mussels. Compositing samples may help reduce the likelihood of false negative results in the field (i.e. failing to collect the veligers when they are present). Spatial information is lost with sample compositing, however, and this may limit the search for the original location of veligers (or possible adults).

1.c.2. Adult and Juvenile Settlement Substrates

Bivalves in freshwater that are firmly attached to a hard surface are cause for concern. Dreissenid mussels are one of the only freshwater mussels capable of adhering to hard surfaces using byssal threads (Figure 1). Another invasive freshwater mussel, *Limnoperna fortunei*, is also capable of adhering to hard surfaces via byssal threads; these mussels have not been detected in the United States. Additionally, brackish water mussels such as *Mytilopsis leucophaeata*, produce byssal threads and firmly attach to hard surfaces, and these on rare occasions, have been found in freshwater river reaches. Lastly, *Corbicula* spp. juveniles can weakly attach to hard surfaces using threads, but this phenomenon is temporary and associated with only small *Corbicula* spp. mussels. A bivalve firmly attached to hard surfaces in a freshwater habitat constitutes a fouling threat, regardless of the species, and byssal thread formation in freshwater can be used to quickly identify specimens that require greater scrutiny.

The density of settled mussels is greatest on the undersides and sides of objects as well as in nooks and areas of surface irregularity. Settlement is an active process that involves initial settlement, metamorphosis, and translocation. Dreissenid mussels do not appear to discriminate surfaces on which they initially settle. After completing metamorphosis, however, the mussels translocate to preferred substrates such as the undersides of objects and other protected locations. It is important, therefore, to inspect all surfaces while focusing on the nooks and crannies.



Figure 1: Quagga mussel showing byssal threads.

1.d. Data Reporting

Early detection monitoring results can be reported and viewed online through the Zebra and Quagga Mussel Monitoring Program coordinated by the Center for Lakes and Reservoirs at Portland State University. The Center for Lakes and Reservoirs maintains the online, interactive Zebra and Quagga Mussel Monitoring Map, and this displays data stored in the Portland State University online database, and shows who is monitoring what water bodies using what methods, and the status and date of the last reports regarding these efforts. The map can be accessed from the following URL:

[<http://www.clr.pdx.edu/projects/volunteer/zebra.php>](http://www.clr.pdx.edu/projects/volunteer/zebra.php)

Oregon Department of Fish and Wildlife staff performing monitoring will have the option of reporting settlement substrate and existing surface inspection results directly to the online database, and/ or reporting to either Rick Boatner, the Aquatic Invasive Species Coordinator with Oregon Department of Fish and Wildlife or Steve Wells using the contact information below.

Positive results for the presence of dreissenid adults and juveniles will be verified by Portland State University staff according to shell morphology. If a questionable organism is found (e.g. bivalve attached to hard surface), carefully remove the suspect specimen and place into a sealable plastic bag. Place bagged specimen in refrigerator for short term storage. Immediately call or email Steve Wells using contact information below for further instructions on species identification. You may be asked to take a digital photograph, compare to reference shells provided, and/ or mail specimen to Portland State University.

The Portland State University Dreissenid Veliger Laboratory verifies positive results for the presence of dreissenid veligers, excluding blind matrix samples, with interlaboratory comparisons. Digital photographs and shell measurements of the suspect specimen(s) are immediately sent to a minimum of two separate laboratories for verification. Laboratories contacted for verification will be asked for their opinions on the identification of the suspect organisms. General habitat information may be shared, e.g. collected from run-of-river reservoir, but all names and site identifiers will be kept confidential. Sample splits will be sent to these laboratories if requested. Upon confirmation of a positive veliger sample, sample results, including opinions of independent laboratories and that of Portland State University and associated digital photographs, will be immediately sent to Rick Boatner.

