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Aquatic Invasive Species Management Program

Field Sampling and Laboratory Standard Operating Procedures

Revised April 2019

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List of Acronyms

AIS	Aquatic Invasive Species
FWP	Montana Fish, Wildlife & Parks
NGO	Non-Governmental Organizations
ZM	Zebra mussel
QM	Quagga mussel
AC	Asian clam
NZMS	New Zealand mudsnail
CLPW	Curlyleaf pondweed
FR	Flowering Rush
WRP	Western Regional Panel

Document Purpose

These protocols were first developed specifically for Montana Fish, Wildlife & Parks (FWP) field crews but have since been adapted to be used by other agencies, non-governmental organizations (NGO's), and volunteer groups as interest in AIS monitoring and early detection has increased. Montana as well as other western states are currently working together to standardize both field sampling techniques and laboratory testing techniques, particularly regarding Dreissenid mussel early detection.

There is a need for consistent sampling and data collection, preservation and storage, etc. FWP is currently working with the Western Regional Panel Lab Standards Committee on the standardization of these methods across western states. These protocols are reviewed and updated at least annually.

It is critically important that all entities conducting AIS monitoring and early detection for any AIS species coordinate these efforts through FWP so that resources can be allocated to ensure the state is comprehensively searched, and so efforts are not redundant. All AIS early detection and monitoring data can be found at: http://data.mtfwp.opendata.arcgis.com.

AIS program annual reports can be found at http://cleandraindry.mt.gov/Resources

This document contains a variety of methods for the early detection of a variety of invasive species. The biggest cost to the AIS program is travel to survey sites; therefore, crews try to get as much information as possible when visiting a sampling site. Sampling schedules are laid out prior to the start of the field season to minimize unnecessary travel costs.

Aquatic Invasive Species Bureau

Mission

The Montana Fish, Wildlife & Parks (FWP) Aquatic Invasive Species (AIS) Bureau works to implement the AIS Management Plan through coordination and collaboration. The goal of the AIS Management Plan is to minimize the harmful impacts of AIS through the prevention and management of AIS into, within and from Montana. Objectives of the program include: prevention of new AIS introductions, early detection and monitoring, control and eradication, and outreach and education. This document outlines the protocols for the Early Detection and Monitoring program to help ensure consistency of implementation. Note: these protocols are dynamic and will change

based on the status of invasive species within the state, new threats, developing technology and methods, and available resources.

Program History

Montana's Aquatic Invasive Species Early Detection and Monitoring Program has been in place since 2004. Early detection allows Montana Fish, Wildlife & Parks biologists to locate small or source AIS populations, while monitoring allows FWP to study population trends when time and resources allow. FWP monitors all taxa of aquatic invasive species, including *Dreissena polymorpha* (zebra mussel [ZM]) and *Dreissena rostriformis bugensis* (quagga mussel ([QM]), *Corbicula fluminea (*Asian Clam), *Potamopyrgus antipodarum* (New Zealand mudsnail [NZMS]), *Myriophyllum spicatum* (Eurasian watermilfoil [EWM]), *Butomus umbellatus* (flowering rush [FR]), and *Potamogeton crispus* (curly-leaf pondweed [CLPW]) as well as other species not known to occur in Montana. The AIS program is an inclusive approach to all invasive species including aquatic plants and invertebrates. Because all invasive species are sampled for, sampling occurs in both lentic and lotic systems. Invasive fish species are not a focus of the program, but the AIS program relies heavily on FWP Fisheries biologists in the detection and control of these invaders. Vertebrates, such as mammals, amphibians and reptiles are another focus; however, AIS program staff will rely heavily on FWP wildlife staff in dealing with these types of invaders. FWP fisheries staff also assist AIS program staff with invasive species monitoring and early detection, native species monitoring, and water quality data collection.

In 2017, the program expanded into a new bureau within the Fisheries Division. This expansion created a new bureau chief, integrated the FWP Fish Health Lab, as well as increased staffing in each facet of the bureau including in monitoring, laboratory and watercraft inspection stations. This expansion separated the Early Detection and Monitoring Program from the watercraft inspection station program to allow for more job duty specialization going forward. The Early Detection and Monitoring Program of the AIS bureau includes 4 permanent staff and 7 seasonal staff.

FWP Annual Sampling Plan and Partner Coordination

Annual Sampling Plan Development

Plankton sampling for *D. polymorpha* and *D. rostriformis bugensis* (ZM, QM), and *C. fluminea* veligers (microscopic larvae) has increased each year, in part due to an increase in volunteer or partner sampling efforts as well as an increasing FWP effort. To aid in AIS monitoring, FWP employees – including fish health staff and regional biologists

and technicians – have been trained in AIS species identification and often contribute to or assist program staff with sampling effort. FWP staff are often sampling high-risk waters for other purposes, and additional AIS sampling increases overall efforts with less travel cost for AIS staff in Helena. Overall early detection and monitoring efforts have increased steadily over the years. Early detection and monitoring are an essential aspect of any effective aquatic invasive species program. FWP is constantly evaluating means to increase statewide sampling while not detracting from the quality of the samples to achieve more comprehensive sampling. The AIS Bureau attempts to host planning and coordination meetings across the state to help improve overall statewide sampling.

FWP assesses the risk for AIS introductions to waterbodies annually. Montana FWP utilizes a matrix as a base to prioritize all waters in Montana for monitoring. Physical habitat features, social fisheries demographics, and fish species composition data were utilized along with professional opinion of field staff to assign Montana water bodies to categories indicating risk levels for AIS introduction (e.g., Extreme, High, Medium and Low risk categories). The culmination of these data resulted in a map for graphical depiction (see Dreissenid Mussel Invasion Potential Map in Appendix E). While these tools were developed using mussels, the vectors for spread of invasive species are very similar, so the invasion potential for mussels also reflects high priority waters for other invasive species taxa and is utilized to develop an annual sampling plan for all Montana locations.

Coordination and Outreach

In addition to sampling conducted by FWP staff, FWP also provides support (equipment, monetary, personnel, training, etc.) and encourages other government entities, Non-Government Organizations (NGO's) and volunteers to conduct their own monitoring. FWP will continue to coordinate and support those activities to bolster the state's overall program and reduce unnecessary redundancy. AIS program protocols include monitoring for all aquatic invasive species taxa whenever possible. While multiple other agencies and organizations assist in monitoring throughout the state (usually with plankton sampling), FWP routinely monitors for all taxa while conducting standard monitoring. Any type of AIS or native species aquatic sampling is supported. FWP has provided training and support to other agencies and groups conducting AIS early detection efforts locally.

In 2019, FWP re-implemented coordination meetings with partners and stakeholders and partners both to report on the prior year sampling as well as to coordinate and plan sampling for the upcoming season.

AIS Sampling Methods

Montana utilizes a variety of techniques in monitoring for AIS species including sampling for plankton, invertebrates, and macrophytes. All of Montana's monitoring protocols have been scientifically reviewed, are updated annually, and are coordinated with neighboring states.

Plankton Sampling

Plankton sampling involves the collection of microscopic organisms in the water column using specialized, fine mesh nets and analyzing those samples at the FWP Aquatic Invasive Species Laboratory in Helena and the FWP satellite laboratory in Kalispell. Cross-polarized light microscopy (CPLM) is the method utilized by the laboratory to detect the larvae (veligers) of invasive bivalves such as Dreissenid mussels and Asian clams. Currently, this is the most widely accepted early detection method used for both its cost and efficacy (Frischer, et.al. 2011). Montana uses Polymerase Chain Reaction (PCR) testing and/or the amplification of environmental deoxyribonucleic acid (eDNA) as a confirmation of microscopy findings for verification. Any DNA tests are conducted by independent laboratories as the FWP AIS laboratory does not have the equipment or training to conduct this type of analysis in-house.

Invertebrate Sampling

Invertebrate sampling involves the use of kick nets, rock picking and substrate samplers detect and monitor invasive species while identifying native species. Fish pathogens, such as whirling disease, are considered AIS and therefore FWP Fish Health Laboratory in Great Falls conducts fish pathogen testing in conjunction with other AIS monitoring.

Macrophyte Sampling

FWP continues to sample for macrophytes during all AIS sampling events. Sampling occurs from early summer until plants begin to die off with colder water temperatures. Typically, sampling occurs from June to October though sampling dates can fluctuate with temperatures and spring runoff. While sampling, FWP notes presence of all aquatic plants and identifies them to species when feasible. Sampling protocols include littoral point sampling, point-intercept sampling, snorkel surveys, and sampling entire stretches of rivers focusing on depositional areas where plants would settle and

establish. While the priorities for the program have shifted away from plants, FWP will not move away from its all-taxa approach to invasive species monitoring.

Environmental DNA (eDNA)

Due to the dynamic nature of invasive species and their rarity in an environment to which they have been newly introduced, sampling techniques for these species are constantly evolving. For example, eDNA or environmental DNA sampling is not a new technique for all application as it is used in the medical field and in forensics as well as with Asian carp detection, but it is an emerging technique regarding early detection of invasive species of invertebrates. In 2017, FWP along with partners (United States Geological Survey, Pisces Molecular, LLC., Flathead Biological Station) conducted Dreissenid mussel sampling using both eDNA and plankton sampling at the same time at Tiber Reservoir to compare the two methods side by side. This information was then presented as a case study at a science advisory panel. The outcomes of this panel will be utilized in FWP's future sampling plans. The results of this panel can be found in the 2018 FWP Monitoring Report in Appendix I (MISC, 2018).

Fish Hatcheries

The movement of fish could also be a substantial vector for transferring AIS. FWP moves large numbers of fish through both its hatchery and wild fish transfer programs. Hatcheries cannot receive certification to sell or move fish without passing an AIS inspection. To accomplish this, the FWP AIS Bureau's Fish Health Laboratory and the Early Detection and Monitoring Program Laboratory inspect all federal, state and commercial hatcheries annually as well as source waterbodies for any transfer of wild fish stock. These AIS inspections include both on-site AIS surveys and disease/pathogen testing in fish.

FWP's Aquatic Health Advisory Committee is also working to streamline the process in which out of state hatcheries can import fish within the state to align more closely with FWP's internal hatchery certification.

Sampling Specific Protocols

Plankton Sampling Protocol for Veliger Detection

Zebra/Quagga mussel veligers (the planktonic larval life stage of these two species of Dreissenid mussels) are free-floating, of microscopic size, and sometimes very abundant (at times greater than 1 million/m^3) with an established population of reproducing, adult mussels. In infested waters, zebra/quagga mussel veligers will generally be

present in the water column after the water temperature has risen and remains above 8.89°C (48°F). That is generally the threshold when reproduction begins (see sample timing below for optimal sampling temperature ranges). Reproduction will continue until water temperatures drop consistently below that temperature. Juvenile zebra/quagga mussels will settle onto optimal habitat after an approximate two to four-week larval stage (depending on water quality and other factors). Optimal habitat for zebra mussels is generally hard structures though quagga mussels may settle in softer substrate. Typically, they would be found attached using byssal threads (the tiny, anchor "ropes" juvenile mussels make) to structures such as piers, docks, on or underneath rocks, or any other hard substrate that is present. There are no native species of clam or mussel that use byssal threads to attach to surfaces in Montana. An attached mussel in Montana is cause for alarm.

Plankton tow sampling is a form of early detection monitoring for Dreissenid mussel larvae and *Corbicula fluminea* (Asian clam) larvae. Plankton (small and microscopic organisms that drift or float in the water column) are collected by slowly pulling a fine-mesh (64-65µm) net through the water column either in a targeted direction. The plankton collected are then analyzed in a laboratory for the presence of veligers using cross polarized light microscopy. To optimize the potential for detecting veligers, plankton tows should follow a standardized sampling method, sample a large volume of water (Counihan and Bollens, 2017), and target the times and locations where veligers are most likely to occur. Of equal importance, samples must be preserved and handled properly to maintain their integrity, so analysis yields accurate results. Montana follows the protocol for veliger sampling developed by the Western Regional Panel on Aquatic Nuisance Species.

Waterbody Classification

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

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

- **Infested –** A water body that has an established population of AIS.

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

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

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

Sampling Timing and Water Temperature

- Adult Dreissenid mussels can survive in waters 0.55-30°C (33-86°F).
- Adult mussels will begin spawning when temperatures reach and stay above 9°C (48°F).
- Peak spawning occurs around 16˚C (60.8°F) and 18˚C (64.4°F) for Dreissenid mussels.
- Peak spawning occurs between 15.5˚C (59.9°F) and 27˚C (80.6°F) for Corbicula.
- The higher the frequency of sample collection during peak spawning temperatures, the higher the chances of early detection, assuming there is a population of reproducing adult mussels.
- Water temperatures at depth may significantly vary from surface temperatures. Sample and record water temperatures at depth to determine if conditions are conducive for sample collection.
- Veligers may congregate at a thermocline if one exists. If you have equipment to detect and sample the thermocline, do so. If not, be sure to sample as much of the water depth as you can so that you are including the thermocline if one exists.

Sampling Location

Sampling at multiple sites throughout a waterbody increases the potential for early detection, as do multiple tows per site. Larval Dreissenid mussels are free-floating (planktonic) in the water column. Adult mussels release gametes (eggs and sperm) into the water column where fertilization occurs, and larval mussels grow until big enough

to settle out of the water column. Therefore, collecting water samples near areas where adult mussels are likely to occur will increase the chances for an early detection of mussels due to their broadcast spawning method of reproduction. The distribution of veligers can be highly localized.

Sites most likely to be a source for introduction are given the highest priority. These sites include the highest use sites such as around boat ramps and docks, marinas, and hydrologic areas where veligers may accumulate such as downwind areas and eddies. These sites can be identified in unfamiliar systems by the accumulation of surface debris (such as leaves, pollen or floating debris).

Always try to sample inlets and outlets of systems when possible as well (such as the mouth of tributaries as well as dams). A little research into the system before you sample it may increase the likelihood of detections rather than just randomly sampling sites. FWP crews will generally be given these points prior to sampling an area,

In river and stream systems, focus sampling in the main current and around boat launch areas and marinas with high use. Be sure to collect samples downstream of these areas and look for eddies. Sampling in lotic systems is especially important below dams.

Volume

- Montana FWP utilizes the Wildco® Veliger net which has a 20-inch (0.25 m radius) opening, and is about 80 inches long.
- If you calculate the length of the tow in meters, you can calculate the volume of the tow by using the following formula:
    - V (Volume of water sampled in m^3) = π (3.14159) x r^2 (radius in m of your net) x L (length – or distance net was towed in m).
    - This essentially calculates the volume of the cylinder of water that you sampled.
- Therefore, it is important to record the depth, distance or length of each tow.
- The volume of water required to effectively sample for a potential rare invasive species is too large to accomplish efficiently with the resources we have. That is why FWP utilizes targeted sampling to try to sample where mussels may be introduced first. This is also why partner sampling can add to the overall sample effort in the state.

Equipment and Supplies

- Plankton net with 61-64 µm mesh size
- Rope for deploying net
    - Long enough for anticipated depths and marked for depth graduations as preferred by sampler. FWP ropes are marked in 1-meter increments.
- Clean sample bottles with leak-proof, screw-on lid (Nalgene 125mL-500mL preferred)
- Wash Bottles for rinsing samples into sample jars
- Preservative (95-100% ethanol or 190-200 proof)
    - DO NOT USE DENATURED ALCOHOL or FORMALIN
- Buffer Solution (1 L prepared 4% NaHCO)
- 5 mL pipette
- GPS unit (units set to decimal degrees (dd.ddddd) with batteries
- Clipboard
- Data Sheets (see Appendix C)
- Bottle Labels
- Tape for affixing labels to sample bottles
- Pens, Pencils
- Sharpies
- Container for decontamination of equipment.
    - Sized appropriately for equipment. FWP uses 55-gallon drums for acetic acid (at office) and Murdoch's Little Giant Rubber Feed Pans - 15 Gallon(field decontamination) https://www.murdochs.com/products/pets/small-animals/accessories/little-giant-rubber-feed-pan/

Figure 1: Wildco® net used by FWP

- White vinegar (5% acetic acid)
- Household bleach (~6% hypochloride)
- Spray bottle 1L
- Measuring cup
- Gallon size Ziploc bags
- pH paper or meter (if testing samples)
- Storage container for samples (box, cooler, etc.)
- Blue ice or gel packs (if refrigerating samples)
- Cooler/Container for sample storage
- Boat – when possible (appropriate Personal Protection Equipment)

Procedure for collection of veligers in plankton tows

Samples can be collected either by boat at multiple locations on one waterbody; or from the shoreline, either from docks or wading. FWP crews will have specific details for sampling each water body prior to departure of home base.

High sampling frequency increases likelihood of finding veligers. Western state protocol requirements vary from state to state. Consult your sampling schedule and maps for specific information on locations and numbers of samples to be collected at each waterbody. The higher the sample volume collected at each location, the better. This is especially when sampling is targeted spatially and temporally. Larger waterbodies will require samples from a higher number of sites. The number of tows per site will depend on the turbidity of the water at that location as well as suspended algae and the net diameter and depth of each tow. Aim for high risk sites and increase sampling frequency when possible. Sampling can occur anytime during the day but should only occur after water temperatures reach and stay above 8℃ and preferably during times when turbidity is low. High turbidity reduces detection ability and significantly increases lab processing time. During high flows and/or runoff, turbidity will be increased and sampling during these times should be avoided. During such times, sampling in rivers and streams will likely be postponed until flows return to normal and efforts will focus on flatwater systems. Vertical plankton tows are best in deep water and horizontal tows are best for rivers and streams. Samples should be strictly from the water column and should not contain substantial amounts of sediment unless the system has high amounts of suspended sediment regularly. Avoid dragging net along bottom of waterbody. If net is accidentally dragged across the bottom, dump out contents and try again.

