British Columbia Dreissenid Mussel Lake Monitoring Field Protocol

BRITISH COLUMBIA | Ministry of Environment and Climate Change Strategy

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1. INTRODUCTION

Monitoring is critical for early detection of new invasive species incursions in 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.

This protocol serves as an update to the 2016 BC Aquatic Invasive Species Sampling Method with an expansion to the Dreissenid mussel early detection monitoring section. The protocol has been updated to reflect the best available science and protocols being used across the Columbia River Basin region. 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.

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). Adult mussels can be found as deep as 55 m (180'), but the depth of maximum abundance is usually between 2-4 m (Mackie et al. 1989, Mackie and Schlosser 1996).

When sampling for adult Dreissenid mussels pay attention that any native mussels present should not be disturbed (Figure 3).

[1] The Zebra and Quagga Mussel Rapid Response Plan for British Columbia can be downloaded from https://www.for.gov.bc.ca/hra/invasive-species/Publications/Prov_ZQM_EDRR_Plan.pdf

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

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

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

3. DREISSENID VELIGER SAMPLING

Veliger samples are collected using a plankton net and conducting vertical and/or horizontal tows. Vertical tows are the preferred method but horizontal tows can be conducted when a vertical tow is not feasible, for example, if a boat is not available and the water depth is too shallow from the dock. Plankton nets with a mesh size of 64µm (max 65µm), and 30cm to 50cm diameter net mouth opening must be used (Figure 4).

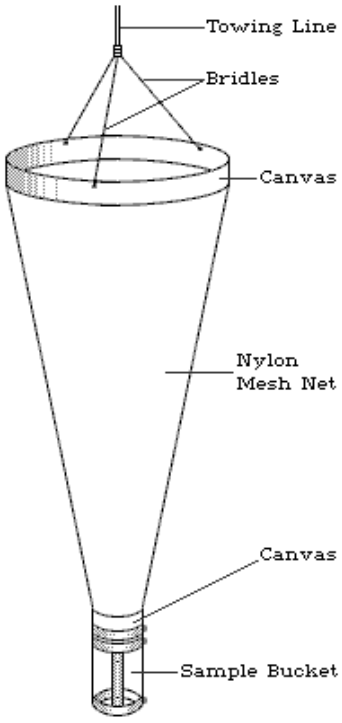

Figure 4. Plankton net used for collecting veliger samples

3.1 WHERE TO SAMPLE

Veligers have been found throughout the water column, ranging from near the surface to depths greater than 122-m (400-ft). The water depth where peak veliger density occurs can vary within and between water bodies. The veligers undergo a diurnal vertical migration, remaining in deeper water during the day and ascending in the water column during the night. When choosing sampling locations, try to select the number and location of sites in such a way to represent the entire water body.

3.1.1 WATERBODY DISTRIBUTION

Rivers: main stream, near dam, near marinas and boat launches, behind islands or downstream of large obstructions that cause eddies, in downwind bays, and along shore in areas of eddies and downwind positions.
Lakes/Reservoirs: near dam and outflows, open water areas, downwind positions (e.g., in a particular bay), near shore areas such as marinas and boat launches, and other areas of eddies.

3.1.2 VERTICAL (WATER COLUMN) DISTRIBUTION

Rivers and non-stratified lakes/reservoirs: entire water column for vertical tows at discrete spatial locations, and 10-m (33-ft) depth for horizontal tows at discrete water depths.
Stratified lakes/reservoirs: tow depths of 31 meters or just above thermocline to surface for vertical tows, near and just above the thermocline across a large horizontal spatial area for horizontal tows.

Samples should be collected from a boat, if possible, at a minimum of three sites in each waterbody. The three sampling sites should be fairly close to hard substrate (i.e. habitat such as rocks or piers) but deep enough to sample. A boat allows the sampling to be independent of land-accessible structures (e.g., docks). Samples should be collected in near shore and open water areas, in different bays or basins and focused on areas near boat launches and marinas, near outflows (e.g., intakes for powerhouse), near inputs (e.g. aqueduct entering a reservoir), in downstream and downwind positions and other areas where plankton collects (e.g., eddy). Additional samples can be taken in bigger bodies of water where there may be multiple fingers, bays, or multiple boat launches.

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 the vertical or horizontal plankton tow procedures outlined below. Mark on the lake map where samples were collected and collect GPS coordinates (lat/long). These same sites should be use for each of the sample periods and if the same site cannot be re-visited then the new sample location must be recorded (GPS lat/long).

3.2 WHEN TO SAMPLE

Veligers can exhibit spatial and temporal patchiness in the water column and high sampling frequency (weekly or biweekly) increases the likelihood of collecting veligers. Dreissenid mussels spawning can start at temperatures as low as 9°C, and preferably after the water temperature has been maintained at 12°C for one to two weeks. The timing of the sampling period will vary by geographic region based on suitable temperature. Sampling should typically occur from May to October period, with the focus of effort in the July to September period. 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.

3.3 SAMPLING METHODS

Vertical plankton tows are recommended to collect a depth-integrated sample from the river/lake bottom to water surface. Horizontal tows are recommended to capture a larger horizontal spatial component at a specific depth, and should be targeted in the upper sections of the water column, i.e., 5- and 10-m (16- and 33-ft) depths. Ideally a combination of vertical and horizontal tows should be conducted, but preference should be given to vertical tows if both methods cannot be used.

A minimum of three plankton tows should be conducted at each sample site and combined into one sample container (Figure 5). More than three plankton tows may be collected to increase the likelihood of collecting veligers. 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. Collect each plankton tow in a different area of the site to further increase the likelihood of collecting veligers. 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. If multiple tows are taken from one sample site use the average temperature from each of the tows.

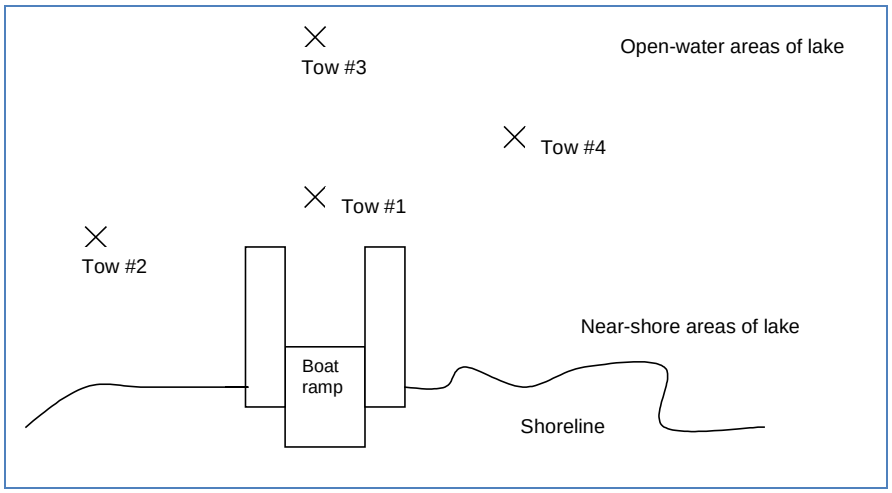

Figure 5. An example of plankton tow collection at a site location

The volume of water that is sampled in each tow should be recorded whenever possible and is calculated based on the diameter of the net opening and the distance towed. A minimum sample volume of 1,000 L is recommended. Record the depth and distance of the tow. (Caution: To assure accuracy of the sample volume, do not let the retrieval speed exceed the filtration rate of the net, otherwise a pressure wave develops, and water is forced to the surface prior to the net emerging from the water). Remember that veligers from spawning zebra and quagga mussels are more commonly found in deeper water so sample accordingly.

In order to prevent the net from clogging it is important to determine the appropriate volume of water to sample and this can generally be determined by the trophic status of the lake. Although it is only an indicator, Secchi disk depth is the simplest and one of the most effective tools for estimating a lake's productivity/trophic status. A Secchi disk is a circular plate divided into quarters painted alternately black and white (Figure 6). The disk is attached to a rope and lowered into the water until it is no longer visible. Higher Secchi readings mean more rope was let out before the disk disappeared from sight and indicates clearer water. Lower readings indicate turbid or coloured water. Clarity is affected by algae, soil particles, and other materials suspended in the water. However, Secchi disk depth is primarily used as an indicator of algal abundance and general lake productivity. Secchi disk readings vary seasonally with changes in photosynthesis and algal growth.