2. SAMPLING METHODS

2.a. Equipment

2.a.1. Veliger Plankton Tow

- Plankton net (simple, conical plankton tow net, 64 μm pore size-*the mesh size is critical*, 1 ft (0.3 m) diameter net opening, removable, weighted cod-end piece) (Figure 2)
- Line for deploying the net (100 feet on spool or about 30 m), marked on one meter intervals
- Sample bottles (polyethylene material, 500 mL or 1.1 pt volume, screw lid)
- Decontamination materials: Large bucket (>5 gal), white vinegar or 5% acetic acid solution (>5 gal), 1% beta-iodine solution, tap water (several gal), and two spray bottles containing 5-7% solution of household bleach, and tap water (do NOT use lake or river water), towels, and scrub brush
- Preservative (absolute ethanol (ETOH) OR liquor ≥ 151 proof = 75.5%, e.g. Everclear. Do NOT use denatured ethanol or isopropyl alcohol (rubbing alcohol))
- Field data sheets (waterproof paper), labels, and waterproof marker and pencils
- pH indicator strips
- Baking soda (sodium bicarbonate)
- Sealable plastic bags (e.g. Ziploc)
- Cooler with cubed or crushed ice
- Global Positioning Satellite unit (GPS) (*recommended*)
- Tweezers or small spatula (*recommended*)
- Boat (*recommended*)
- Multiprobe water quality instrument (e.g. Hydrolab®) (*recommended*)
- Measuring tape or ruler (*optional*)

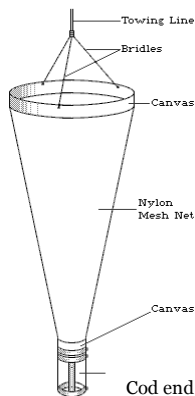


Figure 2: Simple conical plankton-tow net.

2.a.2. Adult and Juvenile Settlement Substrates

- *Settlement substrate (abs and pvc pipe sections suspended along a rope that is woven through a plastic mesh)
 - Sealable plastic bag (e.g. Ziplock)
 - Surface scraper (i.e. mesh metal bucket attached to pole) (*optional*) (Figure 3)
 - Field lens (10x total magnification) (*optional*)
- * Equipment provided by Portland State University

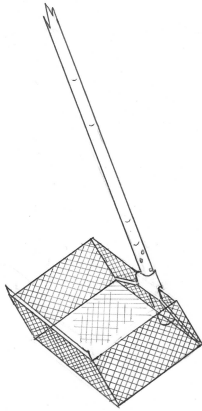


Figure 3: Surface scraper.

2.b. Sample collection

2.b.1. Veliger Plankton Tow

2.b.1.a. Vertical Plankton Tow

1. Secure the cod-end piece and check that the line is securely attached to plankton net.
2. Lower the net, cod-end first, 30.5 m (100 ft) below water surface, or to 1 m (3.3 ft) above the sediment, whichever is deeper. Record the depth the net is lowered. Ropes are marked on one meter intervals.
3. Keep net at this depth for 20 seconds and then manually retrieve using a hand-over-hand technique at a rate of 0.5 m/ s (1.5 ft/ s). Slow and steady retrieval is the key to collecting a good plankton tow.
4. Rinse the net by raising the net so that the cod end of the net is at the water surface. Rinse organisms into the cod end of the net by lowering the net back into the water, keeping the opening above the water surface. Then quickly pull net straight up; this action will move collected plankton into the cod-end piece. Repeat this procedure several times to ensure that all the organisms inside the net are in the cod end. If net is severely clogged, swirl cod-end piece, when still attached to net, to force excess water out and concentrate plankton in the cod end.

