British Columbia Dreissenid Mussel Lake Monitoring Field Protocol

BRITISH COLUMBIA

Ministry of Environment and Climate Change Strategy

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Front cover photo credit: Ministry of Environment & Climate Change Strategy.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Glossary

Epilimnion: The upper, wind-mixed layer of a thermally stratified lake (Water on the Web 2011).

Eutrophic lakes: A very *biologically productive* type of lake due to relatively high rates of nutrient input (usually phosphorus and nitrogen) which leads to excessive plant growth - algae in the open water, periphyton (*attached* algae) along the shoreline, and macrophytes (the higher plants we often call *weeds*) in the nearshore zone (Water on the Web 2011).

Hypolimnion: The bottom, and most dense layer of a stratified lake. It is typically the coldest layer in the summer and warmest in the winter (Water on the Web 2011).

Lentic: Body of standing water, ranging from ditches, seeps, ponds, seasonal pools, basin marshes and lakes.

Lotic: Refers to bodies of water with flowing water (e.g. rivers, streams)

Metalimnion: The middle or transitional zone between the well mixed epilimnion and the colder hypolimnion layers in a stratified lake. This layer contains the thermocline (Water on the Web 2011).

Secchi Disk: A disk with a 4-6 inch radius, divided into 4 equal quadrates of alternating black and white colors. It is lowered into a section of shaded water until it can no longer be seen and then lifted back up until it can be seen once again (Water on the Web 2011).

Spring turnover: Period of complete or nearly complete vertical mixing in the spring after ice-out and prior to thermal stratification (Water on the Web 2011).

Thermal Stratification: when a lake is broken into distinct horizontal layers due to changes in water temperature which leads to differences in density (Water on the Web 2011).

Thermocline: The depth at which the temperature gradient is steepest during the summer; usually this gradient must be at least 1^{0}C per meter of depth (Water on the Web 2011).

Veliger: the microscopic free-swimming larvae (veligers) stage of the dreissenid mussel life cycle.

1. INTRODUCTION

Monitoring is critical for early detection of new invasive species incursions in British Columbia (BC) and is an important first step in the Provincial Early Detection Rapid Response (EDRR) plan[1]. Zebra (*Dreissena polymorpha*) and quagga (*Dreissena rostriformis bugensis*) mussels (hereafter referred to as Dreissenid), are two freshwater invasive species that are not currently found in BC but pose significant environmental and economic risks if they were to be introduced. For more information about zebra and quagga mussels please visit www.gov.bc.ca/invasivemussels.

BC is a signatory on the *100th Meridian Initiative's Columbia River Basin (CRB) Interagency Invasive Species Response Plan: Zebra Mussels and other Dreissena spp*. (Heimowitz and Phillips 2008). Under this Plan the Province is committed to prevention efforts to mitigate the risk of Dreissenid mussels in BC and across the Pacific Northwest. As part of this commitment the Province has been conducting early detection monitoring for Dreissenid mussels since 2011. BC is one of the many jurisdictions across North America conducting early detection monitoring and active prevention efforts for Dreissenid mussels. Furthermore, as a signatory on the CRB Interagency Invasive Species Response Plan the Province is committed to following the minimum standard protocols for Dreissenid mussel prevention efforts including watercraft inspection and decontamination protocols and early detection lake monitoring protocols.

The Dreissenid mussel life cycle consists of three primary life stages; veligers, juveniles and adults and the appropriate sampling method varies depending on the target life stage for early detection monitoring efforts. The sections below outline the appropriate field sampling methods for the different target life stages of Dreissenid mussels. The applicable protocols pertaining to field sample preservation and equipment decontamination for Dreissenid mussel monitoring are also outlined below.

In 2018 the Habitat Conservation Trust Foundation (HCTF) launched a granting program to fund lake monitoring in BC for the presence of Dreissenid mussels. This granting program is in partnership with the BC Ministry of Environment and Climate Change Strategy (ENV). The grants provide funding for sampling for the presence of Dreissenid mussels and must follow the protocols outlined in this document. This includes protocols for conducting plankton tows, deploying substrate samplers, and preserving and shipping of samples to the ENV designated lab.

This protocol serves as an update to the *2021 BC Dreissenid Mussel Lake Monitoring Protocol* to reflect the minimum recommended protocols being used by other jurisdictions in the Columbia River Basin. ENV staff have worked closely with HCTF to update this protocol based on feedback received from the 2021 granting cycle. To complete the 2022 HCTF grant application this protocol must be read in detail. Please refer to the HCTF Invasive Mussels Monitoring Grant guidelines and application instructions for complete instructions on filling out the grant application.

[1] The Zebra and Quagga Mussel Rapid Response Plan for British Columbia can be downloaded from https://www2.gov.bc.ca/assets/gov/environment/plants-animals-and-ecosystems/invasive-species/invasive-mussels/prov_zqm_edrr_plan.pdf

2. DREISSENID MUSSEL IDENTIFICATION

Dreissenid mussels are small, triangular bivalves. Adults are typically 1- to 3-cm (0.4- to 1-inch) in shell length and juveniles range between 350-µm to ~5-mm in size. Shell color varies but they usually have black and white stripes, although some are all dark and others are cream colored to light orange (Figure 1 and Figure 2). When sampling for adult Dreissenid mussels pay attention that any native mussels present should not be disturbed (Figure 3). For more information on zebra and quagga mussels please visit the provincial website.

Figure 1. Invasive Dreissenid mussels with scale reference (Photo: BC ENV)

Figure 2. Size comparison between invasive zebra and quagga mussels (top) versus native mussels (bottom) (Photo credit: BC ENV)

The BC Species and Ecosystems Explorer contains information on mussel species present in BC, including their federal and/or provincial threat status. The Rocky Mountain Ridged Mussel (*Gonidea angulata*)

(Figure 3) is native to BC and it was listed as Special Concern under SARA in 2003 and assessed as Endangered by COSEWIC in 2010. Its presence is limited to the Columbia River system and its tributaries, including the Okanagan and Kootenay rivers. It is trapezoidal in shape, ~12.5 cm long and ~0.4 cm wide. Any live specimens of a species at risk should not be disturbed. Collect photographs, and the location (GPS coordinates preferred) and other information of the specimen and submit it to the BC Conservation Data Centre (CDC) as soon as possible (see Appendix A for reporting information). Additional information on identifying native freshwater mussels in BC is available on the CDC website and more information on other native freshwater molluscs in BC can be found here.

Figure 3. Mountain Ridged Mussel (*Gonidea angulata*) native to BC. Photo credit: The Xerces Society

Marine (Blue) Mussels

The blue mussel (*Mytilus edulis*) is a marine mussel that only survives in salt water. Just like the dreissenid mussel the blue mussel has byssal which allows them to attach onto solids surfaces including boats. Watercraft traveling from saltwater to freshwater could transport blue mussels into freshwater. Dead marine mussels have been mistaken for dreissenid mussels due to the small size of juvenile blue mussels (< 5cm). The blue mussel can be identified by the following characteristics (see Figure 4):

- Outside shell is glossy bluish or bluish black, sometimes pale brown.
- Inside is generally violet in color.
- Pear-shaped and ventrally flattened shell.
- Typically full size is 5-10cm, but can range from 2cm to 20cm.
- Presence of byssal threads

Blue mussels do not survive in freshwater but it is still important to practice Clean, Drain, Dry when moving your boat from salt water to freshwater. **If you observe any suspected invasive mussels contact the Provincial Invasive Mussel Defence Program immediately by calling the Conservation Officer Service Report All Poachers and Polluters Hotline 1-877-952-7277 (RAPP).** This allows the trained Provincial inspectors to respond quickly and take the appropriate steps to identify the mussels and prevent any unnecessary concern.

Figure 4. Marine blue mussel (*Mytilus edulis*) on the left and right.

Other invasive species:

There are two invasive species already present in BC; the invasive freshwater clam (*Corbicula fluminea*) and the New Zealand mudsnail (*Potamopyrgus antipodarum*) that could be mistaken for dreissenid mussels during adult substrate sampling.

Invasive freshwater clam (*Corbicula fluminea*):

- Native to Southeast Asia and parts of central and eastern Australia, Africa, Indonesia and Turkey.
- Shell is triangular shaped and usually less than 2.5 cm but up to 6.5 cm in length, and yellow-green to light-brown in color with elevated growth rings.
- They are found in brackish to freshwater rivers, lakes, streams, canals and reservoirs, in the sediment surface or slightly buried in silt, sand or gravel substrates.
- They are filter-feeders, and prefer flowing water and they have a low tolerance for polluted or near freezing water temperatures.
- Confirmed locations in BC in the Lower Fraser River, the Pitt and Coquitlam Rivers, southern-Vancouver Island and Shuswap Lake.
- A factsheet is available for download on the BC Inter-Ministry Invasive Species Working Group website
- Juvenile *C. fluminea* can resemble the native fing ernail/pea clam species (Sphaeriidae family) but the shells of the native clam are thinner and oval shaped and brown to gray in color.