The Montana FWP AIS lab will delay processing dirty samples or samples with copious amounts of sediment due to the extra time required to process them. If samples cannot be collected without substantial amounts of sediment, please include an explanation as to why with the sample information (such as elevated levels of suspended sediment during times of high runoff or high algae content due to a bloom in algae growth). Samples will also be delayed if they are unusually large (> 500 mL). Otherwise samples will be processed according to the developed prioritization matrix (Table 1), which is related to the risk assessment/prioritization of Montana waters as well as in the order in which they are received. It is up to the discretion of the lab manager to prioritize samples. The lab manager may prioritize samples differently when there is compelling cause to do so (such as an early or suspect detection of mussels).

Table 1 Prioritization matrix used by MT AIS Lab to determine prioritization of samples received (higher number prioritized first).

Risk 5 and Hatcheries	5
Risk 4	4
Risk 3 Lake	3a
Risk 3 River	3b
Out-of-State	3c
Risk 2	2
Risk 1	1

The risk assessment/prioritization of Montana waters will determine which waters are sampled and when as well as frequency of sampling. Some waters will be sampled multiple times during a season. The sampling plan will be reevaluated each spring prior to the start of the sampling season. During the sampling season, actual sampling may vary from the plan due to unforeseen circumstances (such as staffing, equipment, new detections of AIS within the state, etc.).

Plankton Sample Collection

- Secure the cod-end/bucket to the net and attach net to rope. Use a rope you have already marked with delineations of depth, so you know what depth or distance you are sampling.
- Sample retrieval can be any of the following:
    - Lower the net as deep as possible without hitting the bottom and record the depth (vertical tow).
    - Tow the net behind a boat or walk with the net at a specific depth and pull the net parallel to the water's surface (horizontal tow).
    - Throw the net out as far as possible, allow it to sink near the bottom (without hitting the bottom) and pull toward you at an angle (oblique tow).
    - Pull the net along the bottom (without hitting the bottom) or along high-risk habitat, such as hard surfaces (targeted horizontal tow).
- Make sure to record the detail as to the type of tow you conducted, the length of the tow, where you took it and at what depth.

- Very slowly and steadily (at a rate of about ½ meter per second) pull the net back to the surface. Utilize the waterbody to rinse plankton into the cod-end by dipping the net back into the water without letting the opening dip below the surface and pulling up quickly.
- Unscrew the cod-end piece without spilling contents and use a squeeze bottle to rinse contents further in this piece, if necessary. Concentrate the sample as much as possible before pouring into sample bottle by swirling the contents in the cod-end piece. Samples should not be excessively large if concentrated correctly. Aim for less than 500 mL.

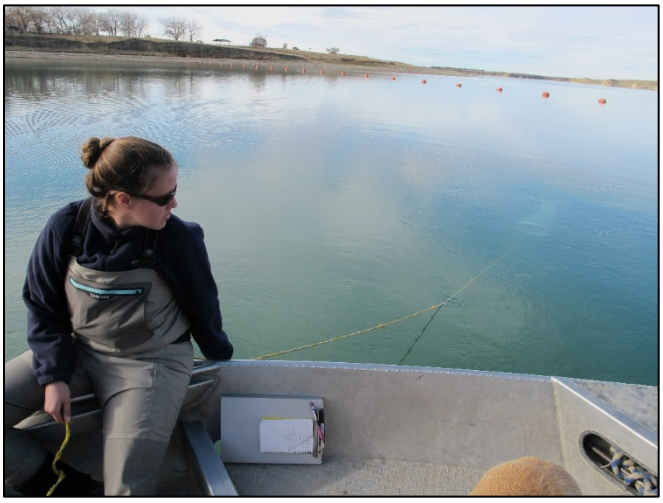

Figure 2: Example of horizontal plankton tow sampling off the bow of a boat.

- Preserve sample immediately after collection in a sample jar with 1-part sample to 3 parts ethanol so that final ethanol concentration is about 75% (if shipping to FWP lab), or as directed by destination lab.
- Buffer the sample by adding 5mL of buffer solution to each sample per 100 mL final sample (including ethanol) volume.
- After collecting samples, rinse both cod end and net separately in waterbody to aid in decontamination. Then rinse both the interior and exterior of net and cod end with fresh, clean water and decontaminate appropriately depending on lab techniques and where else your net may be used and when. Decontaminate nets between sampling in different waterbodies (see protocol in Appendix A). In mussel positive and suspect waterbodies, nets will be decontaminated between each sample collected.

For horizontal tows in rivers and streams, the steps are the same except the easiest way to get samples is to wade into main channel and hold net in place for approximately one minute and retrieve sample from cod-end piece as above. The cleaner the water, the longer the net can be held before retrieving sample. In rivers with considerable amounts of suspended sediment, sample times should be reduced. Record time on data sheet. Or, walk along holding the mouth of the net in front of you to collect a horizontal sample in slow moving or slack water.

Labeling and Data Recording

Record the following data on both the sample bottle label and the field data sheet (see Appendix C). Place clear tape over sample bottle label before adding alcohol. Do not write data directly onto sample bottle.

- Date collected (mm/dd/yy)
- Waterbody name
- Specific location name or description (such as name of fishing access site, boat ramp, address, geological feature, etc.)
- GPS coordinates (in decimal degrees (dd.ddddd) and projection NAD83)
- Length and depth of tows
- Number of bottles from sample
    - If splitting large sample into multiple bottles record how many bottles are from the same sample (1 of 3, 2 of 3, 3 of 3, etc.)
- Type of tow (vertical, horizontal, oblique, targeted horizontal.)
- Name of person collecting sample and their affiliation
- Preservative used and concentration
- Other data that can be useful when collecting plankton samples:
    - Water temp (with units – degrees Celsius)
    - Time of day (either 24 hour or with AM/PM)
    - pH
    - Weather conditions
    - Wind speed and direction
    - Turbidity (secchi disk in meters, or turbidity tube as indicated with type of tube you are using)
    - Calcium
    - Conductivity

For eDNA sample collection, FWP will follow USDA/USFS protocol or as indicated by receiving lab (Carim, 2016). eDNA/PCR tests are not conducted in-house and will be contracted out to other labs. Those contracts may vary from year to year depending on sampling plan. Sampling protocol for receiving lab will be used.

Sample Preservation

Preserve samples using 95% - 100% ethanol (ETOH) immediately after collection. DO NOT use denatured ETOH. Isopropyl alcohol can be used for microscopy but not for PCR analysis. Fill the sample bottle with 3/4 alcohol and 1/4 sample.
Polymerase Chain Reaction (PCR) plankton samples need to be preserved with a final

concentration of 70 percent ETOH, kept cold, and shipped to the laboratory within one week. Those labs that process PCR, eDNA, or qPCR samples generally require higher concentrations of preservative. Check with laboratory on preservation techniques when not shipping samples to the FWP AIS Laboratory.

FWP follows the WRP standard for buffering samples with baking soda to maintain sample pH levels around 7 (Western Regional Panel on Aquatic Nuisance Species, 2018). This prevents samples from becoming too acidic and dissolving the shells of the developing bivalves. To prepare the 4% baking soda solution:

- Desired volume in ml x 0.04g baking soda = grams of baking soda to add
- FWP prepares buffer solution by filling a 1L Nalgene bottle with deionized water and adding about 6.67 tsp of baking soda.
- That buffer solution is provided to sampling crews who will add 5mL of buffer solution to each 100mL of sample volume in the field.
- Ideally the pH of the sample should be at or above 8. In the field, sample pH is tested with strips and in the lab, sample pH is tested with a meter.

Adult Mussel Detection

To enhance early detection, monitoring for adult mussels will be conducted along with plankton tow sampling. Monitoring for adult mussels can be achieved by conducting frequent inspections of artificial substrate samplers, sediment sampling, or physical surveys of surfaces along shoreline, multiple habitat types, and structures located in high use areas (see invertebrate sampling protocol).

Figure 3: Fieldmaster® Aquaview underwater viewing tube.

Veligers will begin to settle out of the water column after about 2-3 weeks after spawning. Their shell will begin to look like an adult mussel's shell after about 3-5 weeks after spawning. Adult mussel surveys should occur in high-risk sites where you will conduct plankton sampling such as where boating traffic is prevalent, habitat is suitable and where veligers would potentially settle (see previous section on plankton sampling). Adult mussel surveys are both visual and tactile searches of existing submerged surfaces (natural and man-made) and shoreline areas. Search areas you can access safely. Mussels will not generally settle in places that are easily observed so you will need to look under rocks and docks in shaded and sheltered areas. Surface scraper devices may aid in the examination of these surfaces. Adult sampling can occur

throughout the season since adult mussels can survive in water during the cold winter months (though they will not reproduce). Underwater viewers may be helpful in searching for adult mussels. These devices can be easily made or purchased but consist of what is essentially a tube or bucket with a clear bottom.

Adult/Juvenile mussel search areas should include:

- Docks
- Boat ramps
- Buoys
- Buoy mooring chains/cables
- Dock floats
- Rocks
- Cement structures
- Shoreline areas
- Other hard structure areas

Figure 4: Wildco Ponar Grab Sampler

Sediment Sampler

Occasionally, FWP may use dredge or Ponar grab sampler techniques to sample for adult mussels in areas that are unreachable from shore. This type of sampling is usually conducted from a boat. Target habitats will be navigated to and dredge or ponar grab samples will be collected from the benthos and examined in the boat. These devices are lowered to the bottom of a lake or stream and bottom organisms are collected by releasing a trigger which closes the device and grabs a section of benthos material which can then be brought back to the boat and examined.

Artificial Substrate Sampling

Artificial substrate samplers for adult Dreissenid mussel are generally used for two purposes: to detect a new population of Dreissenid mussels or to monitor an existing population. The construction of the sampler will vary based on the purpose of the sampling and the species targeted. However, the placement and timing of use does not change according to species or purpose.

These can be purchased or made and there are various types. Some examples of these types include: the Hester-Dendy sampler of different sizes and shapes, Portland samplers, and the Wildco® Zebra Mussel Sampler. These samplers are generally anchored just above the substrate in areas where mussels are likely to be detected and not disturbed by people. Always try to set substrate samplers as early as possible as mussels will not settle on them until a biofilm has deposited first. Utilizing both light

and dark colors is a good idea as depending on the species of Dreissenid present – settlement may not occur on only one color.

Sampler construction

Polyvinyl chloride (PVC) is the preferred settling substrate for Dreissenid mussels and should be used for the primary settling surface of any sampler. Black PVC has been shown to be favored by quagga mussels, while white PVC is preferred by zebra mussels. Anchors and rope are required for most types of samplers to suspend samplers a specified distance from the bottom of a lake or stream.

In general, PVC pipe is used for early detection. Pipe increases surface area with no direct sunlight, and often mesh is inserted in the center of the pipe to facilitate periphyton growth and in turn increase algae and zooplankton concentrations that act as food for Dreissenid mussels. Holes are often drilled into the PVC pipe to allow for water exchange that increases oxygen and food resources inside the pipe, as well as increases zebra mussel settlement.

Plate structures are often used for monitoring existing populations, as surface area is easier to standardize and calculate. In addition, plate structures are generally used for quantifying densities of existing populations, so flat surfaces allow for easy scraping for quantification methods. Though designs vary, plates are easier to assemble and disassemble in an array of varying sizes of plates using spacers.

Sampler placement

Zebra and quagga mussel veligers have very similar requirements. Research has shown an aversion to direct sunlight, as UV light can affect byssogenesis, survival, and may act as a cue to mussels that they are exposed to predators and harsh currents. Veligers settle out of the water column when they are ready to attach to a hard surface and begin their juvenile stage. Due to limited movement abilities of the planktonic veligers, water currents in lotic systems can have a strong influence on where veligers are deposited. Areas with slow to no current that are somewhat protected from wind and wave action are often preferred in lentic systems, provided that appropriate hard structures are present.

Veligers also tend to prefer to settle on structures that have accumulated a biofilm, which can take days to weeks to accumulate, based on the nutrient availability of the system. This may act as a cue that water level fluctuations do not affect the substrate or that food resources would be available. As with most aquatic life, Dreissenid mussels

require oxygen and cannot survive out of the water for extended periods of time, thus water levels and conditions are crucial factors as well.

Samplers should be placed in relative proximity to human structures. Boat docks or fishing docks are generally good locations for several reasons including the availability for hard structures, the protected nature of these areas from harsh wind or wave action, stability of water levels, and (for new populations) the likelihood that the point of initial introduction occurred at or near a high-use area. However, placement at or near docks should be done carefully to avoid the possibility of human interference through removal or vandalism of the sampler or disturbance during a critical phase such as biofilm accumulation or initial stages of settlement by veligers. In general, adult Dreissenid samplers should be placed in areas that meet the following criteria:

- Shaded area
- Slow moving water/water exchange
- Dock at high-use area
- Out of public sight
- About 1 m from bottom of lake/river
- Deeper water

Finally, Dreissenid mussels can begin to spawn when water temperatures reach about 9°C (48°F), with optimal temperatures from 18-20°C (64-68°F). Thus, samplers should be placed prior to water temperatures reaching 9°C to allow for biofilm accumulation before veligers would enter the water column. Veligers typically settle out of the water column between 2 weeks and 3 months, depending on available resources (such as food and Calcium for shell growth) and water temperatures. A good guideline for Montana would be to place samplers sometime in early to mid-May at a minimum, though samplers could be placed as early after ice-off as you can safely place them. Samplers should again be checked after spawning occurs and veligers have had time to settle and grow. However, do not wait too long to check if samplers were placed on a dock, as docks are often removed at the end of the boating season and desiccated pediveligers may be difficult to identify. A good guideline for Montana would be to check samplers in late September to early October, removing them for the season.

Check samplers thoroughly both visually and tactilely (settling Dreissenids feel like grains of sand or rough sandpaper). A 10x loupe may aid in looking at juvenile mussels. If no mussels are found, clean thoroughly with soap and warm water and store dry for use the next year. If mussels are found or suspect mussels are found, report them

immediately to Montana Fish, Wildlife & Parks by contacting your FWP supervisor if you work for the department or calling (406) 444-5228 or emailing sschmidt@mt.gov. Adapted from ND Game and Fish (Howell, 2017)

Invertebrate Sampling Protocol

Introduction

When sampling for invertebrates, pay attention to bivalves (clams and mussels), gastropods (snails), and crustaceans (crayfish, shrimp and waterfleas) as these are the taxonomic groups to which the highest number of aquatic invasive species of concern in Montana belong. Refer to your invertebrate training materials in your binder for specific information on families to key out to species. It is also important to document to the best of your ability the native species present. Your binder contains information on native species as well. When selecting sampling locations, try to sample multiple sites at one access point or at least all the different habitat types within that area.

Equipment

- Waders/Boots
- GPS (units set to decimal degrees (dd.dddd and projection WGS84); record coordinates to five decimal places, or use data tablet
- Kick Net/Dip Net
- White Opaque Bucket/Tray
- Sample Bottles
- Bottle Labels
- 95% Ethanol
- Data Sheets (see attached) or data tablet
- Pens/Pencils/Sharpies
- Quadrat
- Forceps, tweezers
- Measuring tape or ruler
- Camera
- Hand lens/pocket magnifier/10x Loupe
- Aquatic Invertebrate ID Guide

Procedure

At access point, preferably boat ramp or other high angler pressure areas, sample a minimum of 200 feet upstream and downstream. If minimum distance cannot be sampled due to deep water, fast current, or difficult terrain, sample as much as

accessible. Safety is the highest priority. Do not sample anywhere that appears unsafe. Risks may include but are not limited to high water levels, fast moving water, unstable substrate, inappropriate personal protective equipment (PPE), extreme heat or cold, etc. Note distance sampled on data sheet. Within sample area, sample all available habitats, i.e., vegetation, rocky, cobble, boulder, sediment, sand, silt, riffles, pools, backwater, etc. Follow a zigzag pattern from shore to shore in narrow streams and creeks, or as deep as possible in larger rivers. Make note on data sheet on width of stream at the time of sampling, types of substrate sampled, weather conditions (such as sunny, overcast, windy, rainy, etc.), water level (and how it was gauged), etc. Be sure to include time with all observations. Be sure to include dry shoreline surveys in areas where water levels have dropped as mussel habitat may have been exposed after introduction.

Use a kick net (500-900µm mesh) for sampling in vegetation; sweep net through vegetation to capture any invertebrates. Be sure to keep the open end of the kick net facing upstream when sampling. For rocky substrate, place net so you are standing upstream of net, kick rocks to dislodge invertebrates from rocks so they drift into net. Rocks should also be picked up and inspected for invertebrates if depth and clarity allow, as some invertebrates will evade a kick net. Note sampling technique(s) used on data sheet. All searches should be visual and tactile. Inspect contents of net and identify all invertebrates observed. Net contents, or invertebrates picked off rocks can be placed in buckets or trays for ease of identification (opaque, white buckets or trays are best). Any suspected invasive mussels, NZMS, or other invasive species (such as Corbicula) should be placed in a leak proof jar and covered with alcohol and preserved or with river water and refrigerated until identification can be verified.

Collect voucher samples of all designated representative species and send them to either your supervisor or Stacy Schmidt, 1420 E 6th Ave, Helena, MT 59601. Invertebrate voucher samples should be preserved with 1:1 ratio of source water to ethanol and contained in a 125 mL or smaller sample bottle. The top of the bottle should be screwed on tightly and then secured with electrical tape, placed in a Ziploc bag and shipped appropriately according to carrier specifications.