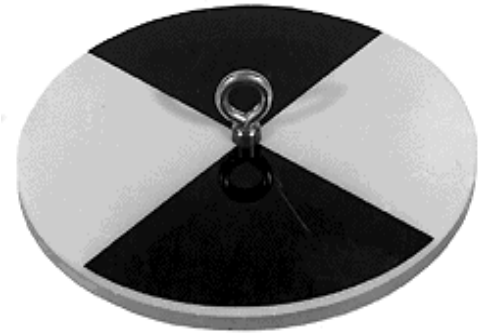

Figure 6. Secchi disk

A highly eutrophic lake will quickly fill the net with plankton and increase the possibility of clogging the net which will lead to under sampling. However an oligotrophic lake requires sampling a greater volume of water to collect a sufficient sample. For example using the standard plankton net (50-cm diameter, 64 micron mesh) the volume of water to collect for the different trophic conditions are:

- Oligotrophic lakes (Secchi > 4m**) - collecting four 5-6 meter long tows, you will have sampled ~4000L of water per site. Consolidate to 1 sample for each site.
- Mesotrophic lakes (Secchi 2-4m) collect four 3-4 meter tows from each site. You will have sampled ~2500L of water per site.
- Eutrophic lakes - (Secchi < 2m) collect four 1.5-meter tows from each site. You will have sampled ~1000L of water per site. Samples from eutrophic lakes are more difficult to analyze, so reducing the sample volume will facilitate the process.

When net clogging occurs, a thin layer of plankton, accumulated at the inner surface of the net, blocks water penetration and instead of going into the net water is forced over the rim and outside of the net. If clogging occurs, first try reducing the length of the tow.

The above tow lengths and water volumes are just examples, actual lengths and volumes should be adjusted depending on the sample site (site depth, location, stratification etc.), and following the sampling protocol for vertical or horizontal tows.

Table 1. Summary of plankton tow sampling recommendations.

Parameter	Recommendation
Water temperature	≥9°C
Locations	Around floating structures, marinas, inlets and outlets, coves, down-wind areas and eddies
Depth	0 – 31 m (stratified) or entire water column (non-stratified)
Number of sampling sites per waterbody	Variable; based on size of waterbody, minimum of 3
Number of tows per sampling site	Variable; based on depth and net size
Total volume sampled	Minimum 1000 liters per site

3.3.1 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 31 m (or appropriate depth depending on thermocline) below water surface, or to 1 m above the sediment (in non-stratified waters) while making sure the bottom of the net does not hit the lake bottom. Touching the bottom will clog the net. When this happens, the sample is of muddy water, which is very difficult or impossible to analyze. Do not dispense any of the bottom material into the sample bottle. If you hit the lake bottom, rinse out the sampling equipment and try shorter tows (e.g. two 1-meter tows instead of the protocol of one 2-meter tow), 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.

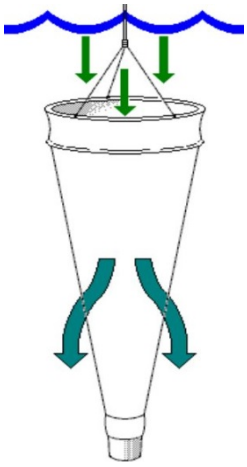

Figure 7. Vertical plankton tow

3. If conducting the plankton tow from a dock and there is only 1-2m of depth available, either conduct several vertical hauls at the same site, or conduct a horizontal plankton tow while walking the length of the dock or using a shoreline toss (see methods below). If possible avoid sampling in aquatic vegetation, but if it is a high use/high boat traffic site sampling should still be conducted.
4. Keep the net at this 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 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.

6. A squirt bottle, filled with either tap water or water from the lake or river, can be used to squirt down the sides of the net. Spray the outside of the net starting at the mouth to rinse organisms into the cod end.
7. Condense and decant your plankton sample into your bottle after each tow to obtain an accurate enumeration of the larval density in your lake. Carefully remove the cod-end piece without spilling collected water and plankton. Condense the sample by swirling the cod-end piece. You may need to use tweezers or a spatula to gently clear the mesh netting in the cod-end piece to allow the water to filter through.
8. Lower the cod-end-piece (separated from the plankton net) into the water, keeping the opening above the water surface. Condense the sample again by filtering out as much water as possible in the field and pour into the sample container. Repeat this procedure until the cod-end piece appears clean. This helps reduce the amount of alcohol that needs to be added and aids in the analyses as well.
9. It is important to record the number and length of tows as well as diameter of net mouth opening, so that the quantity of water sampled can be determined. GPS locations and lengths of tows should be recorded on field datasheets for plankton samples as well as other metadata collected, e.g., water quality, water temperature, Secchi disk readings, weather, etc. (see below and Appendix B).
10. In shallow lakes, you may split the sampling depth (i.e., two one-meter tows with the 50-cm net or five one-meter tows with the 30-cm net), or where it is impracticable to do a vertical tow, collect a horizontal sample at mid-depth.
11. Record survey information in field data sheet (Appendix B) and send it to the specified analytical lab along with the collected samples. Data should be also submitted to the BC Ministry of Environment and Climate Change Strategy (BC ENV). See Appendix A: Reporting information for contact information.

3.3.2 HORIZONTAL PLANKTON TOW

Vertical tows are preferred over horizontal tows however, horizontal tows may be required when a boat is not available or when sampling shallow water or in near shore depths (e.g., when sampling off docks). Trawling is recommended to capture a larger horizontal spatial component at a specific depth, and should be targeted in the upper sections of the water column, i.e., 5- and 10-m.

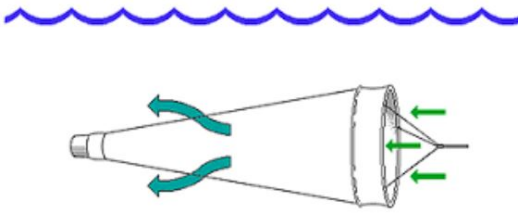

Figure 8. Horizontal Plankton tow

Trawling (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. The net is thrown into the water and allowed to sink to no more than 1 m above the bottom.
3. Lower the net 5- to 10-m (16- to 33-ft) below surface of water and keep net at this depth. Record the start time and the starting location coordinates on field datasheet. Record the distance that the net is towed through the water.

4. 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). Trawling 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 sediment to avoid both snagging and collecting debris.
5. 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.
6. Record the stop time, boat speed and the coordinates of the stop location on the 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).
7. Repeat techniques used for vertical plankton tows to concentrate organisms into the cod end of the net.

Table 2. Recommended minimum horizontal sample tow time (seconds).

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 it to sink below the water surface. While walking the length of the dock pull the net through the water parallel to the dock. If possible avoid sampling in aquatic vegetation, but if it is a high use/high boat traffic site sampling should still be conducted. Repeat techniques above to concentrate organisms into the cod end of the net. Record length of each tow, and number of tows.

3.3.3 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.

Figure 9. Shoreline toss (Photo: S. Wells)

3.4 COLLECTION OF ANCILLARY DATA

It is also important to collect as much metadata as possible at each sample site. The objective is to determine presence and location of thermocline, and to obtain relatively accurate data for water temperature, specific conductance, pH, and dissolved oxygen along depth profiles, as well as collecting other metadata. Most of these data can be measured using a multi-probe unit (e.g., Eureka Manta, AquaRead 900 or Hydrolab Quanta water quality multi-probe sonde). Calibrate multi-probe units

according to their manuals. Record metadata in the field datasheet (see Appendix B). If a temperature or pH probe is not available, measure both parameters at the surface by other available measuring equipment (pen meter, thermometer or pH strips).

1. Anchor boat or tie-off to structure close to sample site such as boom line in front of dam. 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 sunny side of boat. Lower it in to the water until the white quarters are no longer visible. For accurate estimation 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. Slowly raise disk until it reappears, and record this depth. Do not use polarized sunglasses or view finder.
4. Record the depth of the Secchi disk reading on datasheet.
5. Record multi-probe readings at 1-m depth intervals. Start at surface and move down. Keep the unit at least 1-m off the lake bottom.
6. Allow unit to stabilize at each depth (temperature ±0.01°C, depth ±0.1 m, DO ±0.01 mg/L, and pH ±0.01).
7. Record values on datasheet. Report water temperatures in degrees Centigrade.
8. 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 (SpC 7%, pH 0.2 units, and DO 0.2 mg/L).
9. Remove slotted probe cover, and attach probe storage cup with ¼-inch tap water. Do not use DI water. If no tap water is available use lake water or pH standard.
10. If possible, measure Ca++ concentration

If probe is not available pH, temperature or Ca can be measured using Pen meter:

1. Remove the protective cap and insert the electrode into the water.
2. Press the PWR key to turn the meter on and slowly stir until the reading settles.
3. The decimal point will blink while the meter is measuring

3.5 EQUIPMENT FOR PLANKTON TOW

- Plankton net (simple, conical plankton-tow net, 64 um pore size, recommended 0.5 m diameter net opening, but 0.3 m is also acceptable, removable weighted cod-end piece);
- Line for deploying the net (about 31m) 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 isopropyl alcohol);
- 5% Baking soda
- Squirt Bottle
- Field sheets and pen/ pencils;

- Thermometer;
- Permanent marker;
- GPS unit (*recommended*);
- Tweezers or small spatula (*recommended*);
- Boat (*recommended*);
- Multiprobe water quality instrument (e.g. Hydrolab®) (*recommended*) capable of measuring PH and Ca levels, or other portable meters
- pH strips (if multiprobe instrument or pen meter is not available)
- Secchi disk
- Measuring tape or ruler (*optional*)

3.6 VELIGER SAMPLE PRESERVATION

Sample preservation plays a significant role in the accurate identification of veligers in the laboratory analyses. Samples must be preserved in a 70% ethanol solution immediately after collection to ensure sample integrity. Regular ethanol is recommended, isopropyl alcohol is acceptable (99% is preferred over 70%), DO NOT use denatured ethanol.