New Zealand mudsnail (*Potamopyrgus antipodarum*):

- Freshwater snail native to New Zealand.
- Are small with cone shaped shells and 5-8 whorls on shell with a pointed end
- Grow up to 8 mm in length and 4mm wide and range from light brown to black in colour
- Prefer vegetated habitat close to the shore of lakes and low flowing streams with silt and organic substrate. This species has high tolerance to various water quality, temperature (above freezing), and salinity levels.
- Confirmed location in BC is the Somass River near Port Alberni on Vancouver Island.

Figure 5. Invasive freshwater clam (*Corbicula fluminea*) (left) and invasive New Zealand mudsnails (*Potamopyrgus antipodarum*) (right). Photo credit: Portland State University.

Figure 6.Comparison between invasive freshwater clam (*Corbicula fluminea*) top and invasive dreissenid mussels (bottom).

3. DREISSENID VELIGER SAMPLING

3.1 Plankton tows

Veliger samples are collected using a plankton net and conducting vertical and/or horizontal tows. Due to their small size dreissenid mussels will pass through mesh > 65µm therefore to effectively sample for dreissenid mussel veligers the plankton net must have a mesh of 64µm (max 65µm), and 30cm to 50cm diameter net mouth opening must be used (Figure 6).

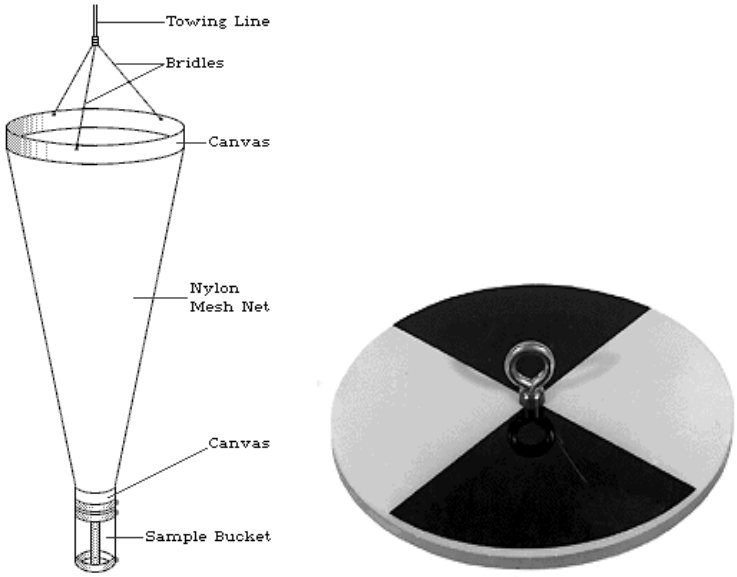

Figure 7. Plankton net used for collecting veliger samples and secchi disk used for measuring secchi depth.

3.2 Where to Sample

The number of sample sites per waterbody should be scaled to the size and complexity of the waterbody. For some of the larger and more complex waterbodies a list of specific priority sample sites have been provided in the table of Priority Waterbodies, these sites are provided as a minimum. A minimum of three site replicates/plankton tows are recommended at each sample site. When choosing locations for site replicates/plankton tows, try to select the number and location in such a way to represent a diversity of areas in the entire water body (i.e. open water and near shore habitats; Figure 7). Site replicate/plankton tow locations should include high use and near shore areas such as marinas, boat launches, docks, at inlets and outlets of the waterbody (e.g. mouth of tributaries; dams) and in downwind areas and eddies which can be identified by the accumulation of leaves, pollen and debris on the surface of the water. Samples should be collected from a boat, if a boat is not available sampling can take place from a dock and preferably from public docks/marinas with high boat traffic. When sampling from a dock please use a vertical plankton tow if depth permits or a horizontal plankton tow in shallower depths. Sampling should be done in a downstream direction to minimize decontamination of equipment and reduce the risk of contamination of upstream sites. Whenever possible sampling should start at upstream sites and move in a downstream direction.

If resources do not permit three site replicates/plankton tows per sample site it is critical that a minimum of 1000L of water is filtered through the net for each sample site.

Plankton tows for each sample site should be combined into one sample container. The number of tows at each sample 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 sample site filtered through the net. The number of tows per sample site to filter 1000 L can be calculated using the equation below.

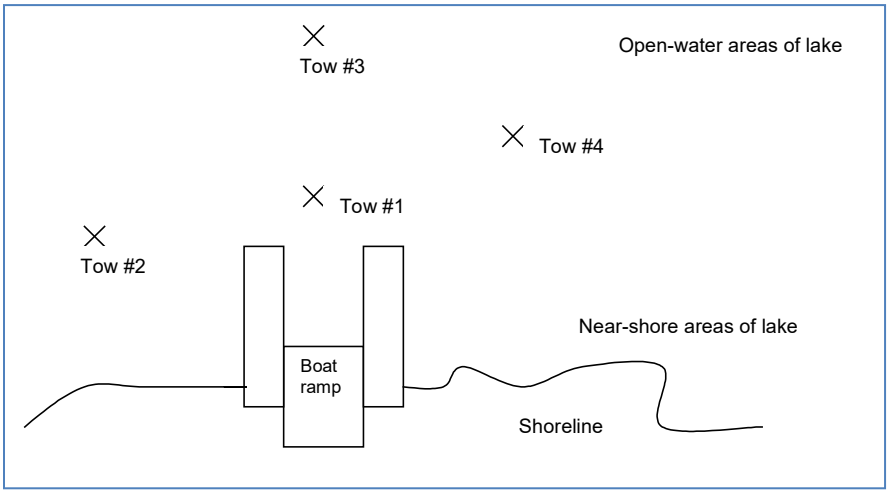

Figure 8. An example of plankton tow collection at a site location

Vertical Tow Volume

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

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

Table 1. 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 1000L Total Volume
13cm (5 in)	0.01m2	100m (328 ft)
20cm (8 in)	0.03m2	33.4m (109 ft)
30cm (12in)	0.07m2	14.3m (46.9 ft)
50cm (20in)	0.2m2	5.3m (17.3 ft)

Net clogging can occur in highly eutrophic (productive) lakes when a thin layer of plankton, accumulates at the inner surface of the net. This blocks water from going into the net water and leads to under sampling. If clogging occurs, first try reducing the depth of the tow and if needed increase the number of tows to reach the minimum of 1,000L filtered per site. The depth of the tow can be adjusted to the secchi depth reading (see section 6 for instructions on taking seechi depth). The actual depths and number of tows should be adjusted depending on the sample site (site depth, location, stratification etc.) to reach the required minimum of 1,000L filtered per sample site.

Horizontal tow volume

An estimate of horizontal tow volume 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.

3.3 WHEN TO SAMPLE

Dreissenid mussels spawning can occur when water temperatures reach ≥9°C. Plankton tow sampling should begin when water temperature has been maintained at ≥9°C for a minimum of two weeks. Generally, sampling should occur from May to October period, with the focus of effort in the July to September when temperatures are optimal for spawning (12°C-18°C). However the timing of the sampling period will vary by waterbody based on suitable temperature levels (12°C-18°C). Veliger sampling can be performed anytime during the day but preferably not immediately following a storm or flooding event. Storm and flooding events can increase water turbidity and hence the time required to process the sample.

3.4 Sampling 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. In waterbodies that stratify (upper and lower water column separated by thermocline -see glossary for definitions) veligers are found above (epilimnion) and below (metaliminoin) the thermocline. Therefore, in waterbodies that stratify both the epilimnion and metaliminoin (above and below the thermocline) should be sampled. The literature supports that veligers are most concentrated from 0-15m. **If stratification levels are unknown the minimum recommended depth for the vertical plankton tow is 15m.** If the depth of stratification is known, then sample should occur above and below the thermocline. For shallower waters, subtract the length of the plankton net, plus .5 meters to keep it from hitting the bottom. See Appendix B for a complete list of equipment for plankton tows and Appendix C for detailed sampling methods.

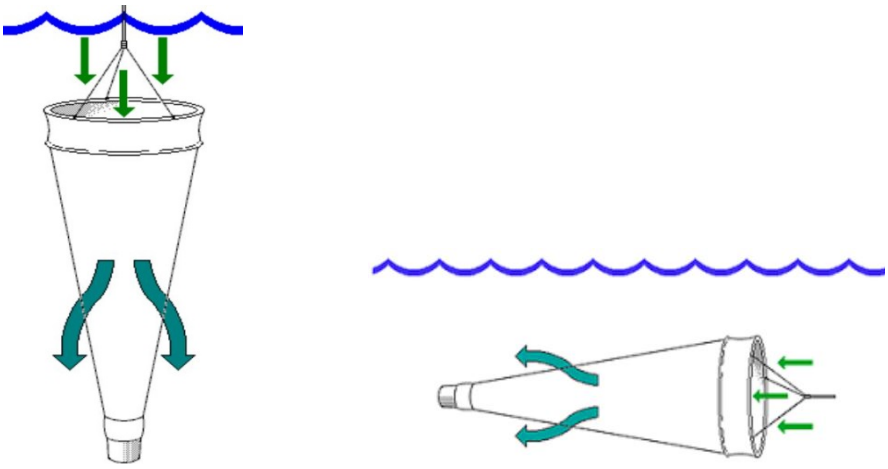

Figure 9. Vertical (left) and horizontal (right) plankton tows.