5. Carefully unscrew the cod-end piece without spilling collected water and plankton. Condense the sample as much as possible before pouring into sample bottle. Condense the sample by swirling the cod-end piece, forcing excess water out of the mesh in the cod end piece. You may need to use your finger, tweezers or a spatula to gently clear the mesh netting in the cod-end piece to allow the water to filter through. Rinse the cod-end piece with a spray bottle several times with minimal volume of water and put rinses into the same sample bottle.
6. It is important to record the number and length of tows so that the volume of river water filtered can be estimated.
7. The volume of water filtered is determined using the formula below, assuming a net filtering efficiency of 100% (i.e. no clogging). If clogging occurs, a pressure wave develops, and water will be forced to the surface prior to the net emerging from the water. If clogging occurs, first try reducing the depth of the tow. If it still occurs, estimate the net filtering efficiency and multiple the corresponding percent by the maximum volume of water filtered (e.g. 80% filtering efficiency means $0.80 \times V_m$).

Maximum volume of filtered water, V_m is

$$V_m = \pi * r^2 * d$$

where r = radius of the net opening (0.3 m) and d = depth to which the net is lowered (20 m). Multiply by 1000 to convert volumetric units from m^3 to L.

8. Place the sample on ice while continuing to collect additional plankton tows.

2.b.1.b. Horizontal Plankton Tow

1. You may attach a weight (1-2 kg or 2-4 lbs) to the line immediately in front of the net opening to help keep the net below the water surface.
2. Secure the cod-end piece and check that the line is securely attached to plankton net. Only use weighted cod-end pieces for horizontal tows.
3. Hold the ring of net, which is the metal loop that holds the net mouth open, using thumb and forefinger. Make large loops of the line and hold loosely with the same hand holding the net.
4. Firmly hold the other end of the line with free hand.
5. Throw the net using a sidearm-style, opening your hand upon release to allow line to feed out with the net.
6. Allow net to sink into water body. A weighted cod-end piece will aid in pulling the net into the water. If an air bubble gets trapped in the net, retrieve the net and start again.
7. Manually retrieve net using a hand over hand technique at a rate of 0.5 m/ s (1.5 ft/ s). Keep the net off the sediment to avoid both snagging and collecting debris.

8. Follow steps # 4 through # 8 used for vertical plankton tows.

2.b.1.c. Plankton Sampling Inside Facilities- Flow Valve

1. Open flow valve and purge system for at least two minutes.
2. Estimate the flow using a flow meter valve or calculate the time needed to fill a 5 gal bucket (gal/ min).
3. Position the plankton net so that the flow of water passes into the mouth of the net. Place the 5 gal bucket underneath the net to collect the water passing through mesh.
4. Record the time that water is entering net so that the volume of water being filtered can be calculated.
5. Follow steps # 7 through # 8 used for vertical plankton tows.

2.b.2. Adult and Juvenile Settlement Inspection

2.b.2.a. Artificial Settlement Substrate Deployment and Inspection

1. Settlement substrates are available from Portland State University. Upon receiving your settlement substrate please be sure that your substrate is complete and includes the pvc (white) and abs (black) pipe sections, and rope woven through plastic mesh. A heavy object may be attached to the washer on the bottom of the substrate string to anchor substrate. Dreissenid mussel shells are included to provide a reference for adult and juvenile settlement inspections.
2. Locate a suitable deployment location that provides a secure structure from which the substrate can be suspended.
3. Lower the substrate into the water to determine the depth at that particular site. The substrate at the end of the rope should rest on the sediment or be as deep in the water as possible. The ideal depth to suspend settlement substrates is 25 ft (7.6 m). Tie the end of the rope to the structure once the required length of rope is known.
4. When checking the substrate, remove it slowly from the water to avoid loss of mussels. Inspection for adults and juveniles is tactile and visual. You are looking for a bivalve attached to a hard surface. Small juveniles may feel gritty to the touch. A hand lens (10x magnification) may be used to assist visual inspections.
5. A biofilm will develop following submersion in natural waters. Do not remove biofilms because mussel settlement is greater on surfaces with biofilms compared to surfaces lacking biofilms. Freshwater sponges, however, should be removed.
6. If you think you have a dreissenid mussel, either carefully remove suspect specimen or remove the entire substrate, and place into a sealable plastic bag. Place bagged specimen in refrigerator for short term storage. Call or email Steve Wells using contact information below for further instructions on species identification. You may be asked to take a digital photograph,

compare to reference shells provided, and/ or mail specimen to Portland State University. Portland State University will reimburse shipping costs.