To make a 70% ethanol solution in the sample container note the volume of sample in the container and then add three times the volume of 95% ethanol to the sample. For example, if your sample bottle contained 2.5 cm of sample, you would add 7.5 cm of 95% ethanol 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 using 70% Isopropyl alcohol, fill a squirt bottle with isopropyl alcohol instead of water and use this to clean the net into the sample container, this will help decrease the amount of extra water added to the sample, and then fill the sample jar with isopropyl alcohol to achieve a 70% alcohol solution.

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”.

Baking soda should be added into the sample to maintain sample pH above 7.0. A pH below 6.8 will result in shell dissolution and the loss of birefringence. To make a 1 liter of 5% baking soda solution, add 50 grams of baking soda to 1000 milliliters (1 L) of water. Add 5 ml of a 5% baking soda solution per 100 ml plankton tow sample then bring the volume to 70% ethanol.

After the addition of baking soda and preservative the pH of the sample should be 8.0 or slightly higher. The pH can be measured in the field with pH pen meter or pH test strips. If the pH is below 8.0, add more baking soda solution. The pH of the sample will also be measured in the laboratory at the time of receiving samples and reported with results. A pH below 8.0 at the time of measuring in the lab means that more baking soda solution should be added at the time of preservation.

Although samples preserved with ethanol may be stored in a cool, dry place for a maximum of three months prior to analysis, it is recommended that samples should be sent to the analytical laboratory within a week for the further analyses. DO NOT store preserved samples in the fridge. 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. Do not wait more than three hours to preserve samples. Keep preserved samples in a plastic container such as a bin or cooler in the back of the car while in transit.

Bias associated with veliger sample collection includes false-positive and false-negative results. False-positive results during sample collection are caused by cross-contamination of field sampling equipment. Multiple sets of gear and decontamination procedures are used to minimize these sources of bias. False-negative results during sample collection are caused by inadequate sample size, inappropriate location and frequency of sampling, and poor sample handling. Efforts should be focused on collecting numerous plankton tows from multiple locations and water depths during several sampling events throughout the peak spawning period to maximize the likelihood of veliger collection. Samples should be immediately preserved in solutions of 70% regular ethanol or isopropyl alcohol and buffered with 5% baking soda to maintain sample pH and specimen integrity.

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.

3.7 SAMPLE LABELING PROCEDURE

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. The label should contain the following information:

- Date of collection
- Name of water body;
- Site location
- Name/agency collecting sample. **Please include the name of the organization responsible for the sample collection – this is important information for year-end reporting, especially if one person is collecting samples for several different organizations.**

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

Additional information that are not on the label but should be recorded in the datasheet (Appendix B) are: site location (lat/long), net hoop diameter, preservative used, buffer used, water temperature, pH, conductivity, Secchi depth, oxygen, calcium, number of tows, type of tow (vertical or horizontal), length of tows, volume of water sampled. Note that the correct format for latitude and longitude is **decimal degrees** and not UTMs. Latitude and longitude must be recorded in separate columns in the data sheet. Report the survey information to the BC Ministry of Environment and Climate Change Strategy, and see Appendix A for contact details.

3.8 PACKAGING AND SHIPPING SAMPLES

Samples should be delivered or shipped to the analytical laboratory within 1 week of collection, by available courier using ground service:

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.

ETOH is a Class 3 flammable liquid and there are restrictions regarding its transport. ETOH can only be transported on the ground/ surface. Do not ship any samples via air and 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. ETOH-preserved samples must be shipped or mailed to the designated analytical lab via ground or surface mail using an available courier and following their specified shipping protocol.

4. ADULT DREISSENID MUSSEL SAMPLING METHODS

The objective of sampling for adult and juvenile 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, zebra 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 adult Dreissenid mussels in BC artificial substrate samplers is the recommended method.**

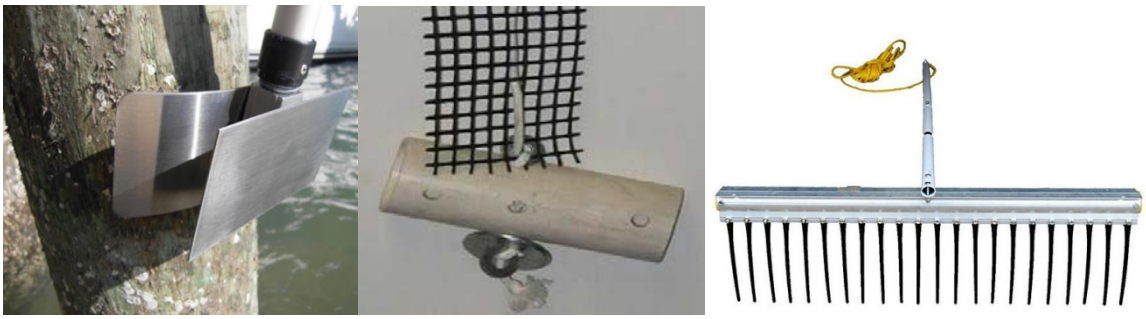

Figure 10. Adult mussels sampling tools: a) surface scraper, b) substrate sampler, c) thatch rake on a rope.

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. Shoreline surveys should be conducted about once every two weeks from ice out to ice on.

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 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 settlement substrate sampling allows for widespread/low cost and low effort monitoring across the province for detection of invasive Dreissenid mussels (Figure 10 b). Substrate refers to any substance in the water that Dreissenid mussels may attach to. Substrate samplers are used to determine if Dreissenid mussels are present in waterbodies or for early detection and monitoring of newly-settled juvenile and adult Dreissenid mussels that colonize substrate surfaces. When placed in waters without known populations of Dreissenid mussels, substrate sampler monitoring documents the arrival and tracks the spread of mussels.

Substrate Sampler Construction:

- The substrate sampler consists of 17 cm sections of white PVC pipe (5-cm diameter) and black ABS pipe (5 cm diameter) with 7 mm diameter holes drilled into the pipe.
- At the middle section of white PVC pipe long 17 cm and diameter 5cm, drill two holes 7mm wide (at the opposite sides of the pipe). Repeat the same with the black PVC pipe with the same dimensions.
- At the end of a long rope (length depends on the depth of the waterbody) suspend white pipe by pulling the rope trough both holes, and tie a small (500g) concrete anchor below the suspended pipe.
- A plastic construction mesh (13 mm diameter mesh) is cut into 3 cm wide strips, and the rope is woven through this plastic mesh strip in between the terminal pipe sections and those located 3 m from bottom. Large flat washers are used above and below the pipe sections to keep them stable.
- A small (500 g) concrete anchor is tied to the end of the rope. The length of rope used depends on water depth of deployment.
- The ideal depth to deploy the substrate sampler is 2-8 m but will vary depending on the depth at the sample site. The key is to keep the end of substrate sampler off the lake bottom. A secure surface structure to tie the surface end of the rope to is the most limiting factor for substrate monitoring; dam booms work best.
- Buoys work well but substrate rope can get tangled with mooring line. Docks and piers are often used, but are typically found in shallower waters.
- Keep in mind that any hard surface works. Concrete, bare steel and certain plastics work well. Biofilm is good for settlement. Surface roughness or irregularities are best.