Due to the recent fish kill on the Yellowstone River in 2016 causing a significant river closure, bryozoan awareness is a high priority for AIS seasonal sampling crews. If you observe bryozoans while sampling, collect a live sample by filling a 125mL sample bottle with source water about half full and scraping as much of the colony into the bottle as you are able. These samples should be kept cold and shipped priority overnight to Stacy Schmidt in Helena. Be sure to notify her prior to shipping these

samples. You will receive training on Bryozoan sampling during the classroom portion of your training and will be provided with reference materials to keep with you during the sampling season.

Any invasive species (invertebrate) locations should be reported immediately to Stacy Schmidt (email at SSchmidt@mt.gov), also provide GPS coordinates for location where they were collected and include photographs that should be sent immediately, and voucher samples shipped overnight. If NZMS are present in a known location, a density estimate should be taken by counting adult and juvenile snails. Count snails in a minimum of three separate square meters (if habitat is uniform) to get an average count per meter. If NZMS are present, try to sample different habitat types within that area to determine if there is a localized habitat preference. Write down all data on data sheets including a description of the location of NZMS, i.e., if they were found upstream or downstream of access point, densities above and below access points, where they were found (i.e., in riffles, vegetation or soft sediment) and the densities in different areas. Any positive or suspected positive find is completely confidential and should only be reported to the designated aquatic invasive species specialist. When a suspect organism is found, always collect a voucher sample and take photographs. Samples should be sent to Stacy Schmidt in Helena (1420 E. 6th Ave, Helena, MT 59620).

On the data sheet, make a note of the other invertebrates found with extra detail paid to snail types. When in doubt about what an organism or plant is, take photographs, collect a sample and report to AIS Specialist. Make note of native species composition on data sheets. With all data recorded on data sheet, be as specific as possible. FWP seasonal staff should be keying out mollusks and crayfish in the field and sending voucher samples for confirmatory evidence.

Other Sampling Techniques

FWP may utilize other techniques as needed. Techniques used in invasive species detection are constant evolving as are the diverse and dynamic types of invaders. For that reason, FWP must stay informed of these emerging techniques and utilize those best suited for invasive species detections in Montana.

The AIS program frequently conducts snorkel surveys when delineating populations of newly detected or unmapped invasive species. Snorkeling is also used as an early detection tool when access to certain areas is limited from boat or shore. The program also currently has one advanced open water diver (with ice specialty certification) and one open water diver. The program would like to expand this to all permanent field staff and to utilize FWP divers from other programs to serve as FWP's own dive

response team. For new mussel detections, the agency relies on the U.S. Fish and Wildlife Dive Team.

Plant Sampling Protocol

Introduction

The importance of proper identification of a plant cannot be overstressed. Inaccurate determinations of plant species mute the survey objective. Surveys should to be completed by personnel that have a background in plant taxonomy or training. In addition, up-to-date taxonomic keys should be used (general reference: http://www.ecy.wa.gov/PROGRAMS/wq/plants/plantid2/index.html; technical reference: http://www.brit.org/brit-press/books/montana**)** along with a voucher collection, so field experts can verify each determination. Having these collections are not only useful for making the correct determination of a specimen but are needed in the case the determination is questioned for accuracy. Lastly, monitoring of aquatic plants using sound science by objective personnel creates a database of useful information for understanding diversity, distribution, and abundance of both native and invasive species. These data can then be used to analyze how effective treatments are on reducing or eradicating aquatic invasive species.

Objective of Plant Sampling:

- Search of new populations of invasive aquatic plants
- Monitor existing populations for expansion and contraction
- Account for native plants, create and maintain a database with the location of invasive and native plants along with their diversity and abundance when possible
- Evaluate the effects of any treatment efforts regardless of method of treatment (e.g., pulling, chemical, biological, flooding, drawdown.)

Sampling Design and Timing

Sampling design will change with the management goals of the water body. For example, sampling techniques are different for river and lake sampling. Sampling in lotic environments commonly utilizes visual observations of the shoreline, whereas sampling in lentic water bodies requires techniques to reach deeper waters (e.g., a rake attached to a rope). The focus of the sampling will have a habitat-prioritized hierarchy. High-risk sites will get more attention such as boat ramps, docks, water downstream of a known infestation, backwaters, oxbow lakes, secondary and tertiary side channels, and most water less than 25 feet in depth.

Keep in mind that a general understanding of the biology will allow for a more complete survey of the waterbody. For example, the timing of surveys is important because some plants such as curlyleaf pondweed lie dormant for part of the summer; thus, early and late summer are best for this species. Eurasian Watermilfoil is best sampled from July into the fall and flowering rush is easier to spot when it is in bloom later in summer.

Macrophyte Sampling Equipment

- Thatch rakes with the handles cut off as close to the rake head as possible. Then an eye bolt will be screwed into the piece of wood remaining and a rope tied into the eye bolt.
- 75' rope (braided is better than twisted rope so it will not unravel).
- GPS Unit (dd.ddddd and projection WGS84)
- Waders/boots
- White tub/tray to examine specimens
- Data Sheets
- Pencils/pens/Sharpies
- Camera
- Hand lens/pocket magnifier
- Ziploc bags for plant samples
- Paper towels
- Secchi Disk (for lake/reservoir sampling)
- Underwater Viewing Tube
- Boat
- Aquatic plant ID guide
- Cooler so samples do not rot
- Plant press for voucher specimens

Sampling Methods

Lake/Pond Sampling

Lakes and ponds are most easily surveyed by boat. The best method to survey lakes is to use a point-intercept sampling method (Bonham, 1989) with predefined points. This allows surveys to be performed across multiple sampling events and years to be able to compare species distributions. This may not always be possible. A littoral survey at regular intervals as well as at specific high-risk locations within the lake could also be used.

Though time consuming, snorkel surveys are useful in locating early invaders (if water clarity is suitable) as well as delineating populations. In addition, surveys along the shoreline on windblown sides can turn up plant fragments.

Figure 5 Example of the sampling rake made from a thatch rake with an attached rope.

Determine Littoral Area and Potential Survey Area

Determining the clarity of the water and subsequently the light availability at different water depths allows you to narrow he potential area for growth of aquatic plants. To do this, motor out into the lake to an area that is deeper than light is likely to penetrate. Utilize your Secchi disk and underwater viewing tube do determine the clarity. Have one person place the end of the tube in the water while the other person slowly lowers the Secchi disk. Continue to look through the tube until you can no longer differentiate between the black and white colors of the Secchi disk. Stop lowering the Secchi disk. Finally, measure how much rope was let out and write it down in your field notebook. Repeat this process three times to get an average. This will guide you to the maximum depth that you will likely encounter vegetation. For most instances, in Montana, this will be no more than 20-30 feet.

Lake Point Sampling

- Navigate the boat to the closest point on the GPS. Try to stop the boat as close as possible to the point you are navigating towards.
- Once you are stopped, throw the rake out to the side of the boat and wait until it hits the bottom of the lake.
- Drag the rake back to the boat at a speed that allows the rake to stay on the bottom (a small 1.5 to 2 lb free weight from a dumbbell may be used in deep water to maintain contact with the bottom).
- Take the plants carefully off the rake and place them into the white tub with some water.
- Use your taxonomic key to identify each plant.
- Record what you are identifying into the field notebook along with depth.
- Once the plants have been identified and recorded save one or two of each species in a Ziploc bag and label the bag to be pressed at the end of the day.
- Perform a minimum of three tosses at each point.

- Any plants that you cannot identify will need to be saved in a Ziploc as well. The label should have the date, species of plant, waterbody, GPS point, and your name.
- Record any invasive riparian plants you find as well.

River Sampling

River surveys are a slightly different because you will be floating in a watercraft. Research the river so you know how to safely navigate water you will be floating through. Some rivers have rapids and diversions that can be dangerous: Be prepared!

The most effective way to survey rivers is:

- Have one person/vessel float on river left and the other on river right. This will cover both sides of the river at the same time. If there is insufficient man power or equipment to do this, a zig-zag method can be used to target the area most likely to contain aquatic plants (i.e. side channels, backwaters, river margins, inside bends, and other depositional areas).
- As you float you will be looking in the water for plants. In most situations, you can easily grab them for identification. A rake can be used if the water is deeper than a few feet. Be careful when using a rake with sharp edges in an inflatable vessel.
- When you encounter backwaters, side channels, or any lateral habitat with slow moving water you should survey them more intensely as these are prime habitat for aquatic plant growth. It is easiest to get out of your boat and walk these channels unless it is deeper in which case you can paddle around or down it to perform the survey. When you can walk these lateral habitats, search out depositional areas while doing a zig zag pattern and collect, identify, and save species as needed.
- Mark with you GPS where you are finding plants and record those species.
- Collect voucher samples and unknown plants as previously described in the lake sampling section.
- If you encounter an invasive plant, use your GPS unit to calculate an area and then estimate the amount of coverage. For example, if you come across some curlyleaf pondweed start the area calculation and walk your polygon back to where you started. Then estimate the percent cover of that species in your field notebook.
- When you run into braided sections of the river just pick a channel and do your best. Some backtracking can help cover all the braids.

High-Risk Site Sampling

There may be times due to logistical, budgetary, or time constraints that limit survey and monitoring work to just higher-risk sites including boat ramps, private dock areas, recreation area, campgrounds, etc. In these situations, survey 200 feet in both directions from the access point. There will be times when you will not be able to survey 200 feet in a direction so survey what is possible in those scenarios. There may be scenarios where you may need to survey more than 200 feet if it is a large recreation area or there is good habitat for invasive plants.

Voucher Samples and Unknown Plant Sample Collection Protocol

Voucher samples of invasive species should be sent to Helena as soon as possible so the plants do not desiccate. Prior to shipping the plant be sure to keep the specimen in a Ziploc bag with water in a cool location out of direct sunlight to prevent the breakdown of plant tissue. To ship the specimen, place the plant in a wet paper towel; then place in a properly labeled Ziploc bag followed by a padded envelope.

Plant Pressing

Figure 6 Plant press used to dry aquatic plant voucher samples.

- When you get done surveying a body of water it is time to press the samples you collected. Use a plant press (Figure 4) to press aquatic plants until they are dried. You will need to press a plant of each species that you locate and identify at each site you sample.
- Place a piece of blotting paper in the press. Get a piece of newspaper and open it up on the blotting paper.
- Place a completed voucher label on the newspaper including species, date, sample point ID, and waterbody.
- Place the plant on the newspaper and carefully spread the plant and the leaves out so anyone can see the important parts of the plant including the roots, stem, leaves, flowers, and seeds.
- Place another sheet of newspaper on top of the plant.
- Place another sheet of blotting paper on top of the plant.
- After that, place a piece of corrugated card board on top of the blotting paper.
- Repeat this process until all your samples are in the press.
- Place the top part of the press and tighten the straps.
- Place in a dry warm place and allow ample time for the plants to dry before removing them from the press.

Laboratory Protocol

Purpose

The Montana Aquatic Invasive Species Laboratory accepts samples from Missouri River Basin states (and the Montana section of the Columbia River Basin) for the sole purpose of detecting the presence/absence of Dreissenid veligers (and Corbicula veligers in Montana and Wyoming samples). Samples are not currently being accepted from outside of the Missouri River Basin states (except for secondary confirmation with prior approval from the lab manager), nor for purposes beyond the detection of Dreissenid veligers in waters not currently known to contain an established population and classified as such. All sample submission guidelines below should be followed to ensure proper and efficient sample processing and to minimize processing time. If the following guidelines are not followed without explanation and prior approval, samples may be discarded, moved to the back of line or returned at sender's expense. Questions about sample submissions or the purposes of the lab should be directed to the lab manager, Stacy Schmidt, at (406) 444-5228 or SSchmidt@mt.gov. More detailed lab protocols are available in the Aquatic Invasive Species Early Detection and Monitoring Program Laboratory Standard Operating Procedures

The Montana Dreissenid Laboratory processes veliger samples from the Missouri Basin states, including: South Dakota, North Dakota, Missouri, Nebraska, Kansas, Wyoming, parts of Colorado and Montana. All samples are processed under the same protocol. The lab primarily detects presence or absence of zebra/quagga mussel veligers and cannot currently do any quantification. The lab strives to provide results within two weeks of receiving samples for priority samples.

Procedure

The sample is measured and then homogenized to suspend particulate matter. Next half of the total volume is poured through a 210-micron filter to remove the large zooplankton and detritus. The filtrate is then run through a 35-micron filter to concentrate it. The filter contents are rinsed into a glass Petri dish with deionized water from a wash bottle. The contents of the Petri dish are then examined under a dissecting microscope using cross-polarized light. Any suspect veligers found are photographed using the dissecting microscope showing the Maltese cross under cross polarized light and photographed using brightfield light. Then, the suspect organism is transferred from the Petri dish to a glass slide using a micropipetter and examined and photographed at higher magnification using a compound microscope using brightfield light. This will allow for closer examination of morphological features. When necessary,

the suspect organism may be then transferred to a glass vial using a micropipetter and shipped to independent labs for microscopy verification or PCR verification. All lab technicians are provided a reference library of plankton photographs and a minimum of one-hundred and eighty hours of training with an experienced and trained technician.

To reduce the chances of sample contamination, each Montana waterbody has assigned filters that are only used for that specified waterbody. Each state will also have its own designated filters. All equipment will be decontaminated for laboratory purposes (washed, soaked in vinegar to dissolve shells and bleached to denature DNA) after each use. See decontamination section for specifics on chemical concentrations and soak times.

The remaining half of the sample is retained for additional testing, if necessary.

Verification Process

If a sample contains a suspected Dreissenid veliger, the lab technician captures digital images (both from the dissecting microscope and compound microscope). The lab manager and another FWP designated expert will analyze the images, and when possible, the original sample. If the sample is still suspected to be a Dreissenid veliger, the digital images are sent to two independent labs/experts for verification. The independent labs used for verification identify positive Dreissenid samples on a regular basis. The remaining half of a sample may be processed either internally or by another lab (for both microscopy and/or PCR) if considered suspect.

A sample is positive if any Zebra or Quagga Mussel veligers are found no matter the quantity (after independent verification using both photographic evidence and the remaining half of suspect sample). This method is not one hundred percent accurate, but it is very useful in early detection. Accuracy is reduced when samples are not preserved correctly, or when there is a large amount of sediment in the sample.

If samples are negative (veligers are undetected), the lab technician will notify the contact person when the samples are completed. If suspect veligers are found, the contact person will be notified by the lab manager and sent the photographs of the organism.

A waterbody will be identified as "suspect" for Dreissenid mussels if:

1) Settled adult Dreissenid mussels are found and verified by two qualified experts **OR**

2) Dreissenid mussel veligers are found and confirmed utilizing **BOTH** of the following methods:

- Microscopy identification of a sample from a qualified expert and concurrence from a second qualified expert: (Montana FWP Aquatic Invasive Species Laboratory, Bodega Labs, BOR, Portland State University) **AND**
- PCR (genetic) identification of a sample by a qualified expert and concurrence from a second qualified expert: (Bodega Labs, Pieces Labs, BOR)

A waterbody will be considered "Positive" for Dreissenid mussels if specimens are verified through the above protocols during two separate sampling events.

Due to issues associated with using environmental DNA (eDNA) sampling for early detection of Dreissenid mussels, a positive eDNA sample will NOT change the mussel categorization of a waterbody. A positive eDNA detection will result in intensified microscopy and eDNA sampling in the area where the positive eDNA sample was found.

Montana FWP is currently working with the Montana Invasive Species Council (MISC) and Department of Natural Resources and Conservation to evaluation the use of eDNA as an early detection or confirmatory tool. See eDNA panel report.

Quality Assurance/Quality Control Measures

It is imperative for any laboratory conducting analyses for the early detection of AIS to undergo QA/QC control efforts to ensure laboratory results can be used to guide managerial action. The standard for the FWP laboratory using cross polarized light microscopy does come with bias. That bias includes false positive and false negative results. See Table 2 for sources of bias (adapted from Wells and Sytsma, 2015).

Table 2: Sources of bias when using CPLM

Sources of False-Positive Errors		
	Problem	Corrective Action/QC Measures
Method	Misidentification	• Equipment (increase magnification, improve photography capabilities) • Training (lab control samples, ID tools) • Duplicate sample analysis (two analysts, sample split) • Photomicrographs shared with independent experts • Molecular analysis on field split
	Contamination	• Field and lab decontamination • Site specific equipment in field and lab
Process	Other planktotrophic organisms (bivalve or other)	• Increase subsampling (multiple sub-specimens) • Training (lab control samples, ID tools) • Identification confirmed by Veliger Lab Manager • Photomicrograph confirmation by outside experts • Addressing research gaps
	Unusual/poor/ limited # of specimens	• Increase subsampling (multiple specimens) • Identification confirmed by lab manager • Photomicrograph confirmation by outside experts
Sources of False-Negative Errors		
Method	Analyst Error	• Blind matrix spiked samples • Training (lab control samples, ID tools)
	Matrix Effects	• Increase aliquot dilution • Improve filtration techniques
Process	Unusual/poor/ limited # of specimens	• Preservation/handling (preservative, pH, T) • Increase subsampling (conc. sample volume)
	Low abundance, clumped spatial distribution	• Sample concentrations • Increase subsampling (conc. sample volume)

For the reasons outlined in Table 2, all laboratory technicians in both FWP laboratories undergo routine testing to ensure they are keeping up on their skills. These tests include blind samples sent from other states as well as analyst performance testing where each laboratory technician evaluates a set of training samples where some samples are

spiked with different densities of veligers. These tests are evaluated on a pass/fail basis. While the Montana FWP AIS laboratory is not a lab that routinely conducts veliger quantification, the samples are either positive (veligers detected) or veligers undetected. Laboratory technicians are expected to pass this test annually and new technicians should pass this test prior to conducting any sample analysis on their own. As part of this test, analysts are also required to identify veliger species, and sketch, photograph and catalogue each veliger with information on size, growth stage and morphological features of each. This testing occurs during the slower winter months.