2.b.2.b. Existing Surface Inspection

1. Locate suitable existing surfaces to inspect. Structures in the water body such as concrete walls, navigation arm, bridge abutments, channel markers, pilings, underwater booms, and breakwaters are excellent choices for the surface scraper. Accessible surfaces (i.e. within arms reach) are good candidates for visual and tactile inspections. These relatively easy-to-access surfaces include the walls of adult fish ladders, metal grates/ fish screens, wood and plant material collected on screens, the undersides and sides of docks, vessel hulls, buoys, and the underside and sides of rocks found in shoreline areas.
2. If sampling from a boat, carefully position vessel near structure to sample (e.g. channel marker) and maintain position either using the motor, securing boat to structure with bow line, or using current and wind to position boat against structure.

WARNING: Be careful not to pin arms between the boat and structure. Beware of conditions on the water.

3. When using the surface scraper, lower it into the water as deep as the pole will allow. Using both hands on the pole, bring the metal rim of the mesh box in contact with the vertical substrate surface and quickly pull up, keeping the metal rim in contact with the vertical surface to be sampled. The sessile communities collected in the mesh are inspected for the presence of bivalves in the field. Questionable organisms are handled according to protocols outlined in section 2.b.2.a. step #6.
4. Repeat step #3 at multiple locations per structure in order to sample a representative portion.
5. When performing visual and tactile inspections on structures, carefully pat surface with the palm of your hand. Do not run your hand along surfaces because of sharp objects. Remove hard protruding objects for visual inspection. Handle questionable organisms according to section 2.b.2.a. step #6.

2.c. Labeling and Associated Parameters

Record the following information on both the sample bottle label and the field datasheet. Use a waterproof permanent marker for bottle label and a pencil for datasheet. Be careful because permanent marker ink will smear when in contact with ethanol. For backup, the author recommends recording the sample bottle information on a piece of waterproof paper using a pencil and inserting into the sample bottle.

- Date of collection

- Water body name
- Sample location (GPS is available or detailed description)
- Number and length of tows
- Type of tow (vertical, horizontal, etc)
- Name and agency of person collecting sample
- Preservative and concentration used (e.g. 70% ETOH)
- Mark sample bottle with two lines using permanent marker, one for the level of sample prior to adding preservative and the final level of preserved sample (sample + preservative).

It is highly recommended to collect the following metadata with plankton samples. Most of these data can be easily measured using a multi-probe unit (e.g. Hydrolab). Calibrate multi-probe units according to their manuals. Record metadata in field datasheets.

1. Water temperature (°F or °C) and depths of reading
2. pH
3. Specific conductance (µS/ cm)
4. Wind speed (two minute average, MPH)
5. Secchi depth reading (ft)
6. Turbidity (if available on multi-probe unit)

2.d. Sample Preservation

Preserve veliger samples and suspect adult specimen(s) immediately after collection to ensure sample integrity. Samples that cannot be preserved immediately after collection should be placed on ice until preservative can be added. Do NOT wait more than three hours to preserve samples. Preserve veliger samples using absolute ethanol (ETOH). ETOH is the preferred preservative for veliger samples. Do NOT use denatured ETOH because it appears denatured ETOH dissolves the calcite in veliger shells much faster than absolute ETOH. Do NOT use Lugol's solution as it contains acetic acid and will dissolve veliger shells. Do NOT use isopropyl alcohol because it may interfere with molecular analytical methods.

Suspect adult and juvenile specimen(s) can be placed into a sealable plastic bag and temporarily stored in the refrigerator. Ethanol (ETOH) is the preferred chemical preservative for adult and juvenile specimen(s). Denatured ethanol and isopropyl alcohol are acceptable chemical preservatives for adult and juvenile mussel samples. When using alcohol as a preservative, use stock that is 70% alcohol and greater, and add enough preservative so that specimen(s) and/ or associated substrate are completely submerged. Other chemical preservatives not listed may be acceptable, but check with Portland State University prior to using.