Horizontal Tows - For waterbodies less than 4m, or that are flowing (drainage ditches, pipes, rivers, streams, etc.), 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. Horizontal tows can be done from a boat (trawling) or from a dock by walking the length of

the dock or using a shoreline toss. See Appendix B for a complete list of equipment and Appendix D detailed sampling methods.

Figure 10. Shoreline toss (Photo: S. Wells)

Table 2. Summary of plankton tow sampling recommendations.

Parameter	Recommendation
Water temperature	≥9°C for a minimum of two weeks
Locations	Around floating structures, marinas, inlets, and outlets, coves, down-wind areas and eddies
Depth	15 m (50 ft) but will depend on depth of waterbody and depth of the thermocline
Number of sampling sites per waterbody	Variable; based on size and complexity of waterbody (some sites provided)
Number of site replicates/plankton tows per sampling site	Variable; when possible a minimum of 3, based on depth and net size, minimum 1000 liters per sample site.

4. ADULT DREISSENID MUSSEL SAMPLING METHODS

The objective of sampling for juvenile and adult Dreissenid mussels is to detect bivalves attached to hard submerged surfaces in freshwater environments. Dreissenid mussels are one of the few freshwater mussels capable of adhering to hard surfaces using byssal threads. The adults can only attach to hard substrate, so in muddy areas they will be found attached to embedded rocks, native clams, or crayfish. In lakes with little hard substrate, dreissenid mussels may initially settle on sticks, logs, shells or plants, or sometimes attach directly to sand grains, and later settle onto each other, eventually forming large mats. Adult Dreissenid mussels can be sampled using a number of different methods such as tactile and visual inspections of existing submersed surfaces and shoreline areas, a surface scraper (Figure 10a),

artificial settlement substrates (Figure 10b), and a thatch rake on a rope (Figure 10c). **A brief description of these methods is provided below but for the purposes of monitoring for juvenile and adult Dreissenid mussels in BC artificial substrate samplers is the recommended method. Shoreline surveys can also be done when checking substrate samplers.**

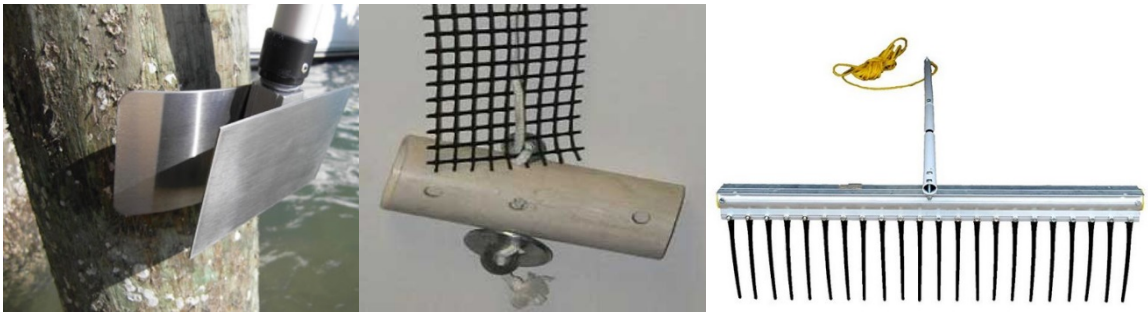

Figure 11. Adult mussels sampling tools: a) surface scraper, b) substrate sampler, c) thatch rake on a rope.

Shoreline surveys and inspections of structures in the water are conducted to identify the presence or absence of adult and juvenile Dreissenid mussels. Visual and tactile inspections of natural and other man-made submersed surfaces (including the undersides of buoys and dam booms, buoy mooring chains, the undersides of dock floats, rocks, logs, shoreline areas and other items) increase the surface area sampled for invertebrate colonization and thereby increase the likelihood of early detection.

A surface scraper can be used to sample submerged portions of hard, smooth surfaces including concrete walls, bridge abutments, pilings, channel markers, underwater booms, floating bathrooms, and dock floats. The surface scraper that is attached to a long pole should be lowered into the water, and then raised while dragging the metal rim along the surface (Figure 10 a). The dislodged organisms will be collected in the attached mesh bucket for inspection at the surface. Repeat at multiple locations per structure in order to sample a representative portion. It is important that a surface scraper is used with caution to ensure that no damage is done to underwater infrastructure.

Submersed macrophytes can be collected to sample for attached Dreissenid juveniles and adults. Aquatic plants can be collected from a boat by throwing a thatch rake attached to a rope (Figure 10 c), allowing the rake to sink and then dragging for approximately 1- to 2-m along the sediment. Macrophyte sampling should occur at locations with plant beds visible from water surface, in areas near marinas and boat launches, and in littoral areas likely to support macrophytes. The collected macrophytes should be visually inspected for bivalves and then shaken in 5-gal buckets of water to detach smaller animals. Bucket water should be poured through a sieve and the sieve and bucket should be inspected for bivalves.

4.1 ARTIFICIAL SUBSTRATE SAMPLER

Artificial substrate sampling allows for widespread/low cost and low effort monitoring of invasive Dreissenid mussels (Figure 10 b). Substrate refers to any substance in the water that Dreissenid mussels may attach to. Substrate samplers are for monitoring newly-settled juvenile and adult Dreissenid mussels that colonize substrate surfaces. See Appendix E for more information on how to build a substrate sampler.

4.1.1 WHERE TO SAMPLE

Two substrate samplers should be deployed at each of the sampling locations in a manner that will not interfere with boater or swimmer activities. Ideally the substrate sampler should be deployed in a covered area with some water flow and as deep as possible (≥6m is preferred but not required; this will vary depending on the depth at the sample site). During the warmest months, surface water temperatures in some areas may exceed the physiological tolerances of dreissenid mussels therefore substrate samplers should be placed at depths of 6 meters or greater when possible. A depth of >6m is preferred but not required, samplers must be placed at the priority locations listed below, even if the depth is less than 6m. Samplers should not be deployed offshore just to achieve the 6m depth. It should also be noted that multiple artificial substrates can be deployed at multiple depths on a single line**.**

Waterbody distribution

- Areas where the water currents and/or wind patterns are likely to concentrate the planktonic larvae, as well as dead adult shells, e.g., near dam or outflow, bays, eddies, etc.
- High boat use areas and points of entry, public boat ramps, marinas, fishing hot spots, resorts, campgrounds, etc.). Main stem, open water areas (on the existing floating objects) and near-shore areas.

The substrate sampler is a small surface area, so it is recommended that other substrates nearby are also checked, such as:

- Submerged hard surfaces including docks, pilings, seawalls, rocks, and logs.
- Shoreline areas including gravel, sand, mud, cobble and woody debris, especially in downwind, downstream, or other positions where shells and other debris are collected.
- Focus on the bottom and sides of submerged objects; in protected and shaded areas such as nooks, crannies and junction of two different surfaces.
- Periphyton may obscure attached bivalves and other specimens.

Remember that any solid surface is a suitable substrate to observe. Rub your hands along some of the submerged surfaces. Dreissenid mussels on the surface will feel like sandpaper.

4.1.2 WHEN TO SAMPLE

Dreissenid veligers begin to settle out of the water column and develop a shell 3-5 weeks after spawning which can occur when temperatures are 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 ≥9°C or cooled to ≤18°C) and veligers begin to settle out of the water column. The timing of sampling will vary by geographic region but typically occurs between May and October. One of the two substrate samplers should be removed and checked monthly from May through October using the methods outlined in Appendix E. The other substrate sampler should be left in the entire monitoring season and then checked at the end of the monitoring season. A physical description and GPS coordinates of each monitoring station must be obtained at initial deployment. See Appendix E for details on how to retrieve the substrate sampler.

Table 3. Summary of Artificial substrate sampler recommendations.

Parameter	*Recommendation*
Timing	beginning 3-5 weeks after water bodies have warmed to ≥9C or cooled to ≤18C
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

5. SAFETY

When conducting any field work in and around the water it is important to ensure that all staff/volunteers have the necessary safety equipment and training to do so safely. Always check weather conditions before going out on the water and do not conduct field work if conditions are unsafe or if someone does not feel comfortable. It is recommended to conduct any work on or near the water with a minimum of two people. When working on or near water ensure that Transport Canada, WorkSafe BC and any other applicable provincial or federal laws/regulations are being followed.

When conducting plankton tows be sure to do so safely. If you are conducting the tow from a boat, first 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 examine both the net and line to be sure the cod end is securely attached, and the tow line is free of tangles. When conducting sampling from the shore ensure that the water current/flow is safe before wading into deeper waters.

6. COLLECTION OF ANCILLARY DATA

It is also important to collect key data (e.g. temp and depth) at each sample site. If a sonde/probe is not available, measure the site information at the surface using available equipment. If there is no boat launch or dock, and you are conducting a shoreline toss or a horizontal plankton tow from the shore, then wade out into the water (if safe to do so) to collect the water quality profile. In water that is less than 1 meter deep, collect one profile reading just under the surface.