Equipment Decontamination

Decontamination is a very important part of your job. We don't want to be the vector for transmitting invasive to new locations. There are a variety of decontamination techniques that Montana FWP utilizes. See Appendix A for specific instructions to decontaminate all sampling equipment. Decontamination of equipment must occur between waterbodies.

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Appendix A – Decontamination Protocol

All personnel working in the aquatic environment should follow this protocol. Aquatic invasive species are a threat to our aquatic resources. Eurasian watermilfoil, New Zealand mud snails and zebra mussels are just a few of the destructive and aggressive invader species that threaten Montana waterways. People and equipment that come in contact with aquatic systems easily transport these exotics. This cleaning and disinfection protocol should be followed by all samplers to ensure that field workers are not transferring aquatic invasive species into new sites. All equipment should be thoroughly cleaned before using it in a different location. Whenever possible, use dedicated equipment for specific locations, particularly if those locations are suspect or positive for dreissenid mussels.

Do not conduct decontamination near sampling locations. Runoff should never reach any waterbody when decontaminating. Any chemical solutions used for decontamination should be properly discarded away from open water, into a drain that goes into a wastewater treatment facility or is appropriately stored and reused. Always decontaminate after returning from the field. Most FWP crews will have decontamination equipment kept at a regional office for use at the end of their work week. Although some trips may require field decontamination. This will be discussed during field training.

Boats and trailers:

Never transfer aquatic animals or plants from one waterbody into another.

- Before leaving any waterbody remove all sediment, vegetation and aquatic animals from your boat and trailer.
- Drain all water from your boat, including from the motor, live well and bilge. Do not transfer any water from one waterbody into another.
- Pressure washing with hot water is preferred, when possible. Chemicals may damage some motor components and other parts. Refer to owner's manuals or manufacturers for specifications on equipment.
- If high-pressure, hot water (120-140°F) is used, no soap, detergents or chemicals are necessary. The higher the temperature used, the less time required. Air-dry your boat and trailer for as long as possible between visits to different sites. It is recommended to leave your boat outside in the sun, after opening and exposing compartments and wet locations. Towel dry areas that tend to remain wet.

Decontamination of Vessels from Mussel Positive or Suspect Waters:

Watercraft Engine

If the watercraft engine is not a closed cooling system configuration (if the engine takes its cooling water from the environment), the following applies:

- A hot water treatment is recommended for engine decontamination.
- Running a chemical solution, such as a bleach solution, through an engine to decontaminate it may violate the terms of the engine's warranty, or otherwise damage the engine, unless specified by the manufacturer.
- Chemical treatments are not well-suited for engine decontamination because the adult mussel is able to sense a toxic external environment and close up for extended periods of time.

Outboard

All outer surfaces of the motor must be cleaned to remove any clinging foreign material by washing with high pressure, hot water. Then, visually inspect, feel by hand, and remove any remaining foreign material. Finally, decontaminate the engine cooling system by either: (1) placing the outboard motor into a barrel filled with 140 °F to 160 °F water and operating the engine for 5 to 10 minutes, or (2) using the appropriate flushing attachment, such as an "earmuff" attachment. Operate the engine according to the "Engine Decontamination Instructions" below.

Inboard/Outboard

All outer surfaces of the inboard/outboard unit must be cleaned to remove any clinging foreign material by washing with hot, high pressure water. Then, visually inspect, feel by hand, and remove any remaining foreign material. Finally, decontaminate the engine cooling system by using the appropriate flushing attachment, such as an "earmuff" attachment. Operate the engine according to the "Engine Decontamination Instructions" below.

Inboard Engine

All surfaces of the propeller, driveshaft, driveshaft bearing supports, rudder, and driveshaft bearings must be cleaned to remove any clinging foreign material by washing with hot, high pressure water. Then, visually inspect, feel by hand, and remove any remaining foreign material. Finally, decontaminate the engine cooling system by using the appropriate flushing attachment. Operate the engine per the "Engine Decontamination Instructions" below.

Engine Decontamination Instructions

Use the appropriate attachment, such as an "earmuff" attachment, to flush the watercraft engine cooling system. Refer to the manufacturer's directions for flushing attachment hookup to the engine.

Stay clear of the propeller and keep other persons away as well during the flushing process.

Set the watercraft transmission in neutral gear.

Start hot water flowing through the engine and wait for water to exit from the cooling system outlet ports as a steady stream of water. If water does not flow as a strong, continuous stream from the outlet ports, there may be some debris or mussels already inside the cooling system that are blocking the free outflow of water. Examine the water intake ports closely and check the intake filter screens for any evidence of mussels or other blockage. After this concern is resolved, resume the flushing procedure.

Check the outflow water temperature with a handheld thermometer, or a handheld infrared temperature reader. If the engine is cold, the outflow water temperature may be much cooler than the required 140 °F required to kill mussels. If this occurs, heat is probably being transferred from the flushing water to the cold engine mass. Wait for the outflow water to reach 140 °F before proceeding.

Some watercraft motor manufacturers allow engines to be operated during the flush procedure, while some do not. In addition, some manufacturers limit the input pressure of the flushing system. For example, certain models of Mercury™ engines specify a flushing system pressure limit of 45 psi. Refer to manufacturer's directions prior to attempting engine flushing.

If the manufacturer allows flushing with the engine running: start the engine and run at the lowest idle speed for 2 minutes. Make sure the required 140 °F temperature is maintained in the outflow water. Also make sure the engine does not reach an overheated condition. On certain engines, it is possible that a low coolant volume in the cooling system will not properly register an overheat condition on the engine temperature gauge; therefore, it is very important to monitor the temperature of the outflow water. When completed, shut down the engine first, and then shut off the water supply. Disconnect all flushing attachments.

Warning:
Most hot water power washers have a flow rate of 4 gallons per minute or less. Be sure

to check the flow rating of your washer! Using less than 4 gallons per minute flow rate when flushing the engine cooling system may cause engine damage if the 2-minute engine run time requirement is exceeded! Operate the engine at only low idle speed during flushing.

If the manufacturer does not allow flushing with the engine running: Proceed per the manufacturer's directions with engine shut down. Make sure the required 140 °F temperature is maintained in the flushing outflow water. When completed, shut off the water supply and disconnect all flushing attachments. Hot water flushing on an engine that is not running can usually exceed the 2-minute limit imposed for an engine that is running.

Waders, nets and all other field equipment used in the water:

- Separate all individual components such as insoles, socks, booties, ankle guards and laces. Wash all components separately.
- Remove all sediment, vegetation and aquatic animals from all equipment. Pay attention to the soles of waders.
- There are a variety of ways to decontaminate equipment. How equipment is decontaminated will depend on what equipment it is and where you are (home base or out in the field) and what equipment you have available to you to use in the decontamination process (such as buckets and tubs). More specific instructions on decontamination of specific equipment will be provided during training.
    - Disinfect all equipment in a 10% commercial bleach solution for 10 minutes or a 5% bleach solution for 1 hour. Items can also be sprayed with a 10% bleach solution and let sit for 15 minutes. Waders may get damaged from bleach solution.
    - Hot water (such as in a bucket or bathtub) can be used and equipment should be soaked for sufficient time to allow components to reach appropriate water temperature (140^{o}F - on contact, 120^{o}F-130^{o}F - at least 2 minutes) and **dried completely** for as long as possible between visits to different sites.
    - Table salt can be used to soak equipment. A 1% solution for 24 hours.
    - Vinegar can be used if equipment is soaked in 100% (5% acetic acid) concentration for 20 minutes. Vinegar can also be re-used if stored properly and maintains a pH of 2-3.
- Do not transfer any water, vegetation or animals between sites.
- If possible, always work from upstream to downstream.

Plankton Nets

All gear should be decontaminated between waterbodies. Ideally, each waterbody should have its own set of sampling gear. But when this is not possible, decontamination of gear is very important. Firstly, when sampling moving systems always start upstream and work downstream, then decontamination between sites on the same system is not necessary and upstream transport of invasive species is eliminated. Visually inspect plankton nets and ropes first to remove large debris such as plant detritus and large insects. Not all nets are made the same so chemicals may destroy rubbers and glues.

With all FWP nets:

1. First rinse net as clean as you can get it.
2. Soak net, bucket and rope in vinegar (acetic acid (5%)) for 24 hours (or a minimum of 2 hours if working in the field). Be sure to test vinegar with pH testing meter or strip to ensure pH level remains around 2-3
    a. This will kill and dissolve shells of potential veligers.
3. Rinse net with clean water.
4. If the waterbody is classified as positive or suspect for Dreissenid mussels, soak net, bucket and rope in 10 % bleach solution for 10 minutes.
    a. This will kill organisms and denature DNA should any PCR testing need to be conducted on samples collected with that net.
    b. This will cause the net to degrade so keep a close eye on net for small pinholes that can be repaired before they turn into large tears that can't be repaired.
    c. Items can also be thoroughly sprayed with 10% bleach solution and left to sit for 10 minutes.
5. Rinse net, bucket, and rope in fresh water.
6. Hang to dry until next use.
7. Store in container provided for CLEAN and DRY nets only.
8. Store dirty nets in dirty containers only. This will prevent cross contamination of nets.

Decontamination Considerations for eDNA, PCR, and Microscopy:

For decontaminating equipment used in DNA analyses (eDNA, PCR, qPCR, etc.) and/or to ensure veliger shells are not transferred between different sites, the following protocol should be used:

- Thoroughly rinse all the items with tap water. Vinegar should be used to dissolve veliger shells:
- Place items to be decontaminated in appropriately sized rubber or plastic tote to be able to submerge and agitate all items.
- Fill the tote with enough household vinegar for a minimum of 2 hours (although 24 hours is preferred)
- Thoroughly rinse items with clean tap water.
- Bleach should be used to denature DNA:
- Spray or soak items with a 10% bleach solution and allow items to sit for 15 minutes (in a spray bottle add ¼ cup bleach then add 2 and ¼ cups well water). Or, a 10% bleach solution can be prepared in a tote or similar container and used to soak items for 15 minutes (2.5L or 0.7 gallons bleach and 49 L or 13 gallons water)

Notes: Vinegar (acetic acid) can be reused multiple times as long as the pH stays around 2-3 (vinegar should be pH tested prior to use to ensure proper level using a test strip or meter). If level is too high, old vinegar should be replaced with new vinegar. Vinegar should also be stored in the container in came in and not your decontamination container.

Due to the new invasive mussel status in Montana waters, in 2017, all FWP sampling nets and small equipment will be decontaminated using vinegar protocols (nets, waders, ropes, etc.) and bleach on waters classified positive or suspect for Dreissenid mussels. Hot water will still be used on large equipment (boats and trailers).

Appendix B – Sample Submission Guidelines

Plankton

1. Place sample in plastic screw-top bottle, 125-500 mL in size. Nalgene sample jars are preferred and electrical tape around the lid/seal is also recommended to prevent leakage.
2. Final sample preservative concentration should be 70% at a minimum. 95%-100% pure ethanol is preferred. Do not use denatured ethanol. Minimize sediment as much as possible. Higher final concentrations of ethanol may be required for samples with excessive amounts of sediment or biological material. Please notify lab of such samples as these samples take longer to process. Improperly preserved samples will be discarded. Please do not ship samples in coolers; if samples are preserved correctly, coolers are not necessary. If samples are shipped in coolers, the coolers may not be returned.
3. Sample jars should be clearly labeled with waterbody name, locality, and date. Copies of data sheets should be included with each shipment and contain location description, GPS coordinates recorded in decimal degrees (dd.ddddd) and projection WGS84 out to five decimal places, date of collection, sample depth, water temperature, sampler, and preservative type and concentration. All samples and results will be kept in the strictest confidence. A copy of the submission form should also be included with shipment and filled out completely.
4. Samples should be shipped as soon as possible after collection, rather than accumulating samples and sending large shipments. This helps to prevent delays in all samples being processed.
5. Upon receipt of results, please respond with an email confirmation. Negative samples will be sent via email notification via the lab technician. Results with suspect veligers will be emailed with pictures from the lab manager to the state coordinator or designated contact person.
6. Please designate one contact person for each state. It will be that person's responsibility to disseminate results within that state.

Sample Information

All sample data provided with the samples is recorded in a lab book and in an electronic database. Data that should be provided with each sample includes: location description, GPS coordinates, date, sample depth, sampler, and preservative. Half of the sample is processed, and the other half of the sample is reserved for further analysis (if necessary). The reserved half of the sample is saved for no longer than one year.

Sample Log In

All samples are logged in as soon as possible after being received by the lab. All samples are recorded on a log sheet for each state. When a sample set comes into the lab, each sample set is assigned a sequential, sample ID number. Each sample set box will be labeled with the sample ID numbers it contains. The site column will contain specific information on the sample's location. Dates for all samples will be logged in when the sample arrives and when the sample is processed. The lab technician who processed the samples will be identified by their initials. A "Notes" column is present for any information that should be included with the log in; such as: positive results, whether the samples are preserved correctly, whether the sample jars leaked, etc.

The log in sheets for the samples will keep a running tally of all samples received by the lab. This will provide an easy reference system for all samples. Samples are processed in the order in which they are received unless rushing is authorized beforehand. Samples will be delayed if they have considerable amounts of sediment or algae or are unusually large (> 500 mL).

Shipping

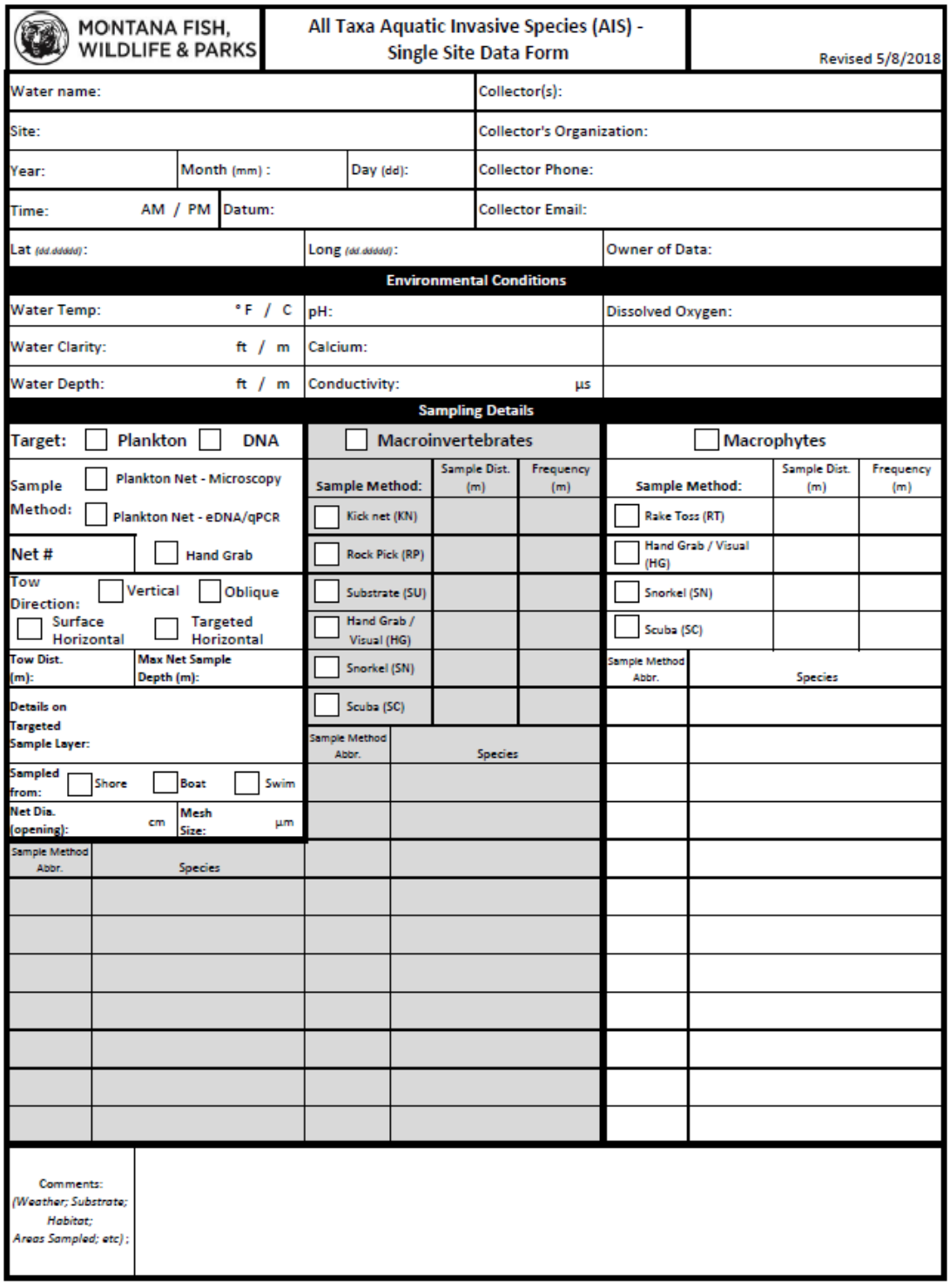
Ship or hand deliver samples as they are collected. FWP sampling crews should have a weekly set of samples to ship or deliver. Use required shipping protocols to follow regulations governing common carriers. Preserved samples do not need to be refrigerated although keeping them cool and in the dark is preferred. Do not leave them in direct sunlight. Inform the laboratory manager when shipping samples. Be sure to include with the samples a hard/paper copy of sample information in case labels are removed during shipping.

Samples must be delivered by hand or mail to Helena Headquarters. If other arrangements are made the Helena AIS Lab must be notified in advance.

Follow carrier (USPS, FedEx, UPS) protocols for shipping with hazardous materials, such as ethanol. Those instructions can be found at carrier websites or at carrier locations.