Preserve veliger samples in a final solution of 70% ETOH. There are monitoring programs that preserve veliger samples in a final solution of 25% ETOH, but most

studies use concentrations between 50% and 70% ETOH. Additionally, veliger samples preserved in a final solution of 25% ETOH must be stored on ice and kept refrigerated prior to and following analysis.

To make 70% solution of ETOH with veliger sample:

1. Make sure sample bottle is $\frac{1}{4}$ or less full. If needed, pour some sample into another sample bottle. Tighten cap and thoroughly mix sample prior to pouring into another sample bottle.
2. Allow sample to settle until water level is constant.
3. Mark the level of sample on the outside of sample bottle using a permanent marker.
4. Using a stock solution of 95% ETOH, add three parts ETOH to one part sample to achieve a final concentration of approximately 70% ETOH. A ruler or measuring tape may be placed alongside the sample bottle to help estimate the ratios.
5. Mark the level of final solution containing sample and preservative on outside of sample bottle using a permanent marker. Tighten cap and shake sample to mix preservative and sample.
6. Measure the pH of the preserved sample using a pH color indicator strip. Immerse the colored sections of the pH indicator strip until there is no further color change (1 – 10 min.). Compare the moist color sections of indicator strip to the references provided on the box. If the pH is less than 6.9, add a small amount (less than 1.0 g) of baking soda (sodium bicarbonate). Secure lid and shake to dissolve baking soda. Measure pH again to ensure pH is above 6.9 but less than 9. If pH becomes too high, add a small amount of white vinegar (5% acetic acid) and measure pH again.

WARNING: Add baking soda and vinegar in small increments to avoid overshooting target pH.

NOTE: The calcite in the veliger shells can erode at pH values less than 6.9 and this impacts plankton analysis. Plankton samples preserved in 70% ETOH collected from Columbia River at Bonneville and John Day and the Snake River at Milner had initial pH values of 5, 5 and 5.5, respectively. After adding 1.0 g baking soda to each, pH values were raised to 7.5.

2.e. Sample Handling and Shipment

Samples preserved using a final solution of 70% ETOH may be stored in a cool, dry place up to three months prior to analysis. A previous study showed that veliger densities in 80% ethanol remained constant up to three months. An ongoing study at Portland State University suggests no change in veliger densities in samples preserved in 70% ETOH and buffered to a pH 7.0 and stored at room temperature for over one year. Samples that lack chemical preservative must be refrigerated prior to analysis. Refrigeration is a

temporary means to preserve biological samples. Avoid placing samples in direct sunlight or freezing conditions. For long term storage, place samples preserved in 70% ETOH in the refrigerator.

Ethanol (ETOH) and other alcohols are Class 3 flammable liquids and there are restrictions regarding its transport. ETOH can only be transported on the ground/ surface. Do not fly in an airplane with ETOH. Keep preserved samples in a plastic container such as a bin or cooler in the back of the car while in transit. ETOH can be mailed but there are training, certification, labeling and shipping requirements. Mail ETOH-preserved samples via ground or surface mail using USPS and/ or FedEx according to the protocols below, which allow exemptions for training and certification. USP will not accept Class 3 liquids.