1. Anchor boat or tie-off to structure close to sample site. Record GPS location on datasheet.
2. Attach the slotted probe cover to multi-probe unit sensor. Immerse probes in lake and turn unit on. Allow unit to equilibrate and record values on data sheet.
3. Deploy Secchi disk on shady side of boat. For accurate estimate of depth, the line must be vertical when the measurement is taken; additional weight may be necessary to hold the disk down in a current. Lower the Secchi disk in the water until the white quarters are no longer visible and note this depth, then raise the disk until the white quarters reappear record this depth. The mean of these two values is your Secchi disk depth reading to be recorded on the datasheet. Do not use sunglasses or a view finder.
4. Record multi-probe readings at 1-m depth intervals. Start at surface and lower by 1-m intervals until at least 1-m off the lake bottom.

5. Allow unit to stabilize at each depth (temperature ±0.01°C, depth ±0.1 m, DO ±0.01 mg/L, and pH ±0.01).
6. Record values on datasheet. Report water temperatures in degrees Centigrade.
7. Continue to obtain profile. Raise unit to 2-m depth and record values a second time. Compare first and second measurements to assess instrument drift. Repeat profile if outside acceptable range

7. FIELD EQUIPMENT DECONTAMINATION

The purpose of decontaminating field equipment when sampling for invasive mussels is twofold, the first is to prevent the accidental transport of these 'aquatic hitchhikers' on waders, boats, trailers, nets and other equipment into new waterbodies. The second reason for decontamination is to prevent cross-contamination of field sampling equipment when traveling between waterbodies which can lead to false-positive results for a waterbody. Multiple sets of gear and thorough decontamination procedures are used to minimize these sources of bias.

7.1 GENERAL RECOMMENDATIONS

Inspect, **clean, drain and dry** all gear and boats following use. When leaving a waterbody, remove any visible plants and animals from your gear and boat –follow the **CLEAN, DRAIN, DRY** procedures. It should also be done away from the lake where run-off will not go into any water body, stream or drain. DO NOT clean the gear with water from the site as you might just re-contaminate it, unless you use additional decontamination procedures afterwards.

CLEAN-Thoroughly inspect boat (hull, drive units, trim plates, transducers), trailer and components (rollers, bunk boards, axles, etc.), equipment (i.e., water pumps, hatchery equipment, siphons, nets, ropes, traps, etc.) for adult Dreissenid mussels. Remove any mud and dirt since they might contain very small aquatic invasive species such as New Zealand mud snails. Pay attention to hidden, hard to reach areas, gaps, crevices, holes and other inconspicuous places (i.e., around the motor housing, trim tabs, and water intake screens, or pump fittings). All trash, mud, vegetation, should be removed and properly disposed of in the trash. **Any suspected AIS must be reported and submitted to the BC Ministry of Environment and Climate Change Strategy (see Appendix A for reporting information).**

DRAIN- Whenever possible, areas that hold water should be drained so there is no standing water. Eliminate water from any conceivable item before you leave the visiting area. This includes live wells, bilges, cargo areas, pipes, water pumps, etc.

DRY– Dry all areas of the vessel that may have gotten wet. Drying boats, gear and equipment will help to minimize risk of contamination.

If possible, avoid launching a watercraft into more than one waterbody per day (depending on weather conditions) to allow time for boat and gear to dry. The use of felt-soled waders is strongly discouraged, as they are a major pathway for the dispersal of aquatic hitchhikers, and particularly difficult to disinfect. Rubber-soled alternatives are available on the market, and provide the same non-slip qualities, but are much easier to clean.

7.2 WATERCRAFT

If you are only moving your boat between waterbodies in BC then practicing clean, dry, drain is the necessary procedure. If you are bringing your boat from outside of BC then you must contact the BC Invasive Mussel Defence Program by emailing the program inbox (COS.Aquatic.Invasive.Species@gov.bc.ca) to arrange for inspection and possible decontamination if necessary.

Complete decontamination of watercraft for Dreissenid mussels requires hot water (60° C) and high pressure (3,000 PSI) using specialized equipment and provincial inspectors are trained in decontamination. High pressure may cause damage to some parts of watercraft therefore pressure washers should only be used by someone trained in its operations. The hot temperature (60° C) with appropriate contact time is what kills the Dreissenid mussels and high pressure is used to assist with removing the mussels. Low pressure can be used for decontamination to minimize risk of damage to the watercraft.

7.3 FIELD EQUIPMENT

Field crews can be vectors for the unintentional movement of plants and animals associated with field sampling equipment. For waters that are suspect, positive or infested with Dreissenid mussels having separate equipment for each waterbody is strongly recommended. Decontamination involves both physical and chemical means to prevent transfer of a variety of taxa within and between systems and samples. Decontamination must be conducted such that runoff does not reach any waterbody and should be done in locations that are high and dry and must be conducted at least 60 meters (200 feet) away from any waterbody, ditch or storm drain. **Equipment does not need to be decontaminated between sampling sites on the same waterbody unless sampling in an upstream direction. If sampling in an upstream direction equipment must be decontaminated between sites or separate equipment can be used.** Field equipment that is used in multiple water bodies must undergo full decontamination between each use. If equipment is only used within one waterbody for the entire sampling season and all sampling occurs in a downstream direction, the equipment does not need to decontaminated between each use. The dedicated equipment should be fully decontaminated at the end of every season.

Sensitive Equipment:

Water quality probes and sensors may be damaged by chemicals and should only be rinsed with clean water and dried thoroughly. Please follow the manufacturers instructions for proper cleaning of probes and multi-meters.

General Sampling Equipment:

For decontaminating equipment (net, cod-end, rope, net anchor, sieve, surface scraper, wash bottles, buckets, waders, boots, ropes etc.) a vinegar and bleach soak must be used. The vinegar degrades the calcium carbonate shell resulting in a negative microscopy result while the bleach denatures the DNA. The volume of decontamination solution used (both vinegar and bleach) should be the minimum necessary to fully submerge the equipment. This will minimize the amount of solution required for disposal and reduce costs of decontaminating equipment.

Step 1 – Vinegar soak (5% acetic acid):

- Standard household vinegar should have a 5% acetic acid concentration and can be used for the vinegar soak.

- All sampling gear that comes into contact with the water must be fully immersed in household vinegar (5% acetic acid concentration) for two hours.
- Following the acetic acid soak, thoroughly rinse all equipment with a large volume of clean water this will help to prevent corrosion of equipment
- The vinegar can be reused multiple times and should 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.
- The use of vinegar can present safety hazards if not used properly. Appropriate Material Safety Data Sheets (SDS) should be included and followed in the standard operating procedures
- For disposal, the vinegar must be diluted with a large amount of water to a very low concentration and disposed in a large gravel area far away (>100m) from any drains or natural waterways with no possibility for drainage/seepage into natural waterways.

Step 2 – Bleach soak:

- 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. 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 (Western Regional Panel 2018).
- Smaller items can be fully sprayed with a 10% bleach solution and allow the items to sit for 10 minutes. Larger items must be fully immersed in a 10% bleach solution in a large rubber tote or similar container for 10min
- Bleach is corrosive, and equipment must be thoroughly washed with tap water following decontamination. Allow the items to air dry completely.
- The bleach solution should be discarded after 24 hours.
- 10% bleach should be retained in a plastic carboy and disposed of following protocols for waste disposal. Check with the local municipality regarding proper disposal of chemicals.

Freezing (optional):

- Place equipment in a freezer for 48 hours (when possible) or a minimum overnight between each use. Freezing can be done in addition to the vinegar and bleach soaking but cannot replace it as a decontamination method.

Table 4. Dreissenid mussel decontamination methods for field equipment.

Type of Equipment	Decontaminant	Concentration	Contact Time	Guidelines
Sensitive equipment (probes,sondes, meters)	Tap/distilled water	-	-	Please refer to the user manual for cleaning instructions.
Field equipment	Vinegar & Bleach	5% acetic acid & 10% bleach solution	2 hours for vinegar and 10min for bleach	Immerse equipment into vinegar (5% acetic acid) for 2 hours, wash off thoroughly, followed by 10min soak in 10% bleach solution
Field equipment	Freezing	<0° C	48hrs (minimum overnight)	Equipment must be fully inside a freezer for 48hrs, or minimum overnight

8. PRIORITY WATERBODY LIST

This section provides an overview of the priority waterbodies selected for carrying out plankton tow sampling efforts across the province (Table 5). The risk of ZQM invasion is based on a combination of risk of introduction and risk of establishment and both were used to identify the priority waterbodies for ZQM sampling listed in Table 5. The risk of ZQM introduction is based on human behaviour, such as the potential for introduction through overland transport of watercraft from mussel infested waterbodies. The risk of establishment is based on chemical and physical attributes of the waterbody that make it suitable for ZQM survival and establishment. Minimum dissolved calcium levels are required in a waterbody to support ZQM shell growth and mean minimum summer temperatures are required to support ZQM reproduction (Stanczykowska 1977; Baker et al. 1993; Sprung 1993; Nichols 1996; McMahon 1996). Calcium concentration is considered the most critical environmental variable for ZQM survival and establishment and to delineate risk categories for dreissenid mussel infestation (Neary and Leach 1991; Cohen and Weinstein 1998; Whittier et al. 2008; Wells et al. 2010; Claudi and Prescott 2011; Therriault et al. 2013).