Materials needed:

- 13mm diameter plastic construction mesh (x3 cm wide strips)
- 17cm white PVC pipe (5cm diameter)
- 17 cm black PVC pipe (5 cm diameter)
- 500g concrete anchor
- 4 large flat washers
- 10m long rope

4.2 WHERE TO SAMPLE

Two substrate samplers should be deployed at each of the monitoring stations 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 (2 - 8m; this will vary depending on the depth at the sample site). As overland transport of watercraft can be an important vector for the spread of zebra and quagga mussels, it is recommended that substrate samplers be deployed in areas with high boat traffic (e.g. marinas, docks, pilings, etc.). Avoid placing substrate samplers in areas where there is

strong current. A physical description and GPS coordinates of each monitoring station must be obtained at initial deployment. If possible, it is ideal to get a contact person for each site (the person who will be checking the substrate sampler most often). The substrate sampler is a small surface area, so this is also an opportunity to check other substrates nearby (e.g., dock, pilings, boat hull, surrounding substrate in shallow water or anything in the water) and look for shells on rocks or beaches.

- Target areas around public boat ramps or areas that are likely to have a lot of boating traffic in the vicinity (for example, fishing hot spots, resorts, campgrounds, etc.).
- 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.
- Dreissenid mussels are often found in cracks and crevices of rocks and structures. Small mussels can be attached to plants as well.
- Artificial settlement substrates and submerged hard surfaces including docks, pilings, channel markers, floating bathrooms, buoys, bridge abutments, 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.

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, particular bays, eddies, etc.
- High boater use areas and points of entry, e.g., near marinas and launches
- Main stem, open water areas (on the existing floating objects) and near-shore areas.

Vertical (water column) distribution

- Lake bottom to exposed shoreline areas in well mixed water bodies
- Thermocline to surface in stratified lakes and reservoirs.

4.3 WHEN TO SAMPLE

Sampling should begin when water temperatures reach between 9°C and 12°C, and is maintained for a week or two, which is most conducive to Dreissenid reproduction. The timing of sampling will vary by geographic region but typically occurs between May and September. One of the two substrate samplers should be removed and checked monthly from May through September using the methods outlined below. The other substrate sampler should be left in the entire monitoring season and then checked at the end of the monitoring season (September):

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). 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
4. If no aquatic invasive species are found, return the substrate sampler back into the water where it was found.
5. If a sample is collected, fill out the information sheet found in Appendix C and report survey information to the BC Ministry of Environment & Climate Change Strategy.

4.4 ADULT DREISSENID SAMPLE PRESERVATION

Preserve suspect 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. Samples, placed into a sealable plastic bag with a small amount of water, may be temporarily stored in the refrigerator (1 to 5 days). Do NOT wait more than three hours to preserve or refrigerate samples. Regular ethanol (ETOH) is the preferred chemical preservative but isopropyl alcohol can also be used when regular ethanol cannot be acquired. DO NOT use denatured ethanol. When using alcohol as a preservative, use stock that is 95% regular ethanol or 99% isopropyl alcohol and greater, and add enough preservative so that specimen(s) and/or associated substrate are completely submerged. Freezing is an acceptable method of preserving adult mussel specimens.

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.

5. 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. Dreissenid mussel veligers are microscopic in size and

juveniles can start attaching to hard surfaces as tiny fragments hardly visible to the human eye, so proper decontamination procedures need to be followed.

5.1 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 is recommended to remove all plant fragments and dirt from watercraft and equipment before leaving the site. It can also be done away from the lake where run-off will not going 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.

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.

5.2 WATERCRAFT

While clean, drain and dry should always be practiced it is also recommended that additional decontamination procedures are used to prevent the spread of aquatic invaders from infested waterbodies to un-infested waterbodies, particularly if personnel are moving between watersheds which are not naturally connected. The following procedures can be done when moving between waterbodies within BC. If you are bringing your boat from outside of BC then you must contact the BC Invasive Mussel Defence Program 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 watercraft decontamination to prevent damage to watercraft. 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.

5.2.1 HOT WATER WASH

Hot water decontamination should be performed in a "high and dry" location where the decontamination water will not run off into any water body. All surfaces that come into contact with a water body must be thoroughly decontaminated using low pressure (40-60 psi) hot water (at a minimum of 60° C) ensuring at least 10 seconds of exposure to all surfaces. This includes the watercraft's exterior (hull, motor), interior (live wells) and any equipment such as anchors, PFDs, nets, float cushions/belts, chains, ropes, fenders as well as the trailer and backend of the motor vehicle. The water must be sprayed at no more than 10 cm (4 inches) from the surface being treated, making sure to avoid nozzle touching the contaminated surface. All compartment drains must be open during the interior flushing process to allow all water to drain.

5.3 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 to sample is strongly recommended. Field equipment that is used in **multiple water bodies** should undergo full decontamination involving both physical and chemical means to prevent transfer of a variety of taxa within and between systems and samples. You do not have to decontaminate equipment between sampling sites on the same waterbody. Decontaminations must be conducted such that runoff does not reach any waterbody and should be done in locations that are high and dry. Single use disposal gloves should be worn when working with bleach solutions. The use of vinegar and bleach can present safety hazards if not used properly. Appropriate Material Safety Data Sheets (SDS) should be included and followed in the standard operating procedures.

Veligers easily stick to the walls of the plankton net. Decontamination procedures require that all laboratory and field equipment be soaked in a 5% acetic acid solution (vinegar). The vinegar degrades the calcium carbonate shell resulting in a negative microscopy result. A bleach rinse is added to eliminate any free-floating tissue that may have a positive result by PCR. The vinegar dissolves the veliger's shell but will not denature DNA. The bleach will denature DNA but will not dissolve shells. Therefore, the vinegar must be used before the bleach so DNA will be exposed to the denaturing bleach.

<u>Sensitive Equipment:</u>

- Rinse multi-probe unit sensors with ample fresh tap water. Replace water in probe storage cup with fresh tap water. It is important to note that dissolved oxygen probes and other sensitive electronic sampling gear may be damaged by chemicals used for decontamination and should only be rinsed with clean water.

General Sampling Equipment:

Step 1 - Vinegar:

- All sampling gear (including net, cod-end, rope, net anchor, dredge, sieve, plant rake, surface scraper, wash bottles, buckets, etc.) that comes into contact with the water should be soaked in 5% vinegar (acetic acid solution) for 24 hours (an absolute minimum of 4 hours). The ideal soak time is overnight; however, if it is necessary to use the net at the next sampling location during the same day, a one hour soak followed up with a rinse prior to the next sampling should be the minimum. The same acetic acid bath may be used repeatedly for all sample sites.
- Following the acetic acid soak, rinse the net with a large volume of clean water before using any bleach solution.
- The vinegar solution can be reused multiple times and should be poured back into the original container for storage. The vinegar solution should be periodically checked with pH test strips to make sure the pH level remains at approximately 2-3.

Step 2 – Bleach:

- As an additional recommended precaution, sampling equipment can be sprayed with or soaked in chlorine solution (household bleach). Routinely inspect the net for damage or wear and repair or replace if necessary. Soak the plankton net, cod-end, plankton rope, net anchor, dredge, sieve, plant rake, and surface scraper in the plastic tub containing 10% solution of bleach for 10 minutes, and then thoroughly rinse with clean tap water (the bleach is corrosive so rinse thoroughly with clean water).
    a. For soaking gear a larger amount of 10% solution should be made by adding approximately 5-L of household bleach to the tub containing approximately 50-L of tap water. The bleach solution must be fresh (less than 24 hours old).
- Bleach is corrosive and equipment must be thoroughly rinsed with tap water following decontamination. Allow the items to air dry completely.
- The chlorine 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 a depot near you to dispose of chemicals with minimal environmental impact.

Step 3 (optional) – Freeze:

- As an addition to the above procedure all equipment could be solid freeze overnight to kill any veligers. Note that freezing should only be done in addition to the vinegar and bleach solutions and cannot be used as a standalone decontamination method.

Table 3. Dreissenid mussel decontamination methods for field equipment.

Decontaminant	Concentration	Contact Time	Usage Guidelines, Safety Precautions, Drawbacks
Vinegar	5%	24h (4h minimum)	Dipping **equipment** into 5% vinegar for 24 hours will kill harmful aquatic hitchhiker species. MUST Ensure that solution does not run-off into any waterways.
Chlorine	10%	10 min	Bleach is corrosive and **equipment** must be thoroughly rinsed with tap water following decontamination. MUST Ensure that solution does not run-off into any waterways.
hot water wash	≥60° C Use hottest water available	10-15 seconds per location	Use for decontamination of **watercraft** and **gear** - *caution when using due to possibility of burns/scalding.* Temperature and contact times are crucial for the efficiency. Hot water wash, including thoroughly flushing lower motor unit.
Freezing	<0° C	>4 h	**Boats, gear, and equipment** should be thoroughly frozen. Ambient air temperature should remain below freezing for the entire contact time. No safety precautions.