Appendix C - Sample Submission Forms

These forms can be used to collect the relevant data at each sample site and can be included with samples sent into Helena for analysis.



MONTANA FISH, WILDLIFE & PARKS

All Taxa Aquatic Invasive Species (AIS) - Single Site Data Form

Revised 5/8/2018

Water name:			Collector(s):
Site:			Collector's Organization:
Year:	Month (mm) :	Day (dd):	Collector Phone:
Time: AM / PM	Datum:		Collector Email:
Lat (dd.ddddd):	Long (dd.ddddd):		Owner of Data:

Environmental Conditions

Water Temp: ° F / C	pH:	Dissolved Oxygen:
Water Clarity: ft / m	Calcium:	
Water Depth: ft / m	Conductivity: µs	

Sampling Details

Target: ☐ Plankton ☐ DNA

Sample Method: ☐ Plankton Net - Microscopy ☐ Plankton Net - eDNA/qPCR ☐ Hand Grab

Net #

Tow Direction: ☐ Vertical ☐ Oblique ☐ Surface Horizontal ☐ Targeted Horizontal

Tow Dist. (m):

Max Net Sample Depth (m):

Details on Targeted Sample Layer:

Sampled from: ☐ Shore ☐ Boat ☐ Swim

Net Dia. (opening): cm

Mesh Size: µm

☐ Macroinvertebrates

Sample Method:	Sample Dist. (m)	Frequency (m)
☐ Kick net (KN)		
☐ Rock Pick (RP)		
☐ Substrate (SU)		
☐ Hand Grab / Visual (HG)		
☐ Snorkel (SN)		
☐ Scuba (SC)		

Sample Method Abbr.	Species

☐ Macrophytes

Sample Method:	Sample Dist. (m)	Frequency (m)
☐ Rake Toss (RT)		
☐ Hand Grab / Visual (HG)		
☐ Snorkel (SN)		
☐ Scuba (SC)		

Sample Method Abbr.	Species

Comments: *(Weather; Substrate; Habitat; Areas Sampled; etc)*:

MONTANA FISH, WILDLIFE & PARKS

Aquatic Invasive Species (AIS) Sampling - Multiple Sites per Waterbody Data Form

Page ____ of _____

Revised 05/08/2018

Water name:			Collectors:	Collector's Organization:
Year:	Month (mm):	Day (dd):	Collector Email:	Collector Phone:
GPS Datum:	Owner of Data:			Net Dia (opening): / Mesh Size:

Target Abrbreviation:
P = Plankton
MI = Macroinvertebrates
MP = Macrophytes
eD = eDNA

Sample Method Abbreviation:
PM = Plankton Net - Microscopy
PD = Net - eDNA/qPCR
eHG = eDNA Hand Grab
KN = Kick Net
RP = Rock Pick
SU = Substrate
HG = Hand Grab
SN = Snorkel
SC = Scuba

Tow Direction Abbreviation:
V = Vertical
O = Oblique
SH = Surface Horizontal
TH = Targeted Horizontal

Lattitude & Longitude in dd.ddddd

Site	Start Lat	Start Long	End Lat	End Long	Water Temp (f)	Water pH	Target	Sample Method	Net #	Tow Direction	Sample or Tow Distance (m)	Max Net Sample Depth (m)	Details on Targeted Sample Layer	Boat (B), Shore (SH), Swim (SW)	Sample Frequency (m)	Species Observed (See back for codes)

MONTANA FISH, WILDLIFE & PARKS	Macrophyte Aquatic Invasive Species (AIS) - Multiple Sites per Waterbody Data Form	Page ____ of ______ Revised 05/08/2018

Water name:			Collector(s):
Year:	Month (mm):	Day (dd):	Collector's Organization:
GPS Datum:	Owner of Data:		Collector Phone:
Time Range: Start: AM / PM	End: AM / PM		Collector Email:

Environmental Conditions at Point # ______

Water Temp: ° F / C	pH:	Dissolved Oxygen:
Water Clarity: ft / m	Calcium:	
Water Depth: ft / m	Conductivity: µs	

Sampling Details

Purpose:

Sampling Method Abbreviations: Rake Toss (RT); Hand Grab/Visual (HG); Snorkel (SN); Scuba (SC); Other:

Point #	Latitude *(dd.ddddd)*	Longitude *(dd.ddddd)*	Sample Method Abbr.	Water Depth (ft)	Species	Abundance/ Comments

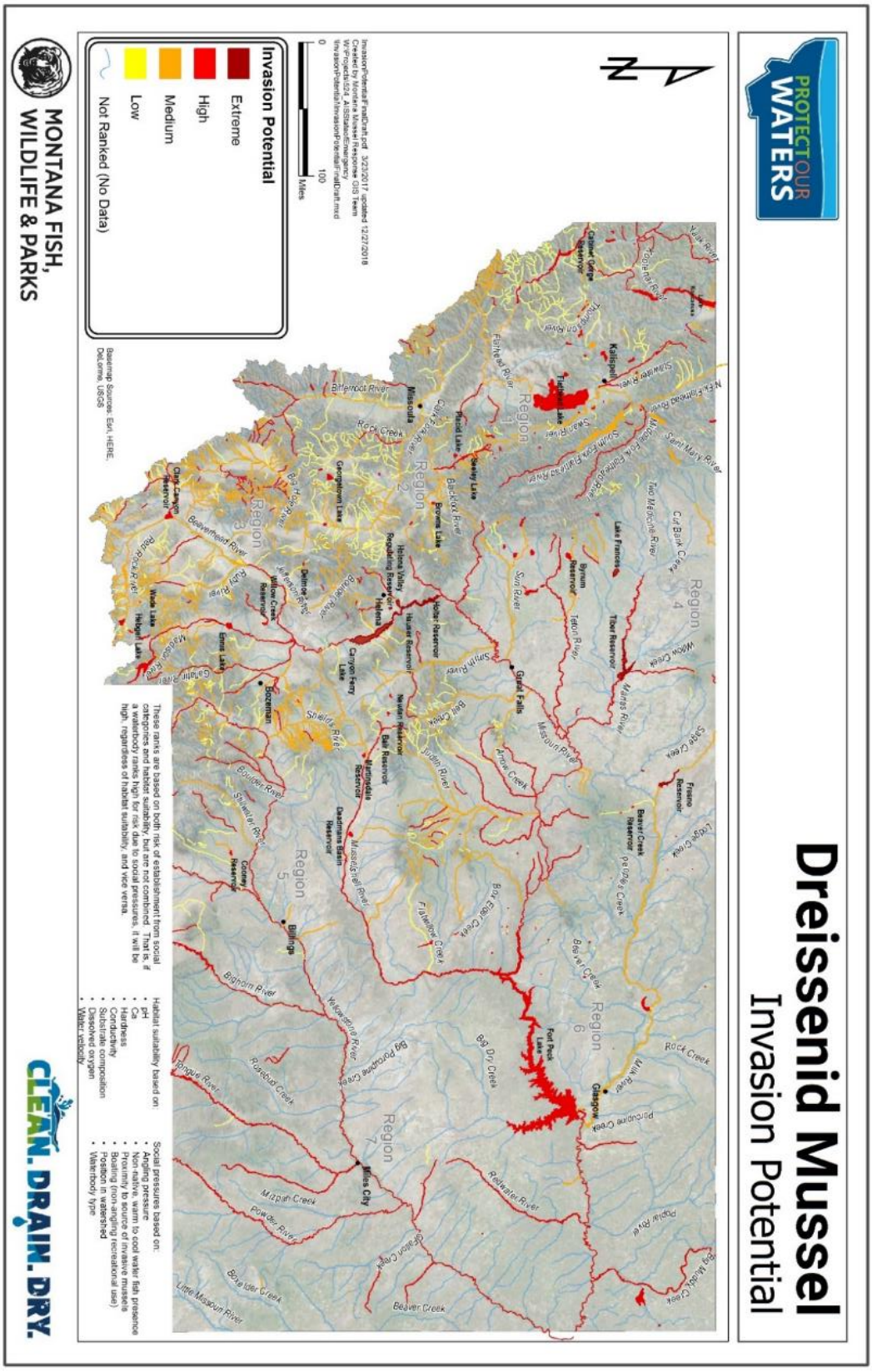
Comments: (Weather; Substrate; Habitat; Areas Sampled; etc);	

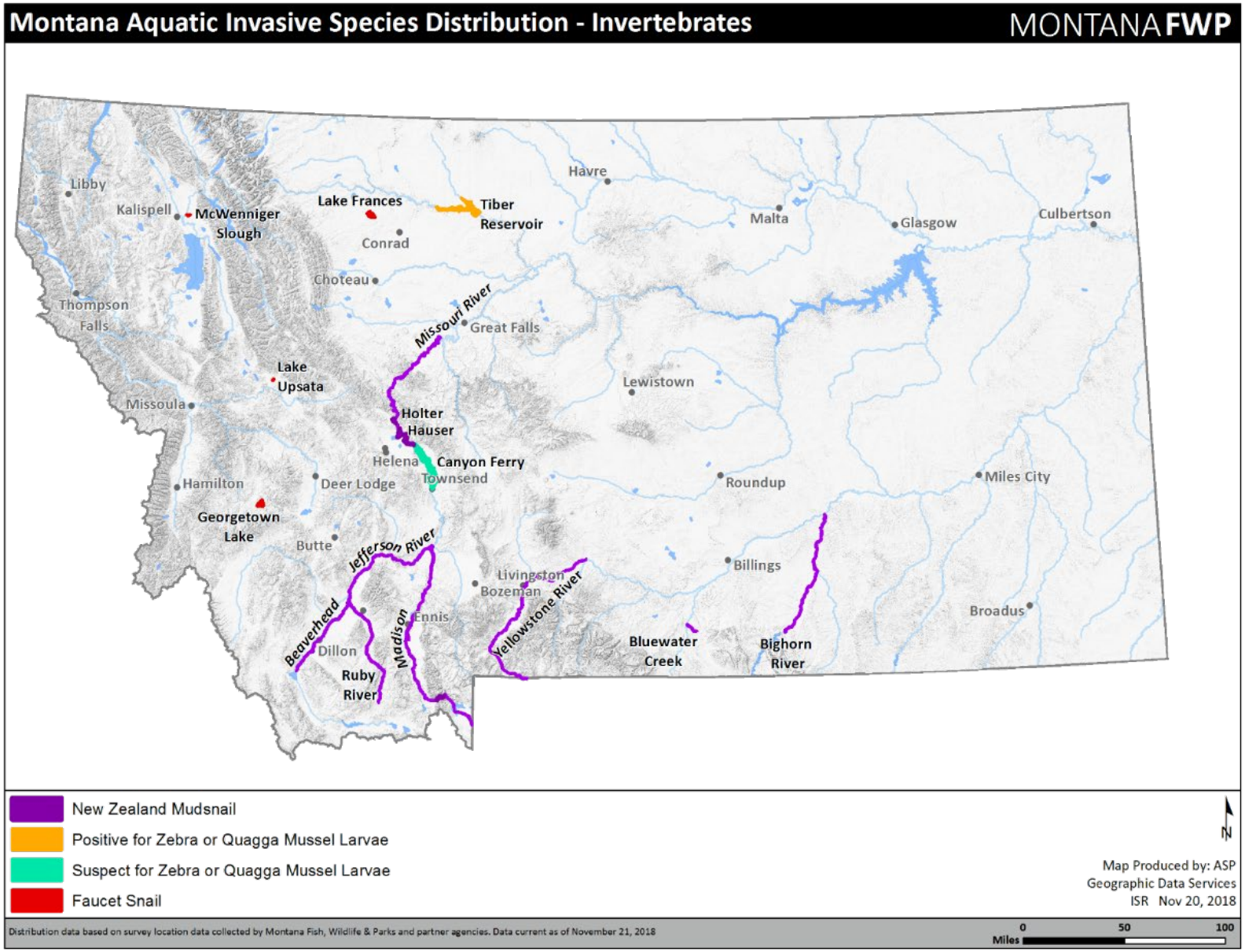
Appendix D – List of Priority Invasive or Introduced Species

Common Name(s)	Scientific Name	Type	Status in MT
Asian clam	Corbicula fluminea	Mollusk-Bivalve	Historically detected, no recent detections
Zebra mussel	Dreissena polymorpha	Mollusk-Bivalve	Unknown - Suspect
Quagga mussel	Dreissena rostriformis bugensis	Mollusk-Bivalve	Unknown - Suspect
Golden mussel	Limnoperna fortunei	Mollusk-Bivalve	Undetected
Black Sandshell	Ligumia recta	Mollusk-Bivalve	Established
White (Creek) Heelsplitter	Lasmigona complanata	Mollusk-Bivalve	Established
Mapleleaf	Quadrula quadrula	Mollusk-Bivalve	Established
European fingernail clam	Spharium corneum	Mollusk-Bivalve	Undetected
Ghost rams horn	Biomphalaria havanensis	Mollusk-Gastropod	Undetected
Mud bithynia, faucet snail	Bithynia tentaculata	Mollusk-Gastropod	Detected
Chinese mysterysnail	Cipangopaludina chinensis	Mollusk-Gastropod	Undetected
Japanese mysterysnail	Cipangopaludina japonica	Mollusk-Gastropod	Undetected
Giant Rams-horn snail	Marisa cornuarietis	Mollusk-Gastropod	Undetected
Red-Rim Melania	Melanoides tuberculata	Mollusk-Gastropod	Historically Detected
New Zealand mudsnail	Potamopyrgus antipodarum	Mollusk-Gastropod	Present
European Ear snail/Big-eared Radix	Radix auricularia	Mollusk-Gastropod	Detected
Mimic lymnaea	Pseudosuccinea columella	Mollusk-Gastropod	Detected
European stream valvata	Valvata piscinalis	Mollusk-Gastropod	Undetected
Banded mysterysnail	Viviparus georgianus	Mollusk-Gastropod	Undetected
Rusty Crayfish	Orconectes rusticus	Crustacean	Undetected

Common Name(s)	Scientific Name	Type	Status in MT
Red Swamp Crayfish	Procambarus clarkii	Crustacean	Undetected
Bloody Red Shrimp	Hemimysis anomala	Crustacean	Undetected
Opossum Shrimp	Mysis diluviana	Crustacean	Detected in Western MT
Water flea	Bosmina coregoni	Crustacean	Undetected
Spiny Waterflea	Bythotrephes longimanus	Crustacean	Undetected
Fishook waterflea	Cercopagis pengoi	Crustacean	Undetected
American Bullfrog	Lithobates castesbeianus	Amphibian	Detected
Rough-Skinned Newt	Taricha granulosa	Amphibian	No recent detections
Red-Eared Slider	Trachyemys scripta elegans	Reptile	Detected
Brazilian Elodea	Egeria densa	Plant	Undetected
Common Reed	Phragmites australis	Plant	Detected
Curly-leaf Pondweed	Potamogeton crispus	Plant	Established
Eurasian Watermilfoil	Myriophyllum spicatum	Plant	Established
Flowering Rush	Botomus umbellatus	Plant	Established
Fragrant waterlily	Nymphaea odorata	Plant	Established
Hydrilla	Hydrilla verticillate	Plant	Undetected
Purple Loosestrife	Lythrum salicaria	Plant	Established
Starry Stonewort	Nitellopsis obtuse	Plant – Algae	Undetected
Yellow Floating Heart	Nymphoides peltata	Plant	Undetected