The risk of ZQM establishment was assessed using calcium data with temperature as a lower bound limiting factor; pH was also assessed but excluded from the analysis due to insufficient data coverage at the provincial level. The risk of ZQM introduction for waterbodies was estimated using the following variables; the Provincial watercraft inspection data for the 2015-2021 seasons; angler use (DFO 2015 National Angler Survey); the number of marinas on a waterbody, and boating restrictions. Multiple datasets were considered in the analysis and not all were included in the final model. Each dataset had limitations, selection was primarily based on best representation of risk and spatial coverage. The waterbody priority list is reviewed on an annual basis to assess if new variables and new data are available for inclusion in the model.

The waterbodies listed in Table 5 have been selected for plankton tow sampling in order to prioritize sampling efforts to the waterbodies ranked at highest risk for dreissenid mussel invasion. Substrate samplers are still encouraged to be deployed in the waterbodies listed in Table 5 to either complement plankton tow sampling or when plankton tow sampling cannot be completed. Substrate samplers are also encouraged to be deployed in waterbodies that are not listed in Table 5 through opportunistic sampling. The Ministry must also prioritize resources towards the frequency of sampling, with the highest priority waterbodies selected for bi-weekly sampling as indicated in Table 5. Resources must

balance the number of priority waterbodies sampled with the frequency and number of sampling locations within a waterbody. Therefore not all waterbodies listed in Table 5 may get sampled annually.

Table 5. Priority waterbodies, including the frequency plankton tow sampling (bi-weekly or monthly) based on the waterbody priority ranking.

Region	Waterbody	Veliger Sampling Frequency	Lat	Long
Thompson-Nicola	Adams Lake	Monthly	51.1889	-119.5812
Thompson-Nicola	Alleyne Lake	Monthly	49.9182	-120.5671
Okanagan	Allison Lake	Monthly	49.6934	-120.6055
Lower Mainland	Alta Lake	Monthly	50.114	-122.9814
Skeena	Atlin Lake	Monthly	59.4761	-133.8107
Skeena	Babine Lake	Monthly	54.7858	-125.9793
Kootenay	Baynes Lake	Monthly	49.2337	-115.2245
Thompson-Nicola	Big Bar Lake	Monthly	51.3099	-121.7968
Cariboo	Bridge Lake	Monthly	51.5043	-120.7306
Skeena	Bulkley River	Monthly	NA	NA
Kootenay	Bull River	Monthly	NA	NA
Skeena	Burns Lake	Monthly	54.2029	-125.661
Cariboo	Canim Lake	Monthly	51.8493	-120.765
Kootenay	Cedar Lake	Monthly	51.2614	-116.9814
Kootenay	Champion Lakes	Monthly	49.1875	-117.62
Peace	Charlie Lake	Monthly	56.3316	-120.9929
Vancouver Island	Chemainus Lake	Monthly	48.9142	-123.7529

Okanagan	Christina Lake	Biweekly	49.1214	-118.2538
Thompson-Nicola	Clearwater Lake	Monthly	52.2644	-120.2298
Omineca	Cluculz Lake	Monthly	53.8796	-123.5779
Kootenay	Columbia Lake	Monthly	50.2303	-115.8529
Kootenay	Columbia River	Monthly	NA	NA
Vancouver Island	Cowichan Lake	Biweekly	48.8729	-124.2627
Lower Mainland	Cultus Lake	Monthly	49.0533	-121.9871
Okanagan	Davis Lake	Monthly	49.8619	-120.7322
Cariboo	Deka Lake	Monthly	51.6504	-120.7915
Cariboo	Dragon Lake	Monthly	52.9494	-122.4208
Kootenay	Duck Lake	Monthly	49.2342	-116.6366
Thompson-Nicola	Dutch Lake	Monthly	51.6519	-120.0559
Kootenay	Elk River	Monthly	NA	NA
Kootenay	Emerald Lake	Monthly	51.4432	-116.5315
Thompson-Nicola	Face Lake	Monthly	50.5431	-120.6347
Cariboo	Fawn Lake	Monthly	51.5518	-120.9817
Kootenay	Flathead River	Monthly	NA	NA
Skeena	François Lake	Monthly	54.012	-125.7053
Omineca	Fraser Lake	Monthly	54.08	-124.742
Lower Mainland	Fraser River	Monthly	NA	NA
Cariboo	Green Lake	Monthly	51.407	-121.2139
Peace	Gwillim Lake	Monthly	55.3519	-121.3177
Thompson-Nicola	Harmon Lake	Monthly	49.9692	-120.7004

Lower Mainland	Harrison Lake	Biweekly	49.5194	-121.8663
Thompson-Nicola	Heffley Lake	Monthly	50.835	-120.0654
Thompson-Nicola	Hihium Lake	Monthly	51.0545	-121.1152
Cariboo	Horse Lake	Monthly	51.594	-121.1101
Cariboo	Horsefly Lake	Monthly	52.4169	-121.0236
Cariboo	Howard Lake	Monthly	51.8132	-120.7956
Okanagan	Jewel Lake	Monthly	49.1743	-118.6099
Kootenay	Jim Smith Lake	Monthly	49.4811	-115.8474
Okanagan	Kalamalka Lake	Biweekly	50.1728	-119.3274
Thompson-Nicola	Kamloops Lake	Biweekly	50.7517	-120.6932
Lower Mainland	Kawkawa Lake	Monthly	49.3873	-121.4012
Okanagan	Kettle River	Monthly	NA	NA
Kootenay	Kicking Horse River	Monthly	NA	NA
Kootenay, Omineca	Kinbasket Lake	Monthly	52.0822	-118.2026
Thompson-Nicola	Knouff Lake	Monthly	50.9912	-120.1223
Kootenay	Kootenay Lake	Biweekly	49.6332	-116.8626
Kootenay	Kootenay River	Monthly	NA	NA
Thompson-Nicola	Lac des Roches	Monthly	51.4771	-120.5701
Cariboo	Lac la Hache	Monthly	51.8239	-121.5438
Thompson-Nicola	Lac Le Jeune	Monthly	50.4805	-120.4782
Kootenay	Lake Enid	Monthly	50.5482	-116.1237
Kootenay	Lake Koocanusa	Biweekly	49.1906	-115.2581
Kootenay	Lazy Lake	Monthly	49.8249	-115.6228

Thompson-Nicola	Leighton Lake	Monthly	50.6192	-120.8453
Thompson-Nicola	Little Shuswap Lake	Monthly	50.8503	-119.6463
Thompson-Nicola	Logan Lake	Monthly	50.4961	-120.8066
Thompson-Nicola	Loon Lake	Monthly	51.1046	-121.2494
Kootenay	Loon Lake	Monthly	49.1145	-115.1067
Okanagan	Louise Lake	Biweekly	49.1433	-118.922
Kootenay	Lower Arrow Lake	Monthly	49.6792	-118.0606
Thompson-Nicola	Lundbom Lake	Monthly	50.0865	-120.6165
Okanagan	Mabel Lake	Monthly	50.5657	-118.7153
Thompson-Nicola, Okanagan	Mara Lake	Biweekly	50.7726	-119.0162
Peace	Moberly Lake	Monthly	55.8207	-121.7654
Kootenay	Monroe Lake	Monthly	49.3642	-115.8665
Omineca	Moose Lake	Monthly	52.9539	-118.9098
Kootenay	Moyie Lake	Monthly	49.3258	-115.8349
Kootenay	Moyie River	Monthly	NA	NA
Thompson-Nicola	Murray Lake	Monthly	49.7933	-121.0059
Peace	Murray River	Monthly	NA	NA
Thompson-Nicola	Nicola Lake	Monthly	50.1802	-120.5294
Kootenay	Norbury Lakes	Monthly	49.5371	-115.4832
Okanagan	Okanagan Lake	Biweekly	49.9007	-119.5471
Peace	One Island Lake	Monthly	55.3073	-120.2939
Okanagan	Osoyoos Lake	Biweekly	49.0412	-119.47

Okanagan	Otter Lake	Monthly	49.5729	-120.7648
Okanagan	Oyama Lake	Monthly	50.1101	-119.2728
Thompson-Nicola	Paul Lake	Monthly	50.7399	-120.1155
Peace	Peace River	Monthly	NA	NA
Kootenay	Pend-d'Oreille River	Monthly	NA	NA
Thompson-Nicola	Peter Hope Lake	Monthly	50.2935	-120.3172
Kootenay	Premier Lake	Biweekly	49.9365	-115.6537
Cariboo	Quesnel Lake	Monthly	52.5599	-120.9839
Thompson-Nicola	Red Lake	Monthly	50.8883	-120.7856
Kootenay	Revelstoke Lake	Monthly	51.5075	-118.449
Thompson-Nicola	Roche Lake	Monthly	50.4746	-120.1522
Kootenay	Rosen Lake	Monthly	49.3997	-115.2569
Thompson-Nicola	Salmon Lake	Monthly	50.2689	-120.0033
Vancouver Island	Shawnigan Lake	Monthly	48.6304	-123.6381
Cariboo	Sheridan Lake	Monthly	51.5161	-120.8944
Thompson-Nicola	Shuswap Lake	Biweekly	50.942	-119.1497
Thompson-Nicola, Okanagan	Shuswap River	Monthly	NA	NA
Lower Mainland	Silver Lake	Monthly	49.3142	-121.4118
Okanagan	Similkameen River	Monthly	NA	NA
Okanagan	Skaha Lake	Monthly	49.4101	-119.5854
Skeena	Skeena River	Monthly	NA	NA
Kootenay	Slocan Lake	Biweekly	49.9358	-117.4226