6. WATERBODY RISK ASSESSMENT RANKING CRITERIA

This section provides a tool for assessing the risk of Dreissenid mussel introduction into a waterbody within BC and how to prioritize early detection lake monitoring efforts based on the risk. The probability of invasion of Dreissenid mussels into a given waterbody within BC should be assessed using climate, habitat and socio-economic factors. In order to prioritize waterbodies for early detection monitoring it is important to evaluate the probability of survival (water quality parameters), the probability of arrival (human activities) and the impact of invasion (e.g. species at risk, hydropower facilities).
The waterbody risk ranking should then be used to identify the appropriate sampling method (plankton tow vs. substrate monitor) and level of monitoring effort that should be done within a waterbody.

6.1 PROBABILITY OF SURVIVAL

An understanding of the zebra mussel's life cycle and its seasonal pattern of development is useful in considering how environmental factors may affect the mussel's distribution. For Dreissenid mussels to become established, the water quality parameters must be suitable for all of their life stages and processes, including juvenile and adult survival and growth, gonad development, gametogenesis, spawning, fertilization, embryonic and larval development, and settlement.

For example in a well-established mussel population with optimal environmental factors, synthesis of gametes typically peaks in the spring and spawning begins in late summer depending on water temperatures (optimum 12-18°C). During spawning large quantities of eggs and sperm are released into the water where fertilization occurs, with a single spawning female potentially releasing tens of thousands to millions of eggs (Mackie *et al.* 1989; Sprung 1993; Mackie & Schloesser 1996; Nichols 1996). After fertilization, pelagic, microscopic larvae (veligers) develop within a few days and acquire minute bivalve shells. The veligers drift with water currents for three to four weeks before settling to a substrate.

Predicting which water-bodies have the highest risk of Dreissenid mussel introduction, will help allocate available resources to the most vulnerable systems. Water quality parameters that have a great impact on Dreissenid life history include calcium, pH, dissolved oxygen, temperature, salinity and turbidity (Mills et al., 1996; Wright et al., 1996; Karatayev et al., 1998; Thorp et al., 2002; Jones & Ricciardi, 2005; Claudi et al., 2012).

6.2 HABITAT SUITABILITY

There are multiple variables that need to be considered when assessing habitat suitability for Dreissenid mussels. For example, calcium concentration is commonly used as a key indicator of Dreissenid mussel survival and growth, suggesting that concentration of >25 mg/l is needed for establishment of mussel population. However if other parameters, such as pH, temperature, oxygen concentration etc. are out of the optimal range, the mussel population will not be able to survive or reproduce despite optimal calcium levels. Therefore a complete assessment of site specific water quality, hydrology and geology will help provide a better determination of risk.

Mackie and Claudi (2010) prepared tables with most common parameters used to predict level of Dreissenid mussel infestation (Appendix D) and potential of adult and juvenile survival, development and growth (Appendix E). Parameters in Appendix D are listed in order of their predictive value from most reliable to less reliable.

Although mean annual values of each of the parameters can be used, temporal (e.g. seasonal) and spatial (e.g. depth, horizontal) variations lend more certainty to the predictions of mussel survival and potential densities. However, means and ranges of these water quality parameters can be very useful for a rapid assessment of infestation potential. Mackie and Claudi (2010) utilized the terms; “chalk variables” (calcium, alkalinity, pH, total hardness), “trophic variables” (nutrients, chlorophyll *a*, Secchi depth and dissolved oxygen) and “physical variables” (conductivity and mean summer water temperature) for their risk assessments.

6.2.1 CALCIUM

Of the chalk variables calcium is by far the most reliable. Calcium is required for shell production, and its concentration is considered the most critical environmental variable for Dreissenid survival and density. It is used as the primary parameter to rank water susceptibility to Dreissenid infestation (Neary and Leach 1991; Cohen and Weinstein 1998; Whittier *et al.* 2008; Wells *et al.* 2010; Claudi and Prescott 2011). Low calcium level can limit the survival of introduced adults and can prevent veligers from developing and reproducing. Dreissenid mussels require calcium for the formation of their shells, which develop shortly after fertilization. The shell is comprised of approximately 40% (dry weight) calcium (Secor *et al.* 1993). Dreissenids have higher calcium requirements than most fresh water mollusks (Claudi and Prescott 2011), and quagga and zebra mussels have variable tolerances for calcium concentrations. Overall researchers have recommended the minimum calcium concentrations of 12-15 mg/L to assess potential Dreissenid distribution (Neary and Leach 1991; Baker *et al.* 1993; Claudi and Mackie 1994; McMahon 1996).

6.2.2 PH

Dreissenid mussels' calcium requirements vary with changes in other environmental factors. Claudi *et al.* (2012) showed that adult shells experienced significant loss of calcium at a pH of 7.1, and at 6.9 the loss of calcium resulted in 40% mortality of adults. The calcium present in Dreissenid shells is primarily in the form of calcium carbonate (Secor et al. 1993), which solubility increases as pH decreases. pH values exceeding 9.5 are lethal to Dreissenids, with the lethal threshold starting at pH 9.0. Optimal pH levels are between pH 8.0 and 9.0. Several studies conclude that zebra mussels' calcium threshold varies with pH, mainly declining with increasing pH (Ramcharan et al. 1994; Hincks & Mackie 1997). Therefore regardless of calcium availability, if pH is too low or high mussel shells will begin to thin and erode as they lose calcium to the external environment (Hincks and Mackie 1997; McMahon 1996; Claudi and Prescott 2011).

6.2.3 TEMPERATURE

Water temperature also has impact on establishing Dreissenid population. However, high water temperature is only likely to serve as a limiting factor in waters that are both warm and shallow. Deep lakes and reservoirs often stratify in the summer, maintaining significant temperature differences between the warmer, upper water level (epilimnion) and the cooler, lower water level (hypolimnion). Some deep water reservoirs with near-surface high water temperatures which is limiting factor for Dreissenid population development, may nonetheless be able to support a large mussel population at lower depths. Water temperature can influence mussel survival, growth and reproduction rates. Shell growth can occur at temperatures as low as 3°C with the typical low range at 6 to 8°C. Eggs can be released at 13°C, but the release rate increases at temperatures over 17°C. Zebra mussels can persist in temperatures up to 30°C with an optimal range of 20 to 26°C (Figure 11).

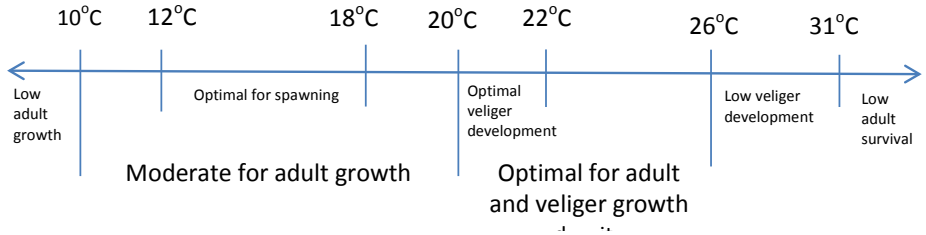

Figure 11. Temperature requirements for Dreissenid mussel population development

Summer temperatures exceeding 26-33°C are considered to be the zebra mussels' upper thermal limit based on long-term lethal temperature effects (Stanczkowska 1977; Strayer 1991; Baker *et al.*1993; Armistead 1995; Mills *et al.* 1996; Cohen 2005). Water temperatures above 6-12°C are needed to support adult growth and spawning during the summer (Stanczykowska 1977; Baker *et al.* 1993; Sprung 1993; Nichols 1996; McMahon 1996).

Additionally, mussels have an upper and lower lethal thermal limit, and water temperature directly impacts their physiological activity, including spawning, development, and growth. Lower threshold (for mean and maximum) of summer temperatures needed for spawning of zebra mussels are 10° and 12°C and 5° and 6°C for quagga mussels. An upper limit of 31°C for both zebra and quagga mussels represent the long-term lethal temperature (Cohen 2007). Overall, mussels can survive at a wide range of

temperatures, and because water bodies stratify during the summer months, mussels would be able to survive and spawn at lower depths even if the surface temperatures reached lethal levels (Cohen 2007). Seasonal and diurnal temperatures in river systems follow atmospheric temperatures more closely than lakes and reservoirs. Rivers are usually in a proportional equilibrium with mean monthly air temperature. Because rivers are shallow and turbulent, thermal stratification is not generally an attribute.