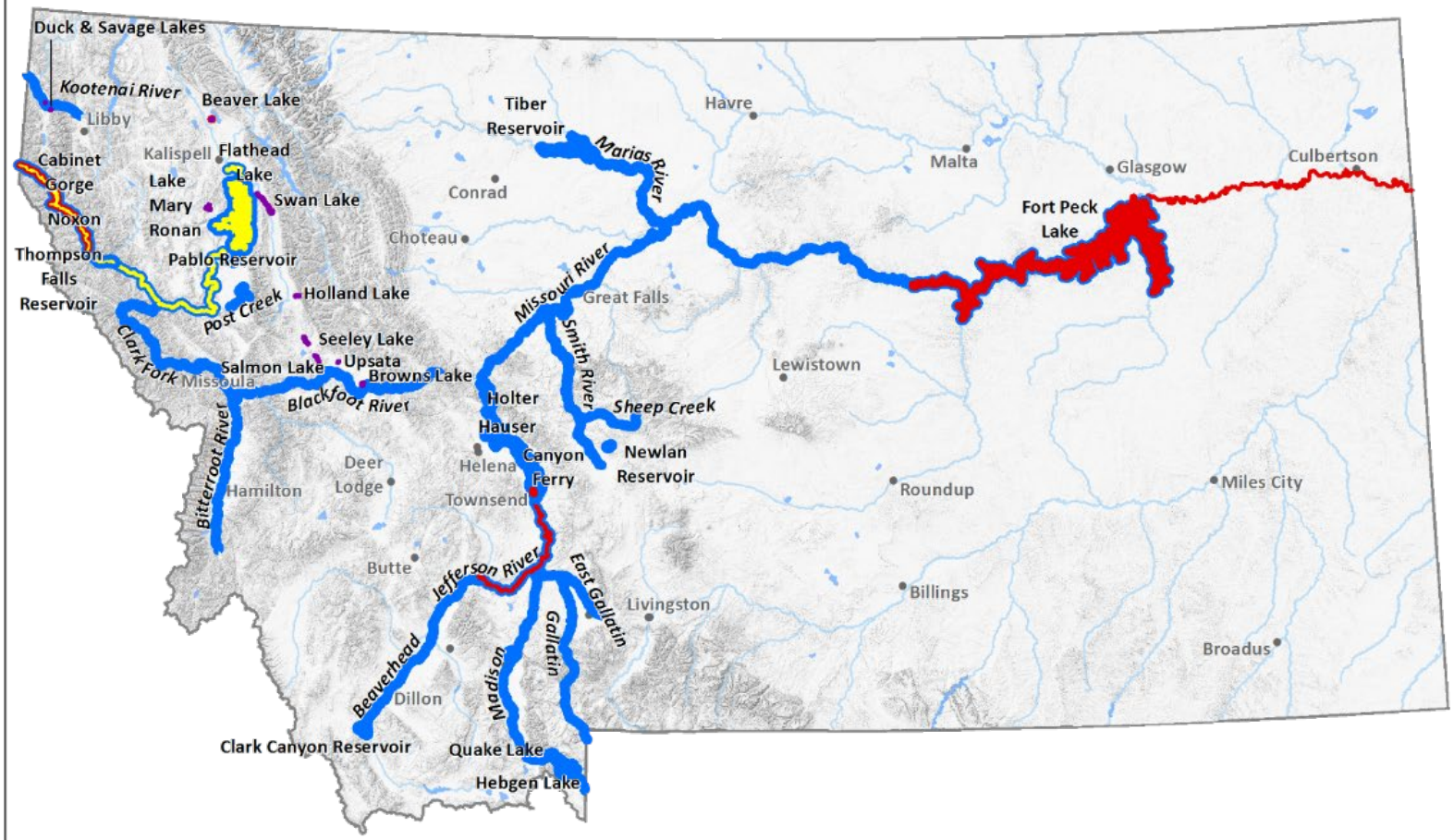
Appendix E – AIS Reference Maps




Montana Aquatic Invasive Species Distribution - Invertebrates
MONTANA FWP
Libby
Kalispell
McWenniger Slough
Lake Frances
Tiber Reservoir
Conrad
Havre
Malta
Glasgow
Culbertson
Choteau
Thompson Falls
Missouri River
Great Falls
Lake Upsata
Missoula
Holter
Hauser
Lewistown
Helena
Canyon Ferry
Townsend
Roundup
Miles City
Hamilton
Deer Lodge
Georgetown Lake
Butte
Jefferson River
Billings
Livingston
Bozeman
Broadus
Beaverhead
Ennis
Madison
Yellowstone River
Dillon
Ruby River
Bluewater Creek
Bighorn River
New Zealand Mudsnail
Positive for Zebra or Quagga Mussel Larvae
Suspect for Zebra or Quagga Mussel Larvae
Faucet Snail
N
Map Produced by: ASP
Geographic Data Services
ISR Nov 20, 2018
Distribution data based on survey location data collected by Montana Fish, Wildlife & Parks and partner agencies. Data current as of November 21, 2018
0
50
100
Miles

Montana Aquatic Invasive Species Distribution - Plants

MONTANA FWP



Fragrant waterlily

Flowering rush

Eurasian watermilfoil

Curly-leaf pondweed

N

Map Produced by: ASP
Geographic Data Services
ISR Nov 20, 2018

Distribution data based on survey location data collected by Montana Fish, Wildlife & Parks and partner agencies. Data current as of November 21, 2018

0 50 100
Miles

Appendix F - Safety

Towing

What will you be towing?

First, you should know what kind of trailer you plan to tow. As part of the Aquatic Invasive Species crew, you will most likely be towing different types of boat trailers using a basic receiver hitch. Your trailer may or may not come equipped with its own set of brakes. There are many factors to consider before towing any type of trailer.

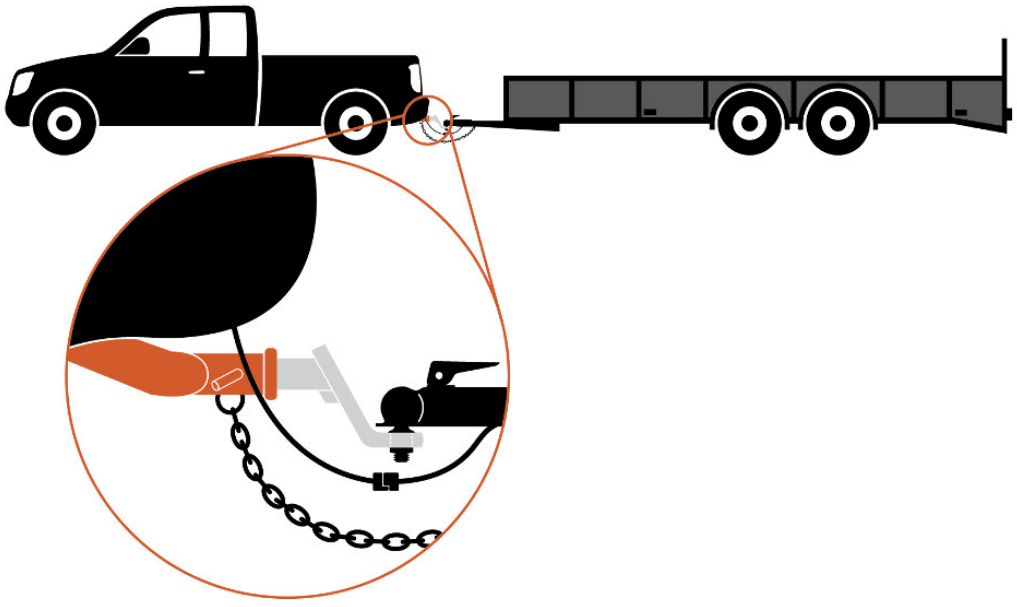
Where will you be towing?

Your towing needs are also dependent on the distance and road conditions along your route. For example, towing a small utility trailer across town is very different than towing a large 5th wheel camper to a remote destination. It is also likely that you will tow different trailers with the same vehicle. You will need to consider how best to equip yourself for these changes.

Trailer Hitch

A trailer hitch is the primary connection component in a towing system that attaches a trailer to your tow vehicle. A trailer hitch requires some extra components, such as a ball mount and trailer ball, to make the connection complete.

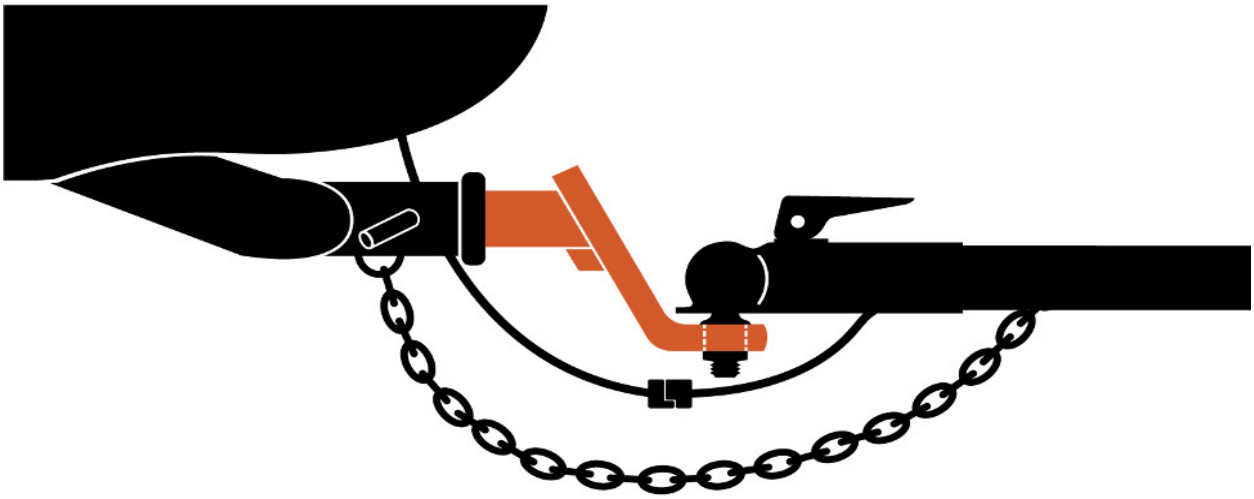
A receiver hitch, perhaps the most common type of trailer hitch, mounts to the frame of the vehicle and provides a receiver tube to accept a ball mount or other insert. Receiver hitches typically fall within one of five classes, based on weight carrying capacity and



receiver tube size. Selecting the correct type of trailer hitch for your vehicle and trailer is critical.

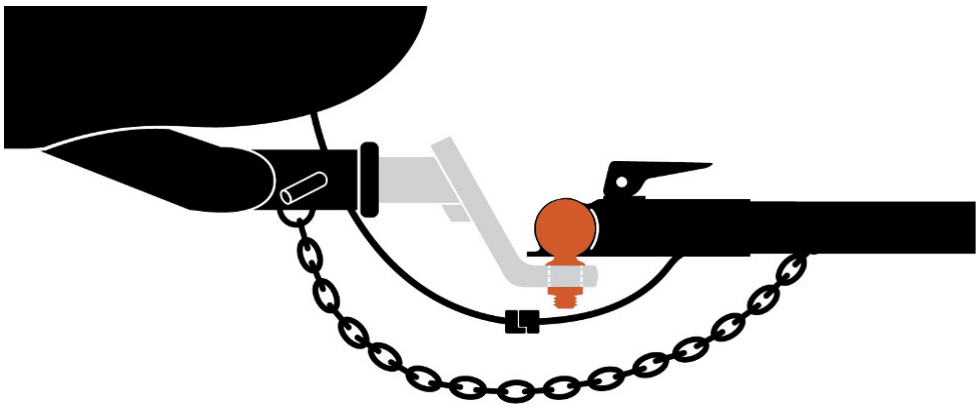
Ball Mount

A ball mount is a metal tube or bar that inserts into the trailer hitch and provides a mounting plate to hold a trailer ball. **Ball mounts are made in a variety of styles and capacities** to accommodate different trailers and coupler heights. A ball mount is held in place in the hitch with a hitch pin & clip or a hitch lock.



Trailer Ball

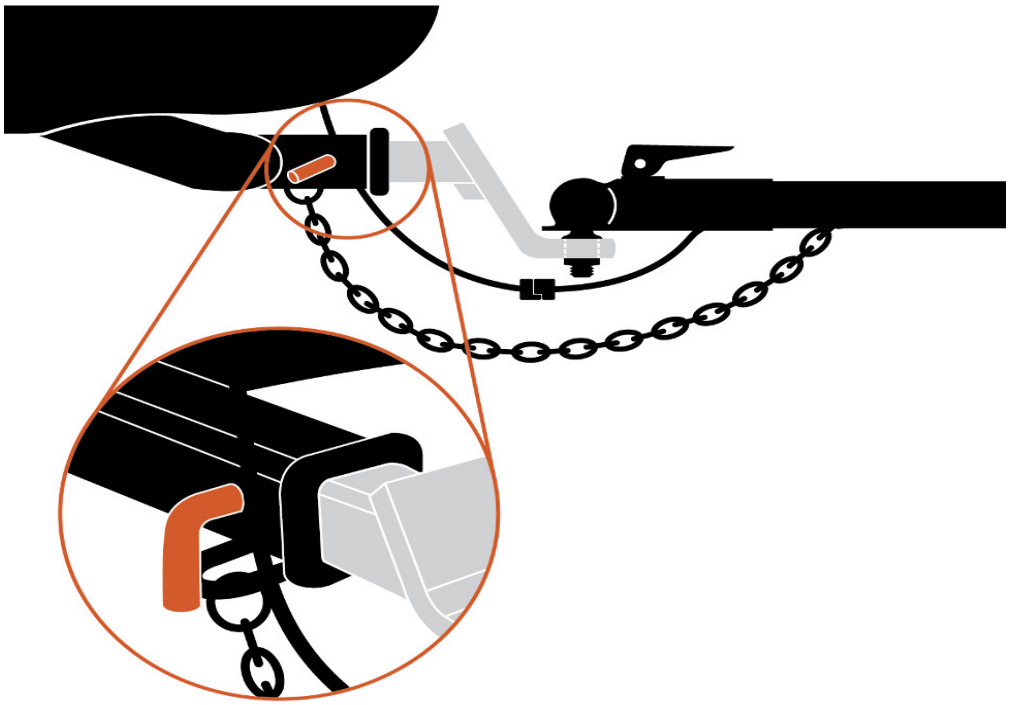
Also called a tow ball or hitch ball, a trailer ball is the immediate connection point between your tow vehicle and trailer. In conjunction with a trailer coupler, a trailer ball allows you to turn corners and travel over bumps and dips without becoming disconnected. The coupler mounts and locks on top of the trailer ball and articulates around it. Trailer balls come in a variety of diameters, including 1 7/8", 2", 2 5/16" and sometimes 3". In general, the smaller the diameter of the trailer ball, the less capacity it



has. However, this may not always be the case. Always abide by the component with the lowest weight rating.

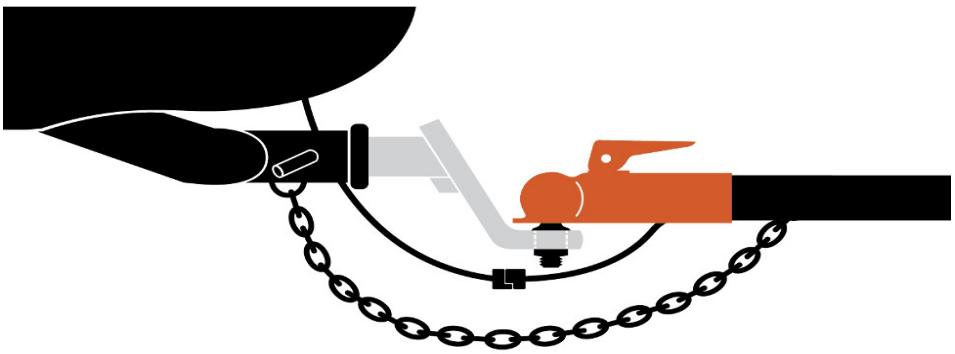
Hitch Pin and Clips

A hitch pin is a small metal rod that holds the ball mount in the hitch's receiver tube. Typically, a hitch pin is bent in an "L" shape and drilled or grooved at one end to accept a hairpin-shaped retainer clip. A hitch pin can also be substituted with a hitch lock.



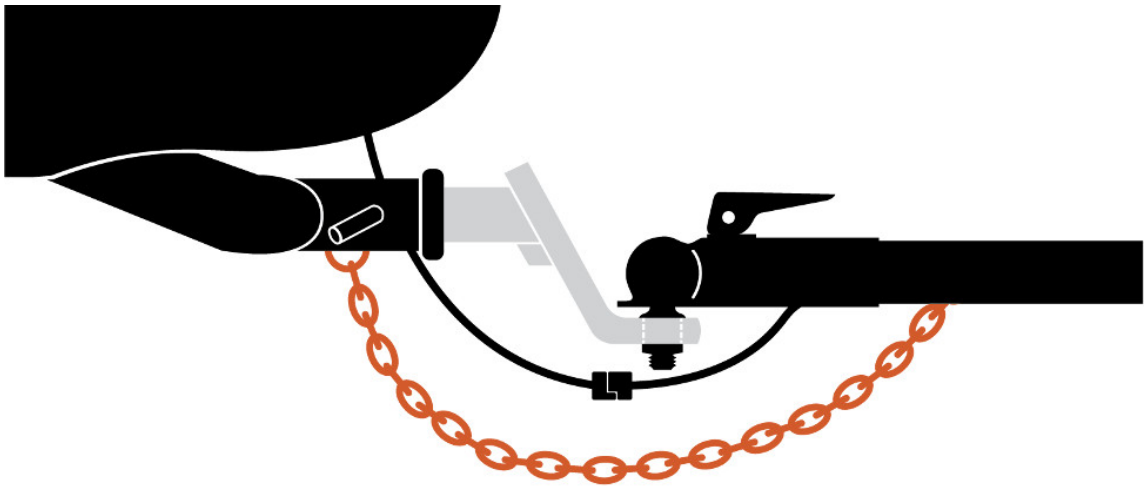
Coupler

The coupler, in conjunction with the trailer ball, allows your tow vehicle and trailer to turn corners and travel over bumps and dips without becoming disconnected. The coupler fits over the trailer ball and is designed to articulate around it.



Safety Chains

A safety chain is a length of chain strong enough to restrain the trailer from complete separation if the hitch or coupler should fail. Safety cables are also an acceptable alternative.



For every trailer, two safety chains should be used and should be set up to crisscross under the coupler. If the coupler becomes disconnected, the nose of the trailer may be caught by the safety chains, providing a measure of control while the tow vehicle stops. Use of safety chains is required by most, if not all, states.

Trailer Wiring & Lighting

Trailer lights are one of the most important aspects of towing, and like safety chains, they are required by law. All towable trailers must have taillights, brake lights and turn signals, at the very least. In order to have working lights, a trailer must be equipped with a wiring system and must be connected to the tow vehicle's wiring system. The connection can be simple or more complex, depending on what features the trailer has.

How to Hook Up Your Trailer

The first step when hitching up your trailer is to back your vehicle up to the trailer. To make this easier, we recommend having a friend help you. Before starting, agree on a set of signals to indicate which way you should turn, when to back up and when to hit the brakes. Have your helper stand on the driver's side of the trailer, about even with the trailer tongue, and make sure you can see him or her clearly before backing up.

Backing Up Your Vehicle

Step 1: Line up your vehicle in a straight line with your trailer. Having a straight shot to the coupler will make it much easier than trying to zigzag your way backward. Your helper should stand off to the side and give you signals of which direction to go.

Step 2: When you are about a foot away, stop and adjust the coupler height. Make sure the coupler will clear the trailer ball. If it is raised too much, lower it until it is only a couple inches higher than the ball.

Step 3: Back your vehicle up the rest of the way. The coupler should be perfectly lined up with the trailer ball. It is important that you go slowly during this step and that you rely on your helper to tell you which way the vehicle needs to go. If things are not lining up, don't be afraid to pull forward and try again.