Kootenay	Slocan River	Monthly	NA	NA
Kootenay	St. Mary Lake	Monthly	49.61	-116.193
Kootenay	St. Mary River	Monthly	NA	NA
Okanagan	Stony Lake	Monthly	50.7125	-118.7031
Omineca	Stuart Lake	Monthly	54.5533	-124.6319
Thompson-Nicola	Stump Lake	Monthly	50.3621	-120.3726
Okanagan	Sugar Lake	Monthly	50.4012	-118.5156
Kootenay	Summit Lake	Monthly	50.1535	-117.6402
Kootenay	Suzanne Lake	Monthly	49.3207	-115.2393
Peace	Swan Lake	Monthly	55.5182	-120.0151
Okanagan	Swan Lake	Monthly	50.3184	-119.2561
Thompson-Nicola	Thompson River	Monthly	NA	NA
Kootenay	Tie Lake	Monthly	49.4149	-115.3185
Kootenay	Toby Creek	Monthly	50.4306	-116.2502
Kootenay	Trout Lake	Monthly	50.585	-117.4366
Thompson-Nicola	Tunkwa Lake	Monthly	50.6088	-120.8576
Kootenay	Upper Arrow Lake	Biweekly	50.53	-117.9177
Kootenay	Wasa Lake	Monthly	49.7797	-115.7354
Thompson-Nicola	White Lake	Monthly	50.8826	-119.2644
Kootenay	Whiteswan Lake	Biweekly	50.1415	-115.4823
Kootenay	Whitetail Lake	Monthly	50.214	-116.0259
Cariboo	Williams Lake	Monthly	52.1154	-122.0696
Omineca	Williston Lake	Monthly	55.6268	-123.54

Kootenay	Windermere Lake	Biweekly	50.4591	-115.989
Okanagan	Wood Lake	Monthly	50.0817	-119.3898
Thompson-Nicola	Woods Lake	Monthly	50.3828	-119.7907

LITERATURE AND SOURCES

Arizona Game and Fish Department Aquatic Invasive Species Decontamination Protocols

Bureau of Reclamation Technical Service Center, Reclamation Detection Laboratory for Invasive and Native Species. Field Protocol: Field Preparation of Water Samples for Dreissenid Veliger Detection.

Bureau of Reclamation Technical Service Centre Reclamation Detection Laboratory for Invasive and Native Species. Impact of Sample Preservation on Detection of Invasive Mussels.

California Department of Fish and Wildlife. 2017. Quagga/Zebra Mussel Artificial Substrate Monitoring Protocol. https://nrm.dfg.ca.gov/FileHandler.ashx?DocumentID=4953&inline

Colorado Department of Natural Resources, Colorado Parks And Wildlife State Invasive Species Program, Aquatic Nuisance Species Sampling and Monitoring Protocol

Minnesota Aquatic Invasive Species Research Center, University of Minnesota (MAISRC) and the Minnesota Department of Natural Resources (DNR). Monitoring for zebra mussels

Portland State University, Wells, S. and Sytsma, M. 2015. Sample Collection Protocols Zebra and Quagga Mussel Early Detection Monitoring

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Therriault, T.W., Weise, A.M., Higgins, S.M., Guo, S. and J. Duhaime. 2012. Risk assessment for three Dreissenid mussels (Dreissena polymorpha, Dreissena rostriformis bugensis, and Mytilopsis leucophaeata) in Canadian freshwater ecosystems. DFO Can. Sci. Advis. Sec. Res. Doc. 2012/174 v + 88 p

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Wells, S. W. and M. Sytsma. 2015. "Zebra and Quagga Mussel Early-Detection Monitoring in High Risk Oregon Waters 2014". Center for Lakes and Reservoirs Publications and Presentations. Paper 32. http://pdxscholar.library.pdx.edu/centerforlakes_pub/32

Western Regional Panel 2018. Minimum Monitoring Guidance For Utilizing Plankton Tows For The Detection Of Dreissenid Veligers. Standard Operating Procedure.

APPENDIX A: REPORTING INFORMATION

Conservation Data Center:

https://www2.gov.bc.ca/gov/content/environment/plants-animals-ecosystems/conservation-data-centre/submit-data

Email: cdcdata@gov.bc.ca

Invasive species information and factsheets:

www.gov.bc.ca/invasive-species

Reporting Information:

Immediately report any suspected invasive mussels to the **Report All Poachers and Polluters Hotline: 1-877-952-7200 (RAPP)**

Ministry of Environment and Climate Change Strategy (ENV) contact:

Cassandra Silverio, Aquatic Invasive Species Specialist

Email: Cassandra.Silverio@gov.bc.ca

Or

Martina Beck, Invasive Fauna Unit Head

Email: Martina.Beck@gov.bc.ca

Habitat Conservation Trust Foundation (HCTF) grant application information:

https://hctf.ca/apply-for-funding/invasive-mussel-monitoring/

Analytical Lab:

The chain of custody (COC) form and shipping information for the lab will be provided by HCTF at the start of the respective sampling season. The COC form must be sent to the lab when shipping samples.

Appendix B: Equipment for plankton tows

- Plankton net (simple, conical plankton-tow net, 63-64 µm mesh size, recommended 0.3 m (1 ft) diameter net opening (0.5m diameter can also be used), removable, weighted cod-end piece.
- Line for deploying the net (about 20m) with 1 meter interval markings;
- Sample container (preferably polyethylene material, 250 to 500 mL volume, screw lid; but any leak-proof container suitable for shipping can be used);
- Preservative (95% regular ethanol or 99% isopropyl alcohol);
- Baking soda solution
- Squirt Bottle
- Field sheets and pen/ pencils;
- Waterproof labels (write in the rain paper);
- Thermometer;
- Permanent marker;
- GPS unit (*recommended*);
- Tweezers or small spatula (*recommended*);
- Boat (*recommended*);
- pH strips or pen meter
- Secchi disk
- Measuring tape or ruler (*optional*)

Appendix C: Vertical Plankton Tow

1. Secure the cod-end piece and check that the line is securely attached to the plankton net. Secure the other end of the line to the boat.
2. Lower the net to planned sampling depth (see Table 2). For waterbodies <15m, calculate the appropriate sampling depth using a depth finder or marked rope.
3. Ensure the bottom of the net does not disturb the lake bottom, touching the bottom will clog the net. If you disturb the lake bottom, discard the sample, rinse out the sampling equipment and try shorter tows, or go to a different area of the lake that will provide enough depth for a good tow. Record the approximate depth that the net is lowered to.
4. Keep the net at the desired depth for 60 seconds and then manually retrieve up vertically using a hand-over-hand technique at a rate of 0.5 m/s. Slow and steady retrieval is the key to collecting a good plankton tow. Care should be taken to pull the net up slowly enough so that no pressure wave is created on the surface of the water. If you are creating a pressure wave, you are under-sampling the water column.
5. Rinse the net by raising the net so that the cod end of the net is just above the water surface. Rinse organisms into the cod end of the net by lowering the net back into the water, keeping the net opening well 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.
6. A squirt bottle, filled with either tap water or water from the lake or river, can be used to wash down the sides of the net. Spray the outside of the net starting at the cod end interface and moving down to rinse organisms into the cod end.
7. Condense the sample by swirling sample the cod-end piece. Carefully remove the cod-end piece without spilling collected water and plankton. Decant your plankton sample into your sampling container after each tow to obtain an accurate enumeration of the larval density in your lake.
8. The sample container should be no more than about 1/4 full to allow room for the preservative. If samples are too large to combine into one sample container use a separate sample container for each tow.
9. Add preservative to the sampling container following the procedures outlined in Appendix F and the procedure in Appendix G for labeling the sample.
10. Record the number and depth of tows as well as diameter of net mouth opening, so that the volume of water sampled can be determined. GPS locations and depths of tows should be recorded on the chain of custody form/field datasheet.
11. Record the water temperature at the maximum depth that the plankton net was set. If the maximum depth cannot be reached record the water temperature as deep as possible from the surface. When multiple tows are taken from one sample site use the average temperature from each of the tows.