6.2.4 OTHER WATER QUALITY PARAMETERS

While calcium, pH and temperature may be the most predictive parameters for potential Dreissenid mussel distribution, other parameters such as total phosphorous, turbidity (Secchi depth), dissolved oxygen content, and conductivity (or salinity) can also limit establishment and abundance. Total phosphorous, turbidity, and dissolved oxygen are parameters that indicate nutrient availability. When total phosphorous, dissolved oxygen and turbidity values are high the biomass of algae is often high. Mussels feed on planktonic algae, therefore trophic indicators like nutrient parameters (total phosphorous and total nitrogen), chlorophyll *a*, Secchi depth, and dissolved oxygen content can be used as a criteria for predicting Dreissenid mussel presence and abundance (Mackie and Claudi 2010). High nutrient, chlorophyll *a*, and surface dissolved oxygen values and low Secchi depth values typically indicate greater algae biomass availability for mussels.

Therriault et al. (2012) conducted a risk assessment of the probability of survival (habitat suitability) and arrival of Dreissenid mussels across Canada including British Columbia. Eastern sub-drainages within British Columbia were found to have the highest **probability of survival** for Dreissenid mussels as determined from calcium concentrations corrected for potential temperature limitations. Sub-drainages along the west coast of British Columbia were found to have low probability of survival, with localized conditions that could be more or less suitable for Dreissenid mussel survival (Appendix F).

6.3 PROBABILITY OF INVASION

The probability of invasion represents the relationship between climate, habitat and socio-economic factors, and all of them should be used for assessing risk. An important factor of invasion success is **propagule pressure**, or the number of individuals invading a new area. Upon their arrival, invaders need to persist in their new habitat, which will depend on environmental conditions in relation to species adaptability. The probability of arrival is defined as a function of propagule pressure and proximity to an invaded habitat. Spreading of Dreissenid mussels in most of the cases is associated with human activities (e.g., trailering of recreational boats). One of the most important factors for the potential introduction of Dreissenid mussels to western Canada is the overland transport of recreational and commercial boats originating from invaded habitats in the United States and eastern Canada.

6.4 WATERBODY RANKING CRITERIA

The vulnerability of the waterbodies should be assessed by evaluating both environmental (especially Ca, pH, temperature and if possible trophic data etc.), and non-environmental (human activities etc.) factors which play a significant role in the successful invasion and establishment of Dreissenid mussels into the waterbody. Ranking factors are divided into three categories: probability of survival, probability of arrival and impact of invasion (Table 4). Not all ranking factors will be applicable to an individual waterbody, therefore only the relevant ranking factors and the best available data should be used for

ranking a waterbody. A minimum of one ranking factor from each category (probability of survival, probability of arrival and impact of invasion) must be used (minimum total of three factors) for calculating the waterbody ranking score.

Table 4.Criteria used to prioritize waterbodies for early detection monitoring efforts for Dreissenid mussels in BC lakes. * If available mean summer temperature should be used instead of the annual mean.

Rank		1 point	2 points	**3 points**	**4 points**
Probability of Survival					
Calcium (mg/L)		0-4	4.1-13	13.1-24	24.1-100
*Water Temp °C	lower limit	0 - 4.4	4.5 -7.8	7.9-13.3	13.4-21.7
	upper limit	>28.3	24.0-28.3	21.8-23.9	
pH	lower limit	0-3.9	4-5.4	5.5-6.9	7-9.9
	upper limit	13-14	11.1-12.9	10-11	
Secchi depth (m)	lower limit	<0.1	0.1-1	1-2	2-4
	upper limit	>8	6-8	4-6	
Probability of Arrival					
Angler Days		<25% quartile	26- 50% quartile	51-75% quartile	> 75% quartile
Waterbody Type or Size (as measured at longest/widest point)		cold water stream(<22°C) or small lake (<2km)	Large river or medium lake (2-4 km)		warm water reservoir(>22°C) or large lake (>4km)
Position Rank		headwaters of watershed	upper end of watershed	lower end of watershed	bottom of watershed
# Boat Launches into waterbody		1	2 to 3	4 to 5	>5
Motorized watercraft		No motorized watercraft			Motorized watercraft permitted
Moorage		No boat moorage facilities			Boat moorage facilities present
Water-based events e.g. fishing/wakeboard/kayak festivals, fishing tournaments		Waterbodies that have no events		Waterbodies that have at least one event per year	Waterbodies that have more than one event per year
Ease of Access		by foot		gravel road	paved road
Proximity to source population		No drainages west of continental divide	West of continental divide	nearby, but may not be as easily accessible	downstream, connected, or within easy drive
Impact of Invasion					
Endangered/ Threatened Species		No known listed species present in waterbody		waterbody has threatened species	waterbody has endangered species
# Hydro-electric facilities and water intakes		1-4 water intakes		5-9 water intakes	≥10 water intakes
Recreation (# of recreation icons in the Backroads map)		1	2 to 3	4 to 5	>5

If there is insufficient data available for a waterbody to generate a score, the risk should be determined using the federal risk assessment by Therriault et al. (2012) and the data provided in Appendix G. The final waterbody ranking score is be calculated by first taking the average of each category and then taking the average across all three categories. Source of data/information must be provided for each ranking factor used.

Score of **3.5 - 4** points: "Critical" waterbodies are either highly susceptible to invasion and/or have significant threatened and endangered species concerns. In a "Critical" waterbody, it is considered essential that robust monitoring continue
Score of **2.6 - 3.5** point "High Priority" waterbodies are considered susceptible to invasion and/or have significant consequences
Score of **1.6 - 2.5** points "Medium Priority" waterbodies are considered to have possible invasion and/or have medium use
Score of **1 - 1.5** points "Low Priority" waterbodies have limited access/ low use and/or less significant impacts. These waterbodies are the lowest priority for monitoring

The waterbody ranking score should be used to determine the appropriate sampling method and frequency of sampling within and across waterbodies in a region. Depending on available resources plankton tow sampling should be prioritized to critical and high priority waterbodies while substrate samplers can be deployed in low to medium risk lakes. Monitoring efforts can also be increased at a low cost by using a combination of plankton tows and substrate monitors to target both adult and veliger life stages of Dreissenid mussels. Frequency of sampling within a waterbody should be prioritized based on the risk of the waterbody and the number of target waterbodies being sampled.

Table 5. Suggested frequency of sampling by waterbody risk score.

	Critical	High Priority	Medium Priority	Low Priority
Plankton tows	**At least monthly** when water temperatures are above 9°C	**At least monthly** when water temperatures are above 9°C	**Every 4-6 weeks** when water temperatures are above 9°C	at least **once per year** based on available resources
Substrate samplers	Check substrates every 4 weeks	Check substrates every 4 weeks	Check substrates every 4-8 weeks	Check substrates every 4-8 weeks
Water quality	Collect a water quality data at the deepest sampling location where plankton tows are collected	Collect a water quality data at the deepest sampling location where plankton tows are collected	Collect a water quality data at the deepest sampling location where plankton tows are collected	*Collect a water quality data at the deepest sampling location where plankton tows are collected

* 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 to collect the water quality profile. In water that is less than 1 meter deep, collect one profile reading just under the surface.

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APPENDIX A: REPORTING INFORMATION

Immediately report any suspected invasive mussels to 1-877-952-7200 (RAPP)

Species identification information:

www.gov.bc.ca/invasive-species

Submit data to:

Martina Beck
Invasive Fauna Unit Head
Email: Martina.Beck@gov.bc.ca
BC Ministry of Environment and Climate Change Strategy

Analytical Lab:

Contact and shipping information for the lab will be provided by BC ENV at the start of the respective sampling season.

APPENDIX B: FIELD DATASHEET FOR VELIGER PLANKTON TOWS

WATER BODY				Preservative				
DATE				Buffer				
COLLECTOR				Net hoop diameter				
				Weather				
					Water quality			
Sampling location	Latitude	Longitude	Depth (m)	Tow: type, length, number	pH	temperature	Ca	Secchi disc

APPENDIX C: FIELD DATASHEET FOR DREISSENID ADULT SAMPLING

WATER BODY				Preservative	
DATE				Weather	
COLLECTOR					
Sampling location	Latitude	Longitude	Depth (m)	Sample site description	Method (substrate sampler, surface scraper etc.)