Connecting the Coupler

Step 4: Lower the coupler onto the trailer ball. With the vehicle in park and the emergency brake engaged, use the trailer jack to lower the coupler until it is resting on the ball. You should also make sure the coupler latch is in the upright, unlocked position before lowering. If you find that the coupler is offset from the ball, raise the jack again and repeat the previous step.

Step 5: Latch the coupler and secure it. With the coupler latch engaged and locked, lift up on the trailer tongue to test the connection. If it comes off the ball, it means that the coupler was not properly set before being latched. Unlatch it and try again. When the coupler is secure, fully retract the trailer jack.

Step 6: Attach the safety chains in a crisscross pattern. This is a very important step. Safety chains are required by law and attaching them in a crisscross pattern underneath the coupler will provide a cradle to catch the coupler if it ever becomes disconnected from your hitch. Your safety chains should each be rated to meet or exceed the gross trailer weight, and they should not touch the ground when attached.

Hooking Up Your Trailer Lights

Step 7: Plug in the electrical connector. You should limit the amount of excess wire between the vehicle and trailer by wrapping the wires around the trailer tongue. They should not be touching the ground. With an adequate amount of wire length, press the trailer-side plug firmly into the vehicle-side socket.

Step 8: Check your trailer lights. With your helper standing in view of the trailer lights, turn them on one at a time to make sure they are working. You should check your right turn signal, left turn signal, hazards, running lights and brake lights. Have your helper call out each lighting function as he or she sees it. If one of your lights are not working, use an electrical tester to make sure there is an active signal at the vehicle-to-trailer wiring connection.

Things to Do Before Towing a Trailer

When you have your coupler hooked up, your connector plugged in and your trailer ready to tow, it is always a good idea to double check your work. Take a moment to go over the following items to help ensure a safe, successful trip.

How to Tow Safely

The leading cause of accidents both in towing and in normal driving situations is driver error, not faulty equipment. Some of the main reasons people get into accidents is because they are not paying attention, they are driving too fast, they are tailgating the person in front of them and so on. The following are some simple safety rules and precautions to help promote safe driving while towing a trailer:

1. Hitch up your trailer correctly. Make sure you have followed the proper procedures for hooking up your trailer. Double check all connections, including the coupler and wiring, and make sure your safety chains are crossed under the trailer tongue and securely connected.

2. Allow plenty of stopping distance. You need to increase your following distance when towing a trailer. It takes longer to stop your towing rig than your tow vehicle alone. Also, you should avoid sudden acceleration, braking and maneuvering.

3. Anticipate problems. Since it takes longer to accelerate, stop, change lanes and turn with a trailer, look ahead farther than you normally would. You can see many problems developing a long way off. Observe traffic flow and be ready to react.

4. Keep an eye out for trailer sway. Crosswinds, large trucks, downhill grades and high speeds can all lead to trailer sway. If you are not careful, your trailer can start swinging back and forth like a pendulum. The best way to address this problem is with a sway control unit. If you experience trailer sway, you can also take your foot off the gas and manually apply the trailer brakes with the brake control. Press the button once and your trailer should align with your tow vehicle.

5. Be extra careful when changing lanes. Changing lanes is a challenge, especially when towing. With a trailer, your blind spots increase, and you cannot accelerate as quickly. You should consider installing tow mirrors to increase your view.

6. Be patient when passing other vehicles. You must allow more distance when passing another vehicle. Passing on a two-lane road should almost never happen.

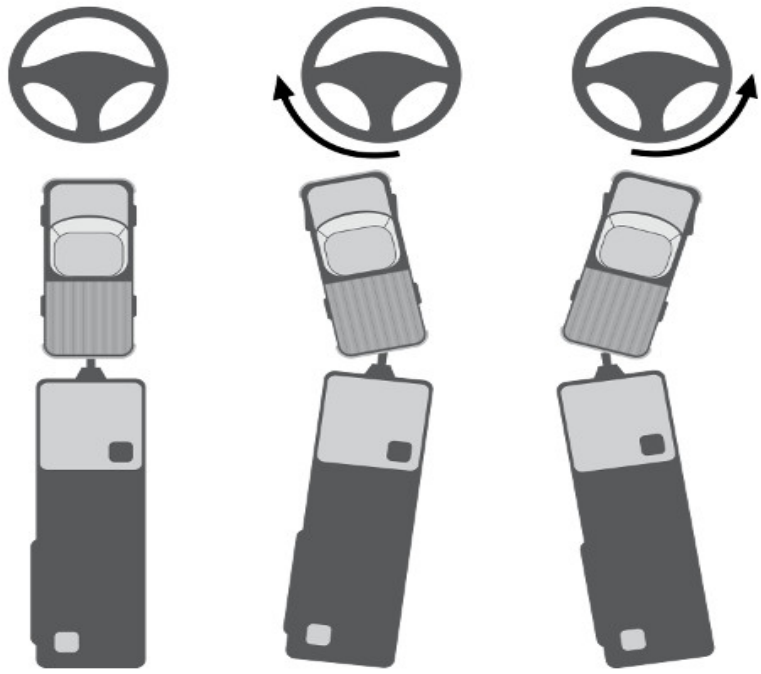
7. Stop gradually whenever possible. Towing a trailer requires extra work out of your brakes. Keep your vehicle and trailer brakes maintained and your brake control properly adjusted.

8. Do not pull in where you cannot see out. It is easy to get stuck with a trailer. You might pull into a small parking lot and must perform a complicated backup maneuver to get out. Parking farther away may be a better option.

9. Be safe with a trailer lock. Trailer theft is a serious problem. A trailer left unattended can easily be uncoupled and stolen while you are away. Use a coupler lock when towing, as it not only keeps your coupler secure but also deters theft.

How to Back Up a Trailer

For most people, one of the most dreaded things about towing a trailer is having to back up. Drivers do all kinds of things to avoid it. **However, the fact is that if you are going to tow a trailer, you are going to have to put it in reverse at some point.** The following tips are intended to help you get started. Remember, backing up with a trailer takes lots of practice.



Tip #1: Hold the steering wheel in the 6 o'clock position. With your hand in this position, it is much easier to visualize which way to steer your trailer. Moving your hand to the left will cause the trailer to go left. Moving your hand to the right will steer the trailer to the right.

Tip #2: Look over your shoulder if you can. If your view is blocked by your trailer, roll down your windows and make sure you have a good view through your side mirrors. Face forward and use your side mirrors to keep track of your trailer's movements.

Tip #3: Think of your vehicle pushing your trailer. Try not to think about them as one complete unit moving together. As you back up, visualize the back end of your vehicle pushing the coupler of the trailer. Think of it as a person pushing the handles of a wheelbarrow. If you want to turn the wheelbarrow to the right, you must move the handles to the left and vice versa.

Tip #4: Make wide initial turns but go slowly. To steer the trailer, you must steer the vehicle, and some inexperienced drivers tend to turn too little. It might feel uncomfortable at first, however, making wider turns will become more familiar with practice. One note of caution: do not move too quickly and do not exaggerate your turns so much that it causes the trailer to jackknife.

Tip #5: Do not jackknife the trailer. This point is worth repeating. A jackknifed trailer can cause damage to both the vehicle and the trailer. When backing up, go slowly and correct excessive turns by steering the tow vehicle the same way the trailer is moving or by pulling forward and trying again.

Towing section adapted from. Source for illustrations
http://www.curtmfg.com/page/understanding_towing.

Appendix G: Water Safety

As part of the AIS sampling crew, you will be working in and around water both from shore and from various types of watercraft. If you are inexperienced in water and boating safety, talk to you your supervisor about taking a U.S. Coast Guard Boating Safety Course or equivalent: http://cgaux.org/boatinged/

Should you need this type of training, your supervisor can assist you in determining which course(s) to complete. You will be paid your normal hourly rate when taking these types of courses.

Preparedness

It is important to be prepared for the worst of conditions. Research your assigned sampling locations before you get there to become familiar with accessibility, access to freshwater for drinking, toilet facilities, etc. Be sure to bring extra dry clothing and to wear appropriate materials for being in and around water. Do not wear cotton clothing as it doesn't dry quickly and can weigh you down in the water. In cold weather, cotton causes you to lose more body heat. Quick drying materials are best (such as nylon). Though you will mostly be working in the warm, summer months, inclement weather can happen anywhere, so be prepared and bring warm layers that will keep you warm even if wet (such as merino wool, fleece and synthetics). A good layering system and the right materials will keep you warm and dry in the worst conditions.

Wear appropriate footwear as well. Wear river shoes that will not fall off in swift water, that grip well in various water conditions (slippery cobble in rivers is usually the worst) and that can be easily cleaned.

Always bring and wear waterproof sunscreen. You are more likely to be burned when working in water as the sun reflects off the water's surface.

Boating in moving water

In general, when sampling in moving water, you will most likely be using inflatable watercraft (such as raft, kayak, inflatable personal pontoon). It is always good to check water conditions before you go – from local fishing websites and from the USGS website: https://waterdata.usgs.gov/mt/nwis/rt.

Cubic Feet per Second: One cubic foot per is about the size of a basketball. So if you drew a line from shore to shore across a river at 5,000 cfs, that line would have 5,000 basketball sized containers of water pass by every second. It is extremely important to know the flow of the river you are working on and to know how an increase or decrease

in flow will change the character of that river. A calm river at average flows can become very dangerous at high flows. High flows also generally come with an increase in turbidity and debris in the water.

You will never be sent out to sample in dangerous conditions, however, know there is always a risk of injury or death when working in and around water. The best thing to do is to be well educated and prepared. If you are not comfortable in certain vessel types or in certain types of water, be sure to let your supervisor know so they can work more closely with you in those types of conditions until you are comfortable.

River Sense: Direction around moving water is decided by standing with your back to the direction of flow and referred to as "river right or river left."

River Right: facing downstream, the right-hand shore is river right. Facing upstream, the left-hand shore is river right.

River Left: Facing downstream, the left-hand shore is river left. Facing upstream, the right-hand shore is river left.

White water hazards include swift sections where it is often impossible to gain a footing, floating and anchored debris that can strike or entrap a person, holes or keepers that can retain a swimmer, and long swims in cold water leading to exhaustion and hypothermia. The basic position for a river swim is on your back with your feet up, knees bent, and looking in the direction you wish to travel. This will prevent foot entrapments and allow you to use your legs to bounce off obstacles and see where you are going.

Communication: When navigating moving water in teams of multiple boats, always maintain sight contact with the boat both ahead and behind you. The most experienced boater should be in the lead with the second most experienced boater acting as a "sweep."

Hand signals

- 1 or 2 arms waving = Help!
- 1 hand on your head = "I'm OK" or "Are you OK"
- Arms crossed over chest = First Aid
- Always point positive = point in the direction boats should go (don't point at hazards)
- Both arms straight out from body = Stop

- One arm or Paddle straight up = Go
- Whistle Signals
    - 1 blast – "Stop! Look Here"
    - 2 blasts – "Upstream"
    - 3 blasts – "Downstream"
    - 3 blasts (repeated) – "Help!"

Equipment: anticipate the worst and plan for the best. Be prepared by wearing a properly-fitting lifejacket and attaching a throw bag on the front for easy access. Practice throwing your rope in non-stressful situations. If working around swift water, wear a helmet to prevent head injuries in case of a fall or surprise submersion. Wear a whistle in case you need to signal for help. A sheathed water knife worn on your PFD can cut a rope or saw through various materials in an emergency.

Swimming in moving water has many risks. Strainers are obstacles that allow water to pass through but will hold or snag a swimmer. Encounters with strainers often result in drowning. Avoid working upstream of fallen trees, wires and fencing. Log jams are particularly dangerous and should be avoided at all costs. If trapped, attempt to push off and up over the strainer. Never stand up in fast moving water in depths deeper than about your shin.

Use the defensive swim technique to conserve energy, avoid foot entrapment and to see upcoming obstacles. Let your PFD float you while facing downstream with your feet on the surface of the water. Be aware that as you lift your head in this position, your tailbone will be lower in the water. To move, point your feet at obstacles you wish to avoid and use a backstroke to pull your body away. Use an aggressive swim technique to move towards shore when safe to do so. Roll onto your stomach and keep your feet at the surface and use a crawl-stroke to move. Keep your face out of the water so you can see where you are going.

Entrapments are caused when a person's foot becomes wedged in a rock or other material while attempting to wade across a river, or to stand too soon. A good rule to follow is not to stand up until the water is shallow enough to 'beach' you.

Holes and keepers are caused by water pouring over rocks and ledges. They can be recognized by smiling or frowning waves or the appearance of a straight line on the near horizon. If you are already in the water, it may be impossible to judge the hole's

shape and velocity. If you are caught tumbling within a recirculating hole, attempt to exit sideways, or by diving deep and out the bottom.

Rescues from moving water can be done successfully using throw bags. Hold the end of the rope firmly and throw the bag directly at the swimmer. Be prepared for the rope to catch by staying low, bracing your feet, and pendulum the swimmer into a safe eddy. A partner can assist by holding onto the back straps of your PDF. It may be necessary to move downriver with the rope in order to avoid landing your swimmer in a new hazard. When attempting a water rescue – always follow the "Hello, Reach, Throw, Row and Go" techniques in that order. If you are not trained as a water rescuer – do not attempt to "Go" or you may become a victim yourself. Call for help instead. When attempting a "Reach" you can use your arms (though don't allow a victim to pull you in as well), an oar, an empty cooler, a life ring, etc. When attempting the "Throw" technique, you should aim to throw the rope over the victim. Practice rope throws in non-emergency situations.

Rope Rules:

1. Never wrap a rope around yourself
2. Never tie a rescue swimmer to a non-releasable rope
3. Never tension a line at 90 degrees to the current
4. Never stand on the downstream side of a tensioned line

River crossings can be done safely by facing upstream, using a long sturdy pole as a third anchor, and shuffling your steps. If assisted by a partner(s), hang onto each other's PFD straps and either shuffle side by side, or with one person in front of the other. With a group of three or more, a circular stance is more useful. If the current is strong and above your hips, or if the river bottom feels unstable, be prepared to retreat and find a safer location to cross.

Initial immersion in cold water results in the "gasp" reflex where the body involuntarily takes in a breath of air… if your head is above water! The next thing is a dramatic temporary lowering of the heart beat by stimulus of a "vagal" response which can lead to a dangerous drop in blood pressure. If the body survives the initial dunking, then the extremities are quickly numbed and rendered useless at grasping and holding.

Hypothermia… or more correctly "Immersion Hypothermia" is an emergent form of the general cooling of the body core. Although it is thought to incapacitate the body in a

matter of seconds or a few minutes, it is in fact a slower process and is influenced by several factors. When dealing with "immersion hypothermia" it is important to note that water conducts and convects heat from the body around 30 times faster than a comparable dry temperature. Action must be effective and efficient. Wet layers must be changed for dry ones. This is where the correct PPE makes a critical difference. Do not be tempted to let wet layers "dry off" as you wear them… this is very energy intensive.

Boating in non-moving water

1. Always check the weather report before you launch for the day. Weather can change quickly while you are out on the water. Be prepared for variable weather conditions and prepare for them. Know the limitations of the type of vessel you are using.

2. **File a float plan with someone you know.** By letting someone know where you plan to be and when you plan to be back, you always have a "safety net" in case unexpected situations arise.

3. Always wear a life jacket. It can't save your life if you're not wearing it!

4. Know the waters you're are navigating. Always refer to area maps and have a good understanding of the water you will be boating on

Appendix H – Material Safety Data Sheets

The Clorox Company
1221 Broadway
Oakland, CA 94612
Tel. (510) 271-7000

Material Safety Data Sheet

I Product: CLOROX REGULAR-BLEACH

Description: CLEAR, LIGHT YELLOW LIQUID WITH A CHARACTERISTIC CHLORINE ODOR

Other Designations	Distributor	Emergency Telephone Nos.
Clorox Bleach EPA Reg. No. 5813-50	Clorox Sales Company 1221 Broadway Oakland, CA 94612	For Medical Emergencies call: (800) 446-1014 For Transportation Emergencies Chemtrec (800) 424-9300

II Health Hazard Data

DANGER: CORROSIVE. May cause severe irritation or damage to eyes and skin. Vapor or mist may irritate. Harmful if swallowed. Keep out of reach of children.

Some clinical reports suggest a low potential for sensitization upon exaggerated exposure to sodium hypochlorite if skin damage (e.g., irritation) occurs during exposure. Under normal consumer use conditions the likelihood of any adverse health effects are low.

Medical conditions that may be aggravated by exposure to high concentrations of vapor or mist: heart conditions or chronic respiratory problems such as asthma, emphysema, chronic bronchitis or obstructive lung disease.

FIRST AID:

Eye Contact: Hold eye open and rinse with water for 15-20 minutes. Remove contact lenses, after first 5 minutes. Continue rinsing eye. Call a physician.

Skin Contact: Wash skin with water for 15-20 minutes. If irritation develops, call a physician.

Ingestion: Do not induce vomiting. Drink a glassful of water. If irritation develops, call a physician. Do not give anything by mouth to an unconscious person.

Inhalation: Remove to fresh air. If breathing is affected, call a physician.

III Hazardous Ingredients

Ingredient	Concentration	Exposure Limit
Sodium hypochlorite CAS# 7681-52-9	5 - 10%	Not established
Sodium hydroxide CAS# 1310-73-2	<1%	2 mg/m[1] 2 mg/m[2]

[1]ACGIH Threshold Limit Value (TLV) - Ceiling

[2]OHSA Permissible Exposure Limit (PEL) – Time Weighted Average (TWA)

None of the ingredients in this product are on the IARC, NTP or OSHA carcinogen lists.

IV Special Protection and Precautions

No special protection or precautions have been identified for using this product under directed consumer use conditions. The following recommendations are given for production facilities and for other conditions and situations where there is increased potential for accidental, large-scale or prolonged exposure.