Appendix D: Horizontal Plankton Tow

Horizontal tow from a boat:

1. A weight (1-2 kg or 2-4 lbs) is attached to the rope immediately in front of the net opening to keep the net below the water surface.
2. To determine the depth, subtract the length of your plankton net, plus minimum .5 meters off the bottom to avoid fouling the net/sample.
3. The net is thrown into the water and allowed to sink to no more than 1 m above the bottom and keep at consistent depth.
4. Record the start time and the starting location coordinates on field datasheet. Record the distance that the net is towed through the water.
5. Use the boat engines and/or the river current to move the net horizontally through the water for three to 5 minutes (depends on boat speed, net mouth opening, eutrophic status of the waterbody), or slowly pull the net back to you at a slow and steady rate as described above (the total length of the tow can be determined using the graduation marks on the tow rope). The tow should be done at low speeds, e.g., 0.5 to 3-Km/h. The boat may be driven directly upstream, essentially keeping the boat in the same approximate longitudinal position and allowing river to flow through net. Trawling can also be done transversely to the current. Reduce the trawling time in productive and turbid waters as net may clog. Keep the net off the bottom to avoid both snagging and collecting debris.
6. Idle or stop the boat engine and manually retrieve the net using a hand-over-hand technique at a rate of 0.5-m/s. 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.
7. Record the stop time, boat speed and the coordinates of the stop location on the COC/field datasheet (Appendix B). The trawling time and boat speed are used to estimate the volume of water filtered (i.e., distance = rate x time).
8. Repeat techniques used for vertical plankton tows to concentrate organisms into the cod end of the net.
9. Follow the procedure outlined in Appendix F for preserving the sample and Appendix G for labeling the sample.

Flow Rate (m/s)	Plankton Net Diameter (50 cm)	Plankton Net Diameter (30 cm)
0.5	33	93
1.0	17	46
1.5	11	31
2.0	8	23
2.5	7	19
3.0	6	16

Horizontal Tow from a Dock

1. If conducting horizontal plankton tow from a dock, lower the net and 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.
2. If possible avoid sampling in aquatic vegetation, but if it is a high use/high boat traffic site sampling should still be conducted.
3. Follow steps 5-8 in Appendix C to concentrate organisms into the cod end of the net. Record length of each tow, and number of tows.
4. Follow the procedure outlined in Appendix F for preserving the sample and Appendix G for labeling the sample.

Shoreline Toss

When a boat is not available or when you are sampling from a dock or other land structures then a shoreline toss can be used.

1. Remove the net anchor, which is secured to a loop in net rope.
2. Screw on the weighted cod end, check that the hose clamp is secure, and that the net rope is secured to steel ring.
3. Hold the net ring using thumb and forefinger of your throwing hand. Make large loops of the net rope and hold loosely with the same hand holding the net. Grasp the loops of the rope in front of the net opening.
4. Firmly hold the other end of the rope with free hand.
5. Throw the net using a sidearm-style, opening your hand upon release to allow rope to feed out with the net.
6. Allow the net to sink into water body. A weighted cod end 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 the 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. Repeat techniques used for vertical plankton tows to concentrate organisms into the cod end of the net.
9. Follow the procedure outlined in Appendix F for preserving the sample and Appendix G for labeling and shipping of the samples.

APPENDIX E: ARTIFICIAL SUBSTRATE SAMPLERS

Either a Portland sampler or a plate sampler can be used for substrate monitoring. This section provides the instructions and materials needed to build both types of samples.

Portland Sampler:

Materials needed

- 13mm diameter plastic construction mesh or plastic gutter guard (x3 17cm wide strips)
- 17cm white PVC pipe (5cm diameter) x 2
- 17 cm black PVC pipe (5 cm diameter) x2
- 500g concrete anchor
- 4 large flat washers
- Rope (nylon or paracord, do not use polypropylene plastic rope as it will degrade over time)

Portland Sampler Construction:

1. Cut the rope to the appropriate length for the depth of the sample site. At the end of the rope tie a small (500g) concrete anchor at the bottom.
2. Cut the white and black PVC pipe (5-cm diameter) into 17cm pieces. The PVC pipe sections will be placed 2ft apart along the length of the rope, so the exact number of PVC pieces will depend on the length of the rope but should be around 3-4 per substrate sampler (2 white and 2 black).
3. In the middle of the 17cm PVC pieces drill two holes 7mm wide (at the opposite sides of the pipe) this will be for feeding the rope through. Repeat the same with the black PVC pipe with the same dimensions. Sand down the edges of the holes to make a smooth surface.
4. On the other side of the PVC pipe drill three 7mm wide holes at equal distance across the length of the 17cm PVC section. Sand down the edges of the holes to make a smooth surface. These holes provide additional surface area for dreissenid mussel to settle on. Nothing will be thread through these holes (see Figure below).
5. Cut the plastic mesh into 17 cm wide strips (the number of strips will depend on the length of the rope).
6. First thread a large flat washer followed by the first 17cm piece of white PVC pipe to the bottom of the rope just above the secured anchor. Thread another large flat washer just above the first pipe section. Weave the plastic mesh strip lengthwise through the rope just above the flat washer and the first pipe section see Figure below).
7. About 2 ft above the top of the first PVC pipe section tie a knot in the rope and thread a flat washer through the rope so it sits just above the knot.
8. Repeat steps 5 and 6 until all the PVC pipe sections and plastic mesh sections have been thread through the full length of the rope section with 2ft spacing between each PVC pipe-mesh section. Alternate between black and white PVC pipe along the rope section.

9. Use a secure surface structure to tie the surface end of the rope off. Docks and piers can be used but these are high traffic areas for recreational activity and increases the risk of theft or damage to the sampler.
10. If theft or vandalism is a concern a small laminated label can be attached to the surface end of the rope with the following:

MONITORING EQUIPMENT – DO NOT REMOVE (you can provide a contact number if available)

Plastic gutter guard (left) and white PVC pipe (right). Photo credit: Home Depot. One PVC pipe and mesh section of the portland sampler (below) that is repeated 3-4 times along the length of the rope.

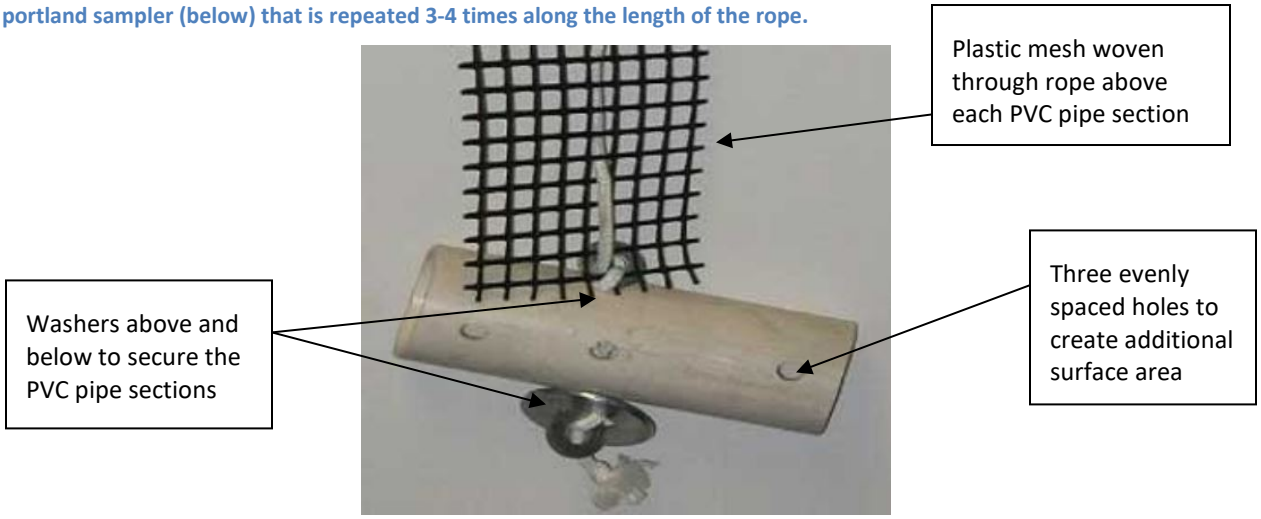

Plate Sampler

Materials needed:

- (4) 6” x 6” x 0.25” black/grey PVC with 1” hole through center
- (5) 1.5” x 1.375” (35mm) exterior diameter PVC or ABS tube
- (1) 8.5” x 0.8125” (21 mm) exterior diameter PVC or ABS tube
- ~6m rope (length will depend on the depth at each sample site)
- Weight to keep plates from floating up
- Laminated label with your contact information

Plate Sampler Construction:

- Tie a weight at one of the rope
- Run the 8.5” tube (21mm exterior diameter) through the rope and secure it just above the weight. This will be the shaft support.
- From the loose end of the rope string on the pieces of PVC plate and PVC spacers through the rope and 8.5” tube, alternating between the short segments of tube (spacers) and the PVC plates, beginning and ending with the spacers (see figure below).
- Tie a knot above the final spacer to prevent the pieces from moving up.
- Attach a label to the rope where the rope is secured to the structure.