APPENDIX D: CRITERIA USED IN DETERMINING LEVELS OF INFESTATION BY ZEBRA MUSSELS IN THE TEMPERATE ZONE OF NORTH AMERICA (from Mackie, 2010)

Parameter	No infestation	Low	Moderate	High
Calcium mg/L	<10	<16	16-24	≥24
Alkalinity mg CaCO3/L	<35	35-45	45-89	>90
Total Hardness mg CaCO3/L	<40	40-44	45-90	≥90
pH	<7.2	7.2-7.5	7.5-8.0 or 8.7-9.0	8.0-8.6
Mean Summer Temperature °C	<18	18-20 or >28	20-22 or 25-28	22-24
Dissolved Oxygen mg/L (% saturation)	<6 (25%)	6-7 (25-50%)	7-8 (50-75%)	≥8 (>75%)
Conductivity S/cm	<30	<30-37	37-84	≥85
Salinity mg/L (ppt)	>10	8-10 (<0.01)	5-10 (0.005-0.01)	<5 (<0.005)
Secchi depth m	<0.1	0.1-0.2 or >2.5	0.2-0.4	0.4-2.5
Chlorophyll a /L	<2.0 or >25	2.0-2.5 or 20-25	8-20	2.5-8
Total phosphorous g/L	<5 or >35	5-10 or 30-35	15-30	10-15
Total Nitrogen g/L	<200	200-250	250-300	300-500

APPENDIX E: CRITERIA USED IN DETERMINING POTENTIAL OF ADULT AND JUVENILE SURVIVAL, DEVELOPMENT AND GROWTH OF ZEBRA MUSSELS IN THE TEMPERATE ZONE OF NORTH AMERICA (from Mackie and Claudi, 2010)

		Low Potential for Adult Survival	Low Potential for Larval Development	Moderate (survivable, but will not flourish)	High (favorable for optimal growth)
Living Conditions	Dissolved oxygen (mg/l)	<3	3 - 7	7 - 8	>8
	Temperature (C)	<10 or >32	26 - 32	10 - 20	20 - 26
Shell Formation	Calcium (mg/l)	<8	8 - 15	15 - 30	>30
	pH	<7.0 or >9.5	7.0 - 7.8 or 9.0 - 9.5	7.8 - 8.2 or 8.8 - 9.0	8.2 - 8.8
	Alkalinity (as mg CaCO3/l)	<30	30 - 55	55 - 100	100 - 280
	Conductivity (umhos)	<30	30 - 60	60 - 110	>110
Food	Secchi depth (m)	<1 or >8	1 - 2 or 6 - 8	4 - 6	2 - 4
	Chlorophyll a (ug/l)	<2.5 or >25	2.0 - 2.5 or 20 - 25	8 - 20	2.5 - 8
	Total phosphorus (ppb)	<5 or >50	5 - 10 or 35 - 50	10 - 25	25 - 35

APPENDIX F: PROBABILITY OF ZEBRA MUSSEL AND QUAGGA MUSSEL INVASION

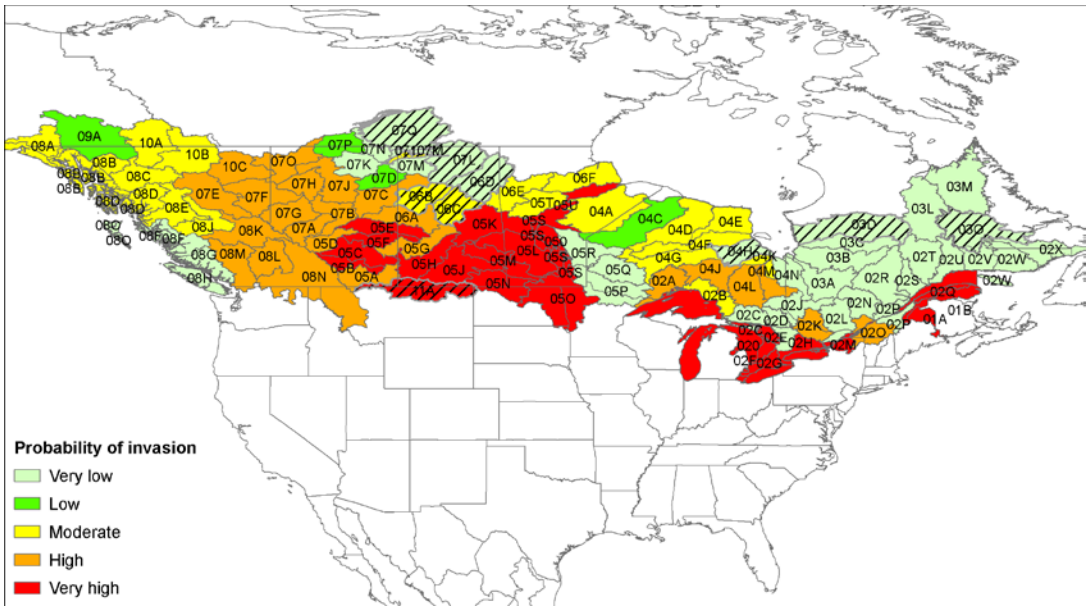

Figure A. Probability of Zebra Mussel invasion based on probability of survival and arrival. Hatched watersheds had less than 5 sampling sites (Therriault et al. 2013).

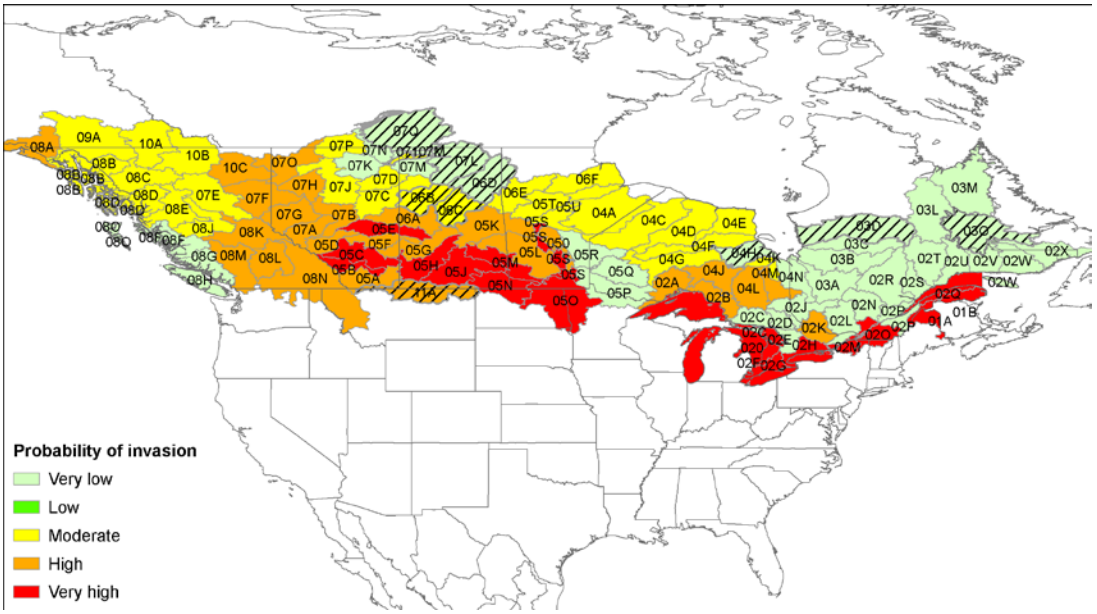

Figure B. Probability of Quagga Mussel invasion based on probability of survival and arrival. Hatched watersheds had less than 5 sampling sites (Therriault et al. 2013).

APPENDIX G1: *Percentage of sites falling into each calcium category for "n" sites and scores for the probability of survival (habitat suitability) for Zebra Mussel per sub-drainage based on calcium concentrations (mg/L; 75th percentile) and corrected for temperature. Sub-drainages were ranked on their suitability for Zebra Mussel survival based on literature accounts* (Therriault et al. 2013).

Province	ID	Sub-drainage	*n*	< 12	12-19	20-25	≥ 26	Probability of Survival
BC	08A	Alsek	5	0%	0%	60%	40%	High
BC	05B	Bow	21	10%	0%	0%	90%	Very high
BC	08F	Central Coastal Waters of B.C.	88	90%	6%	0%	5%	Very low
BC	10B	Central Liard	14	43%	21%	0%	36%	Very high
BC	08N	Columbia - U.S.A.	747	19%	17%	14%	50%	Very high
BC	10C	Fort Nelson	7	29%	0%	0%	71%	Very high
BC	07O	Hay	13	23%	15%	15%	46%	Very high
BC	09A	Headwaters Yukon	38	61%	26%	5%	8%	Very low
BC	08M	Lower Fraser	299	34%	26%	14%	25%	High
BC	08D	Nass – Coast	64	55%	27%	13%	6%	Moderate
BC	08J	Nechako	204	48%	33%	8%	11%	Moderate
BC	08B	Northern Coastal Waters of B.C.	27	19%	37%	26%	19%	Moderate
BC	08O	Queen Charlotte Islands	30	90%	7%	0%	3%	Very low
BC	08E	Skeena – Coast	309	57%	25%	6%	12%	Moderate
BC	07G	Smoky	33	3%	18%	27%	52%	Very high
BC	08G	Southern Coastal Waters of B.C.	92	80%	11%	2%	7%	Very low
BC	08C	Stikine – Coast	62	40%	18%	19%	23%	Moderate
BC	08L	Thompson	520	20%	21%	10%	49%	Very high
BC	07A	Upper Athabasca	9	0%	33%	0%	67%	Very high
BC	08K	Upper Fraser	198	21%	22%	12%	45%	Very high
BC	10A	Upper Liard	23	30%	17%	9%	43%	High
BC	05D	Upper North Saskatchewan	12	8%	8%	17%	67%	Very high
BC	07F	Upper Peace	157	3%	11%	10%	76%	Very high
BC	05A	Upper South Saskatchewan	52	0%	6%	8%	87%	Very high
BC	08H	Vancouver Island	562	82%	10%	2%	5%	Very low
BC	07E	Williston Lake	68	19%	38%	10%	32%	Very high

APPENDIX G2: *Percentage of sites falling into each calcium category for "n" sites and scores for probability of survival (habitat suitability) for Quagga Mussel per sub-drainage based on calcium concentrations (mg/L; 75th percentile). Sub-drainages were ranked on their suitability for Quagga Mussel survival based on literature accounts* (Therriault et al. 2013).