USPS Protocols for mailing ETOH:

1. Samples must be in plastic containers with a screw lid. There can be multiple containers but the total volume of the entire package CANNOT exceed 473 mL. Secure screw lids.
2. Place all containers into a sealable plastic bag (e.g. Zip Lock) and then place this bag into another sealable plastic bag.
3. Place sealed bags and sample containers into box and add cushioning material such as grocery bags or scrap paper. Seal this box with clear packing tape. The box does NOT need to be a specific type of box so long as it is sturdy.
4. Place this box into another box and add cushioning material as needed. The outer box does NOT need to be a specific type of box either, so long as it is sturdy. Seal box with clear packing tape.
5. Make sure the return address sticker is on the side of the box with the information shown below:

Surface Mail Only Consumer Commodity ORM-D Flashpoint = 55.6°F
--

Mail via USPS domestic surface transport as Standard Mail or Parcel Post to:

Center for Lakes and Reservoirs, ESR
PO Box 751
Portland Oregon 97207-0751
Attn: Steve Wells

FedEx Protocols for mailing ethanol:

1. Samples must be in plastic containers with a screw lid. The volume in each container cannot exceed 30 mL (1 oz). Secure screw lids.
2. A maximum of 16 containers per box. The total volume in all the containers can NOT exceed 500 mL.

3. Place all containers into a sealable plastic bag (e.g. Zip Lock) and then place this bag into another sealable plastic bag.
4. Place sealed bags and sample containers into a box and add cushioning material such as plastic grocery bags or scrap paper. Seal this box with clear packing tape. The box does NOT need to be a specific type of box so long as it is sturdy.
5. Place this box into another box and add cushioning material as needed. The outer box does NOT need to be a specific type of box so long as it is sturdy. Seal box with clear packing tape.
6. Make sure the return address sticker is on the side of box with the information shown below:

This package conforms to 49 CFR 173.4

7. Mail via FedEx ground transport to:

Steve Wells
Center for Lakes and Reservoirs
Science Bldg 2, Room 246
1719 SW 10th Ave
Portland OR 97201

2.f. Decontamination

Field equipment must be decontaminated at the site to prevent transfer of organisms within and between systems and samples. It is strongly recommended that each water body being sampled have a dedicated set of equipment. The plankton net, however, will always require decontamination to prevent the cross contamination of other samples. The plankton net, cod-end piece and affiliated rope are decontaminated by spraying with 5-7% bleach solution (spray twice in 10 minute period), thoroughly rinsing with fresh water, and totally submerging and soaking in a solution of 5% acetic acid (i.e. white vinegar). Bleach denatures protein and acetic acid dissolves the calcite in the shells of veligers. The ideal soak time is 24 hours and the minimum soak time is two hours. Equipment is thoroughly rinsed with clean water in a spray bottle before and after the vinegar soak. The acetic acid bath may be reused.

Multi-probe sampling units (e.g. Hydrolab) are thoroughly rinsed with fresh water. Mud and debris are rinsed off the unit using tap water in a spray bottle, and then the entire unit is soaked in fresh water and shaken dry. Probes are stored in fresh tap water.

The boat bilge, hull, through-hull fittings, anchor, anchor lines, and bow line are decontaminated using a combination of scrubbing, oxidizing and non oxidizing chemicals

and towel drying. To make a 5-7% bleach solution (i.e. approximately 0.05 mL of active chlorine per L of water assuming 10% of bleach solution is active chlorine), add 7oz (a little less than 1 cup) of household bleach to 1 gal (16 cups/ 128 oz) of water. The 5 to 7% bleach solution is carefully poured into a spray bottle and applied to hull and through-hull fittings on pavement or concrete a minimum of 200 ft (61 m) from open water. Scrub surfaces with brush to loosen and remove debris. Rinse with tap water. Engine cooling water is decontaminated by lowering lower engine unit into a bucket containing 1% beta-iodine solution, and running engine for two minutes. Fill bucket so that iodine solution is level with cavitation plates. Fresh towels are used to remove remaining debris and to dry hull, through-hull fittings and propulsion system following the application of the bleach solution, scrub brush, and iodine solution. Iodine solution is poured into bilge and allowed to sit for a minimum of 30 minutes. Ropes, anchors and anchor lines can be soaked in iodine solution in boat bilge or in a large bucket (>5 gal) containing either a bleach or acetic acid solution. Bleach and acetic acid are corrosive and equipment must be thoroughly rinsed with tap water following decontamination.

3. CONTACT INFORMATION

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