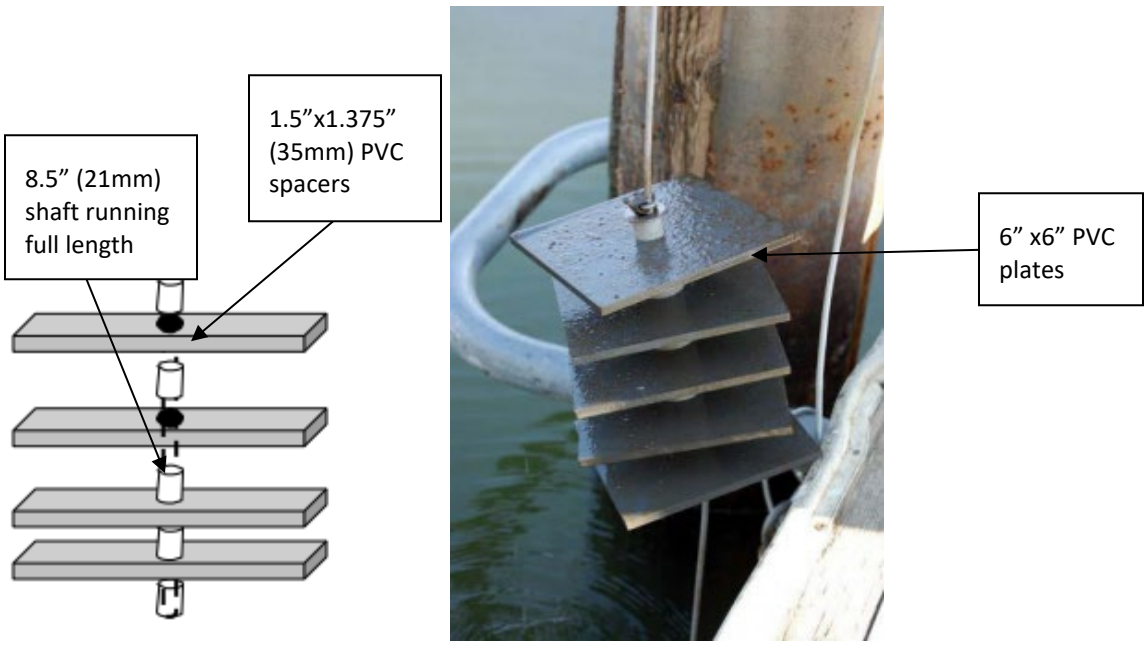

Plate Sampler (photo credit: California Department of Fish and Wildlife)

Retrieving the substrate sampler:

1. Retrieve substrate sampler from water carefully – place in a bucket for close inspection.
2. Inspect the sample closely for zebra and quagga mussels. Juvenile mussels are very small, but have a rough sand-paper feel relative to the substrate sampler. Adult Dreissenid mussels are most likely to be found in dark areas, in corners or crevices.
3. **If you suspect that the sampler is contaminated with Dreissenids DO NOT return it to the water.** The suspected Dreissenid specimens should be photographed with an object/ruler in the photo for scale. The suspected specimens should be collected into a vial, with water, and then kept cool in a refrigerator OR be preserved in regular ethanol or isopropyl alcohol for expert verification. **Report it immediately to the BC Ministry of Environment & Climate Change Strategy** (see Appendix A for contact information).
4. Examine the bucket for other suspect aquatic invasive species such as Eurasian Water Milfoil, Flowering Rush, or New Zealand mudsnails. For more information on how to identify these species visit gov.bc.ca/invasive-species
5. If no aquatic invasive species are found, return the substrate sampler back into the water where it was found.
6. The substrate sampler tracking form provided by HCTF must be filled out at the start and end of the season when the samplers are deployed and each time the substrate sampler is checked. At the end of the season submit the completed form to HCTF.
7. Follow the procedure outlined in Appendix F for preserving the sample and Appendix G for labeling and shipping of the samples.

APPENDIX F: SAMPLE PRESERVATION

Sample preservation plays a significant role in the accurate identification of veligers in the laboratory analyses. Plankton tow samples must be preserved in a 70% alcohol concentrated solution immediately after collection to ensure sample integrity. Do not wait more than three hours to preserve samples. Regular ethanol (95%) is recommended, but 99% isopropyl alcohol is acceptable - DO NOT use denatured ethanol. Avoid placing samples in direct sunlight or freezing conditions. Samples that cannot be preserved immediately after collection should be placed on ice until preservative can be added. Keep preserved samples in a plastic container such as a bin or cooler in the back of the car while in transit.

To buy regular ethanol in British Columbia a Purchase Permit is required, more info can be found at the following link:

https://www2.gov.bc.ca/gov/content/employment-business/business/liquor-regulation-licensing/liquor-licences-permits/applying-for-a-liquor-licence-or-permit/ethyl-alcohol-purchase-permit

Regular ethanol (with purchase permit) and isopropyl alcohol can be ordered from Fisher Scientific or other chemical scientific lab suppliers.

4% Baking Soda (sodium bicarbonate) Solution:

If the pH in the sample is below 7 then a baking soda solution must be added to raise the pH above 7. A pH below 7.0 can result in shell dissolution and the loss of birefringence. The pH must be checked and baking soda solution added, if necessary, before adding the preservative. To make a solution that is approximately 4% solution the following concentrations should be used; add 40 grams of baking soda to 1000 milliliters (1L) of 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. For example, adding a level soda bottle capful of baking soda to a 250 ml Nalgene container that is approximately ½ full with water will provide a solution of baking soda close enough to 4% that it can be used to adjust the pH of plankton tow samples.

Plankton tow sample preservation (70% EtOH concentration):

- **Step 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.
- **Step 2:** Check the pH of the water sample, if it is below 7 then the 4% baking soda solution must be added (see above for concentrations). First divide the height measurement from step 1 by 0.95. This measurement (mm) is the level to which the baking soda solution is added to the sample. 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.
- **Step 3**: Next note the new volume of sample water in the container and then add three times the volume of preservative (95% ethanol or 99% isopropyl alcohol) to the sample. For example, if your sample bottle contained 2.5 cm of sample, you would add 7.5 cm of preservative so that the sample bottle contained 10 cm of combined sample and preservative. This is why it is important to not fill the sample bottle more than ¼ full of sample. A measuring tape or ruler may be placed alongside the sample container to estimate the volumes.
    - If the prescribed alcohol to sample ratio (4:1) cannot be achieved after repeated condensing and decanting, then the sample should be split between two (or more)

sample bottles. Label each with the same information, and label one as "Split 1 of 2" and the other as "Split 2 of 2".

- **Step 4:** Check the pH of the sample again after adding the preservative it should now be greater than 7. If the pH is below 7 add more baking soda solution. If the sample is not shipped within 1-2 weeks after preserving, check the pH again before shipping the samples. If the pH is below 7 add more baking soda solution. The lab will also check the pH of the sample upon arrival and add more baking soda solution as needed.
- **Step 5:** Pack all sample bottles and keep cool until they can be shipped to the lab. Do not freeze plankton samples. Freezing damages shells and reduces detection sensitivity.

Adult Dreissenid Sample Preservation

- Preserve suspect specimen(s) immediately after collection to ensure sample integrity. Place in a sample jar or vial and if possible avoid using a plastic bag for samples with shells (to avoid damage).
- Either 95% regular ethanol or 99% isopropyl alcohol should be used and DO NOT use denatured ethanol. Add enough preservative so that specimen(s) and/or associated substrate are completely submerged in the sample container. Do not add a buffer to plant or animal samples that are not plankton tows.
- If preservative is not available freezing is an acceptable method of preserving adult mussel specimens. See Appendix G instruction on labeling and shipping of samples.

APPENDIX G: LABELING AND SHIPPING OF SAMPLES

Labeling Samples

- Sample containers must be labeled. Be sure to write legibly and using a wax pencil or alcohol resistant marker as many permanent markers are ink soluble in alcohol (e.g. Sharpie).
- To prevent the loss of information on the container a label should also be placed in the sample using waterproof paper and pencil. The label should contain the following information:
    - Date of collection
    - Name of waterbody;
    - Site location
    - Name/agency collecting sample.

This information MUST also be recorded on the form. Below is an example of a label on a sample container:

Date: 06/24/2018
Waterbody: Columbia River
Location: Chinook Boat Landing Boat Ramp
Sampler: John Doe/name of the organization

Packaging and Shipping Samples

Plankton tow samples must be sent to the lab throughout the sampling season, please follow the instructions provided by HCTF regarding frequency of shipping samples. Ethanol/isopropyl alcohol preserved samples must be shipped or mailed to the designated analytical lab via ground mail and following the appropriate Transport Canada shipping regulations. Ethanol/isopropyl alcohol is a Class 3 flammable liquid and there are restrictions regarding its transport. Keep preserved samples in a plastic container such as a bin or cooler in the back of the car while in transit. Ethanol can be mailed but there are training, certification, labeling and shipping requirements that must be followed. Do not ship any samples via air and do not fly in an airplane with ethanol, ethanol can only be transported on the ground/surface.

1. Samples must be in plastic containers with a screw lid. Secure screw lids with tape.
2. Place sample containers into a box lined with a plastic bag and add cushioning material such as plastic grocery bags or scrap paper. Once all samples are inside, close plastic bag tightly, and tie a knot to close the bag to prevent spills during shipping. Seal the box with packing tape. The box does NOT need to be a specific type of box so long as it is sturdy. DO NOT send samples in coolers.
3. Include a complete return address.
4. Include a paper copy of the chain of custody (COC) form with the samples and email an electronic copy of the COC form to the lab and the ENV contact in Appendix A.
5. Ensure that all fields in the COC form are filled out as instructed and using the correct unit