Province	ID	Sub-drainage	n	< 12	12-32	>32	Probability of Survival
BC	08A	Alsek	5	0%	60%	40%	Very high
BC	05B	Bow	21	10%	24%	67%	Very high
BC	08F	Central Coastal Waters of B.C.	88	90%	8%	2%	Very low
BC	10B	Central Liard	14	43%	36%	21%	High
BC	08N	Columbia - U.S.A.	747	19%	45%	36%	Very high
BC	10C	Fort Nelson	7	29%	14%	57%	Very high
BC	07O	Hay	13	23%	46%	31%	Very high
BC	09A	Headwaters Yukon	38	61%	37%	3%	High
BC	08M	Lower Fraser	299	34%	52%	13%	High
BC	08D	Nass - Coast	64	55%	44%	2%	High
BC	08J	Nechako	204	48%	43%	9%	High
BC	08B	Northern Coastal Waters of B.C.	27	19%	70%	11%	High
BC	08O	Queen Charlotte Islands	30	90%	7%	3%	Very low
BC	08E	Skeena - Coast	309	57%	35%	8%	High
BC	07G	Smoky	33	3%	52%	45%	Very high
BC	08G	Southern Coastal Waters of B.C.	92	80%	13%	7%	Very low
BC	08C	Stikine - Coast	62	40%	40%	19%	High
BC	08L	Thompson	520	20%	40%	40%	Very high
BC	07A	Upper Athabasca	9	0%	44%	56%	Very high
BC	08K	Upper Fraser	198	21%	44%	35%	Very high
BC	10A	Upper Liard	23	30%	48%	22%	High
BC	05D	Upper North Saskatchewan	12	8%	75%	17%	High
BC	07F	Upper Peace	157	3%	36%	61%	Very high
BC	05A	Upper South Saskatchewan	52	0%	42%	58%	Very high
BC	08H	Vancouver Island	562	82%	15%	2%	Very low
BC	07E	Williston Lake	68	19%	60%	21%	High

APPENDIX G3: *Probability of Zebra Mussel arrival, survival, and invasion per sub-drainage. The probability of invasion is based on the probability of survival (calcium suitability corrected for temperature) and probability of arrival (propagule pressure corrected for proximity to an invaded watershed). The risk to the environment is based on the probability of invasion and impacts to the environment* (Therriault et al. 2013).

Province	ID	Sub-drainage	Calcium Suitability	Temp corr.	Probability of Survival	Propagule Pressure	Prox corr.	Probability of Arrival	Probability of Invasion	Risk to Environment
BC	08A	Alsek	Very high	-1	High	Low	0	Low	Moderate	High
BC	05B	Bow	Very high	0	Very high	High	0	High	Very high	High
BC	08F	Central Coastal Waters of B.C.	Very low	0	Very low	Low	0	Low	Very low	Low
BC	10B	Central Liard	Very high	0	Very high	Very low	0	Very low	Moderate	High
BC	08N	Columbia - U.S.A.	Very high	0	Very high	Moderate	0	Moderate	High	High
BC	10C	Fort Nelson	Very high	0	Very high	Low	0	Low	High	High
BC	07O	Hay	Very high	0	Very high	Low	0	Low	High	High
BC	09A	Headwaters Yukon	Moderate	-1	Low	Low	0	Low	Low	Low
BC	08M	Lower Fraser	High	0	High	Moderate	0	Moderate	High	High
BC	08D	Nass - Coast	Moderate	0	Moderate	Low	0	Low	Moderate	High
BC	08J	Nechako	Moderate	0	Moderate	Low	0	Low	Moderate	High
BC	08B	Northern Coastal Waters of B.C.	High	-1	Moderate	Low	0	Low	Moderate	High
BC	08O	Queen Charlotte Islands	Very low	0	Very low	Moderate	0	Moderate	Very low	Low
BC	08E	Skeena - Coast	Moderate	0	Moderate	Low	0	Low	Moderate	High
BC	07G	Smoky	Very high	0	Very high	Moderate	0	Moderate	High	High
BC	08G	Southern Coastal Waters of B.C.	Very low	0	Very low	Low	0	Low	Very low	Low
BC	08C	Stikine - Coast	High	-1	Moderate	Low	0	Low	Moderate	High
BC	08L	Thompson	Very high	0	Very high	Moderate	0	Moderate	High	High
BC	07A	Upper Athabasca	Very high	0	Very high	Moderate	0	Moderate	High	High
BC	08K	Upper Fraser	Very high	0	Very high	Low	0	Low	High	High
BC	10A	Upper Liard	Very high	-1	High	Low	0	Low	Moderate	High
BC	05D	Upper North Saskatchewan	Very high	0	Very high	Moderate	0	Moderate	High	High
BC	07F	Upper Peace	Very high	0	Very high	Moderate	0	Moderate	High	High
BC	05A	Upper South Saskatchewan	Very high	0	Very high	Moderate	0	Moderate	High	High
BC	08H	Vancouver Island	Very low	0	Very low	Moderate	0	Moderate	Very low	Low
BC	07E	Williston Lake	Very high	0	Very high	Low	0	Low	High	High

APPENDIX G4: *Probability of Quagga Mussel arrival, survival, and invasion per sub-drainage. The probability of invasion is based on the probability of survival (calcium suitability) and probability of arrival (propagule pressure corrected for proximity to an invaded watershed). The risk to the environment is based on the probability of invasion and impacts to the environment* (Therriault et al. 2013).

Province	ID	Sub-drainage	Probability of Survival	Propagule Pressure	Prox corr.	Probability of Arrival	Probability of Invasion	Risk to Environment
BC	08A	Alsek	Very high	Low	0	Low	High	High
BC	05B	Bow	Very high	High	0	High	Very high	High
BC	08F	Central Coastal Waters of B.C.	Very low	Low	0	Low	Very low	Low
BC	10B	Central Liard	High	Very low	0	Very low	Moderate	High
BC	08N	Columbia - U.S.A.	Very high	Moderate	0	Moderate	High	High
BC	10C	Fort Nelson	Very high	Low	0	Low	High	High
BC	07O	Hay	Very high	Low	0	Low	High	High
BC	09A	Headwaters Yukon	High	Low	0	Low	Moderate	High
BC	08M	Lower Fraser	High	Moderate	0	Moderate	High	High
BC	08D	Nass - Coast	High	Low	0	Low	Moderate	High
BC	08J	Nechako	High	Low	0	Low	Moderate	High
BC	08B	Northern Coastal Waters of B.C.	High	Low	0	Low	Moderate	High
BC	08O	Queen Charlotte Islands	Very low	Moderate	0	Moderate	Very low	Low
BC	08E	Skeena - Coast	High	Low	0	Low	Moderate	High
BC	07G	Smoky	Very high	Moderate	0	Moderate	High	High
BC	08G	Southern Coastal Waters of B.C.	Very low	Low	0	Low	Very low	Low
BC	08C	Stikine - Coast	High	Low	0	Low	Moderate	High
BC	08L	Thompson	Very high	Moderate	0	Moderate	High	High
BC	07A	Upper Athabasca	Very high	Moderate	0	Moderate	High	High
BC	08K	Upper Fraser	Very high	Low	0	Low	High	High
BC	10A	Upper Liard	High	Low	0	Low	Moderate	High
BC	05D	Upper North Saskatchewan	High	Moderate	0	Moderate	High	High
BC	07F	Upper Peace	Very high	Moderate	0	Moderate	High	High
BC	05A	Upper South Saskatchewan	Very high	Moderate	0	Moderate	High	High
BC	08H	Vancouver Island	Very low	Moderate	0	Moderate	Very low	Low
BC	07E	Williston Lake	High	Low	0	Low	Moderate	High