Hygienic Practices: Avoid contact with eyes, skin and clothing. Wash hands after direct contact. Do not wear product-contaminated clothing for prolonged periods.

Engineering Controls: Use general ventilation to minimize exposure to vapor or mist.

Personal Protective Equipment: Wear safety goggles. Use rubber or nitrile gloves if in contact liquid, especially for prolonged periods.

KEEP OUT OF REACH OF CHILDREN

V Transportation and Regulatory Data

DOT/IMDG/IATA - Not restricted.

EPA - SARA TITLE III/CERCLA: Bottled product is not reportable under Sections 311/312 and contains no chemicals reportable under Section 313. This product does contain chemicals (sodium hydroxide <0.2% and sodium hypochlorite <7.35%) that are regulated under Section 304/CERCLA.

TSCA/DSL STATUS: All components of this product are on the U.S. TSCA Inventory and Canadian DSL.

VI Spill Procedures/Waste Disposal

Spill Procedures: Control spill. Containerize liquid and use absorbents on residual liquid; dispose appropriately. Wash area and let dry. For spills of multiple products, responders should evaluate the MSDS's of the products for incompatibility with sodium hypochlorite. Breathing protection should be worn in enclosed, and/or poorly ventilated areas until hazard assessment is complete.

Waste Disposal: Dispose of in accordance with all applicable federal, state, and local regulations.

VII Reactivity Data

Stable under normal use and storage conditions. Strong oxidizing agent. Reacts with other household chemicals such as toilet bowl cleaners, rust removers, vinegar, acids or ammonia containing products to produce hazardous gases, such as chlorine and other chlorinated species. Prolonged contact with metal may cause pitting or discoloration.

VIII Fire and Explosion Data

Flash Point: None

Special Firefighting Procedures: None

Unusual Fire/Explosion Hazards: None. Not flammable or explosive. Product does not ignite when exposed to open flame.

IX Physical Data

Boiling point ..... approx. 212°F/100°C
Specific Gravity (H_2O=1) ..... ~ 1.1 at 70°F
Solubility in Water ..... complete
pH ..... ~11.9

DATA SUPPLIED IS FOR USE ONLY IN CONNECTION WITH OCCUPATIONAL SAFETY AND HEALTH DATE PREPARED 08/09

SAFETY DATA SHEET

This SDS complies with 29 CFR 1910.1200 (Hazard Communication Standard)
IMPORTANT: Read this SDS before handling & disposing of this product. Pass this information on to employees, customers, and users of this product.

1. Identification

1.1. Product identifier

Product Identity	Vinegar Floor Neutralizer
Alternate Names	Vinegar Floor Neutralizer
Product Code	119-01

1.2. Relevant identified uses of the substance or mixture and uses advised against

Intended use	Cleaner
Application Method	See Label Instructions

1.3. Details of the supplier of the safety data sheet

Company Name	Diamond Products Inc. 1216 Bozeman Ave. Helena, MT 59601

Emergency

24 hour Emergency Telephone No.	Infotrac: 1 800-535-5053 Emergency: (406) 449-6570
Customer Service: Diamond Products Inc.	(406) 449-6570

2. Hazard(s) identification

2.1. Classification of the substance or mixture

Skin Corr. 2;H315 Causes skin irritation.
Eye Dam. 2A;H319 Causes serious eye irritation
Aquatic Acute 3;H402 Harmful to aquatic life

2.2. Label elements

Using the Toxicity Data listed in section 11 and 12 the product is labeled as follows.

Warning

H315 Causes skin irritation.
H319 Causes serious eye irritation
H402 Harmful to aquatic life
[Prevention]:

P264: Wash thoroughly after handling.
P280: Wear protective gloves/protective clothing/eye protection/face protection.
[Response]:
P302+352: IF ON SKIN: Wash with plenty of water.
P305+351+338: IF IN EYES: Rinse continuously with water for several minutes. Remove contact lenses if present and easy to do – continue rinsing.
P337+313: If eye irritation persists get medical advice/attention.
P332+313: If skin irritation occurs: Get medical advice/attention.
P362+364: Take off contaminated clothing and wash it before reuse.
P391 Collect spillage.
[Storage]:
No GHS storage statements
[Disposal]:
No GHS disposal statements

3. Composition/information on ingredients

This product contains the following substances that present a hazard within the meaning of the relevant State and Federal Hazardous Substances regulations.

Ingredient/Chemical Designations	Weight %	GHS Classification	Notes
Acetic Acid **CAS Number: 0000064-19-7**	1.0 - 10	**Flam. Liq. 3;H226** **Skin Corr. 1A;H314** **Eye Dam. 1;H318** **Skin Sens. 1;H317** **Aquatic Acute 3**	[1][2]

In accordance with paragraph (i) of §1910.1200, the specific chemical identity and/or exact percentage (concentration) of composition has been withheld as a trade secret.
[1] Substance classified with a health or environmental hazard.
[2] Substance with a workplace exposure limit.
[3] PBT-substance or vPvB-substance.
*The full texts of the phrases are shown in Section 16.

4. First aid measures

4.1. Description of first aid measures

General	In all cases of doubt, or when symptoms persist, seek medical attention. Never give anything by mouth to an unconscious person.
Inhalation	Remove to fresh air, keep patient warm and at rest. If breathing is irregular or stopped, give artificial respiration. If unconscious place in the recovery position and obtain immediate medical attention. Give nothing by mouth.
Eyes	Irrigate copiously with clean water for at least 15 minutes, holding the eyelids apart and seek medical attention.

Skin	Remove contaminated clothing. Wash skin thoroughly with soap and water or use a recognized skin cleanser.
Ingestion	Do NOT induce vomiting. Dilute product by giving large quantities of water or milk. Call your nearest poison control center for further action and seek medical attention immediately.

4.2. Most important symptoms and effects, both acute and delayed

Overview	Exposure to solvent vapor concentrations from the component solvents in excess of the stated occupational exposure limits may result in adverse health effects such as mucous membrane and respiratory system irritation and adverse effects on the kidneys, liver and central nervous system. Symptoms include headache, nausea, dizziness, fatigue, muscular weakness, drowsiness and in extreme cases, loss of consciousness. Repeated or prolonged contact with the preparation may cause removal of natural fat from the skin resulting in dryness, irritation and possible non-allergic contact dermatitis. Solvents may also be absorbed through the skin. Splashes of liquid in the eyes may cause irritation and soreness with possible reversible damage. See section 2 for further details.

5. Fire-fighting measures

5.1. Extinguishing media

Water or other normal extinguishing media.

5.2. Special hazards arising from the substance or mixture

Hazardous decomposition: Thermal decomposition may yield carbon dioxide and/or carbon monoxide.

5.3. Advice for fire-fighters

Wear self-contained breathing apparatus

ERG Guide No. ----

6. Accidental release measures

6.1. Personal precautions, protective equipment and emergency procedures

Put on appropriate personal protective equipment (see section 8).

6.2. Environmental precautions

Do not allow spills to enter drains or waterways.
Use good personal hygiene practices. Wash hands before eating, drinking, smoking or using toilet. Promptly remove soiled clothing and wash thoroughly before reuse.

6.3. Methods and material for containment and cleaning up

Small spills: Mop up with water.
Large spills: Absorb with inert material and place in suitable containers.
Dispose of in accordance with local, state and federal regulations.

7. Handling and storage

7.1. Precautions for safe handling

See section 2 for further details. - [Prevention]:

7.2. Conditions for safe storage, including any incompatibilities

Handle containers carefully to prevent damage and spillage.
Incompatible materials: Strong oxidizing agents
Keep product containers cool and dry.
See section 2 for further details. - [Storage]:

7.3. Specific end use(s)

No data available.

8. Exposure controls and personal protection

8.1. Control parameters

Exposure

CAS No.	Ingredient	Source	Value
0000064-19-7	Acetic Acid	OSHA	TWA: 10 ppm (25 mg/m3)STEL N/A
		ACGIH	TWA: 10 ppm STEL: 15 ppm
		NIOSH	TWA 10 ppm (25 mg/m3)
		Supplier	No Established Limit

Carcinogen Data

CAS No.	Ingredient	Source	Value
0000064-19-7	Acetic Acid	OSHA	Select Carcinogen: No
		NTP	Known: No; Suspected: No
		IARC	Group 1: No; Group 2a: No; Group 2b: No; Group 3: No; Group 4: No;

8.2. Exposure controls

Respiratory	If workers are exposed to concentrations above the exposure limit they must use the appropriate, certified respirators.
Eyes	Protective safety glasses recommended
Skin	Not normally required.
Engineering Controls	Provide adequate ventilation. Where reasonably practicable this should be achieved by the use of local exhaust ventilation and good general extraction. If these are not sufficient to maintain concentrations of particulates and any vapor below occupational exposure limits suitable respiratory protection must be worn.
Other Work Practices	Use good personal hygiene practices. Wash hands before eating, drinking, smoking or using toilet. Promptly remove soiled clothing and wash thoroughly before reuse.

See section 2 for further details. - [Prevention]:

9. Physical and chemical properties

Appearance	Clear Liquid
Odor	Vinegar
Odor threshold	Not Measured
pH	2.4 at 6%
Melting point / freezing point	Not applicable
Initial boiling point and boiling range	210 - 220°F

Flash Point	Non-flammable
Evaporation rate (Ether = 1)	Not available
Flammability (solid, gas)	Not Applicable
Upper/lower flammability or explosive limits	**Lower Explosive Limit:** Not applicable **Upper Explosive Limit:** Not applicable
Vapor pressure (Pa)	11.4
Vapor Density	2.07 (Air = 1.0)
Specific Gravity	1.05 g/ml
Solubility in Water	Complete
Partition coefficient n-octanol/water (Log Kow)	Not Measured
Auto-ignition temperature	Not applicable
Decomposition temperature	Not available
Viscosity (cSt)	Not available
VOC Content	Not available

9.2. Other information
No other relevant information.

10. Stability and reactivity

10.1. Reactivity
Hazardous Polymerization will not occur.
10.2. Chemical stability
Stable under normal circumstances.
10.3. Possibility of hazardous reactions
No data available.
10.4. Conditions to avoid
No data available.
10.5. Incompatible materials
Strong oxidizing agents
10.6. Hazardous decomposition products
Thermal decomposition may yield carbon dioxide and/or carbon monoxide.

11. Toxicological information

Acute toxicity

Ingredient	Oral LD50, mg/kg	Skin LD50, mg/kg	Inhalation Vapor LC50, mg/L/4hr	Inhalation Dust/Mist LC50, mg/L/4hr	Inhalation Gas LC50, ppm
Acetic Acid – (64-19-7)	90,000.00, Rat	No data available	5620 ppm Mouse	No data available	No data available

Note: When no route specific LD50 data is available for an acute toxin, the converted acute toxicity point estimate was used in the calculation of the product's ATE (Acute Toxicity Estimate).

Classification	Category	Hazard Description
Acute toxicity (oral)	---	Not Applicable
Acute toxicity (dermal)	---	Not Applicable
Acute toxicity (inhalation)	---	Not Applicable
Skin corrosion/irritation	2	Causes skin irritation.
Serious eye damage/irritation	2A	Causes serious eye irritation
Respiratory sensitization	---	Not Applicable
Skin sensitization	---	Not Applicable
Germ cell mutagenicity	---	Not Applicable
Carcinogenicity	---	Not Applicable
Reproductive toxicity	---	Not Applicable
STOT-single exposure	---	Not Applicable
STOT-repeated exposure	---	Not Applicable
Aspiration hazard	---	Not Applicable

12. Ecological information

12.1. Toxicity
No additional information provided for this product. See Section 3 for chemical specific data.
Aquatic Ecotoxicity

Ingredient	96 hr LC50 fish, mg/l	24 hr EC50 crustacea, mg/l	ErC50 algae, mg/l
Acetic Acid – (64-19-7)	79.00, Fathead Minnow	47.00, Daphnia	No Data Available

12.2. Persistence and degradability
Biodegradable
12.3. Bioaccumulative potential
Biodegradable, no accumulation expected.
12.4. Mobility in soil
No data available.
12.5. Results of PBT and vPvB assessment
This product contains no PBT/vPvB chemicals.
12.6. Other adverse effects
No data available.

13. Disposal considerations

13.1. Waste treatment methods
Observe all federal, state and local regulations when disposing of this substance.

14. Transport information

	DOT (Domestic Surface Transportation)	IMO / IMDG (Ocean Transportation)	ICAO/IATA
14.1. UN number	Not Applicable	Not Regulated	Not Regulated
14.2. UN proper shipping name	Not Regulated	Not Regulated	Not Regulated
14.3. Transport hazard class(es)	**DOT Hazard Class:** Not Applicable	**IMDG:** Not Applicable **Sub Class:** Not Applicable	**Air Class:** Not Applicable
14.4. Packing group	Not Applicable	Not Applicable	Not Applicable

14.5. Environmental hazards
IMDG Marine Pollutant: No
14.6. Special precautions for user
No further information

15. Regulatory information

Regulatory Overview	The regulatory data in Section 15 is not intended to be all-inclusive, only selected regulations are represented.
Toxic Substance Control Act (TSCA)	All components of this material are either listed or exempt from listing on the TSCA Inventory.
WHMIS Classification	Not Regulated
US EPA Tier II Hazards	**Fire:** No **Sudden Release of Pressure:** No **Reactive:** No **Immediate (Acute):** Yes **Delayed (Chronic):** No

EPCRA 311/312 Chemicals and RQs:
Acetic Acid (5000 lbs.)
EPCRA 302 Extremely Hazardous:
To the best of our knowledge, there are no chemicals at levels which require reporting under this statute.
EPCRA 313 Toxic Chemicals:
To the best of our knowledge, there are no chemicals at levels which require reporting under this statute.
Proposition 65 - Carcinogens (>0.0%):
To the best of our knowledge, there are no chemicals at levels which require reporting under this statute.
Proposition 65 - Developmental Toxins (>0.0%):
To the best of our knowledge, there are no chemicals at levels which require reporting under this statute.
Proposition 65 - Female Repro Toxins (>0.0%):
To the best of our knowledge, there are no chemicals at levels which require reporting under this statute.
Proposition 65 - Male Repro Toxins (>0.0%):
To the best of our knowledge, there are no chemicals at levels which require reporting under this statute.
New Jersey RTK Substances (>1%):

To the best of our knowledge, there are no chemicals at levels which require reporting under this statute.
Pennsylvania RTK Substances (>1%):
To the best of our knowledge, there are no chemicals at levels which require reporting under this statute.

16. Other information

The information and recommendations contained herein are based upon data believed to be correct. However, no guarantee or warranty of any kind, expressed or implied, is made with respect to the information contained herein. We accept no responsibility and disclaim all liability for any harmful effects which may be caused by exposure to our products. Customers/users of this product must comply with all applicable health and safety laws, regulations, and orders.

The full text of the phrases appearing in section 3 is:

H226 Flammable liquid and vapor.

H314 Causes severe skin burns and eye damage.

H317 May cause an allergic skin reaction

H318 Causes serious eye damage.

This is the first version in the GHS SDS format. Listings of changes from previous versions in other formats are not applicable.

The information herein is presented in good faith and believed to be correct as of the date hereof. However, Diamond Products, Inc., makes no representation as to the completeness and accuracy thereof. Users must make their own determination as to the suitability of the product for their purposes prior to use. No representations or warranties, either express or implied, of merchantability, fitness for a particular purpose or of any other nature with respect to the product or the information herein is made hereunder. Diamond Products, Inc., shall in no event be responsible for any damages of whatsoever nature directly or indirectly resulting from the publication or use of or reliance upon information contained herein.

End of Document

Appendix I – Key Findings eDNA Science Advisory Panel: A discussion on eDNA technology use in invasive species management

Montana Invasive Species Council

Key Findings eDNA Science Advisory Panel: A discussion on eDNA technology use in invasive species management

A six-person panel of aquatic invasive species, monitoring and eDNA experts was assembled in April 2018 by the Montana Invasive Species Council (MISC) to evaluate the use of environmental DNA (eDNA) for dreissenid mussel early detection and provide input and guidance to managers regarding its use in Montana.

Key Challenges and Recommendations by Panelists

Challenges

- Lack of standardized protocols
  - Field collection
  - Lab analysis
  - Communication of results (between researchers/labs and managers)
  - Management response
- Balance of risk and uncertainty
  - Understand the costs of false negatives or false positives to assess risk tolerance
  - Perspective on terms false negatives and false positives
- Detection threshold of eDNA for false negatives is not known and varies with sampling/analysis methods
- A limited number of labs are conducting eDNA analysis for early detection of dreissenids and use different protocols
- No coordinated dreissenid eDNA group to help address gaps and encourage communication
- Few published peer reviewed studies for dreissenid eDNA
- Communicating what a “positive” eDNA sample means

Recommendations

- Develop, refine, and agree upon method/standards with adaptive capacity
  - Decontamination protocols (utilize existing US Fish and Wildlife Service for Asian carp effort)
  - Field collection
  - Lab analysis including Quality Assurance/Quality Control standardization
  - Data reporting requirements and standards
- Develop consistent language (for both within lab and out)

- Develop a communication plan between managers and lab
    - Approach eDNA results as a link in a chain of evidence
    - Clearly define the steps to be taken following a detection. An eDNA detection could result in further sampling or directly lead to a management action, depending on these pre-defined steps
- Coordinate across western partners and cross-border partners via the suggested avenues
    - Coordination among managers: Utilize existing venue of Western Regional Panel on ANS and/or Western Governors Association
    - Coordination between managers and researchers: Establish forum to continue conversation
    - Coordination among researchers: Develop a system to share information
- Identify risk tolerance and map management actions for detection scenarios and trends
- Test assays with round robin process to assist with lab/manager confidence, identify areas for improvement in consistency, and relationship building
- Gene sequence any positive result to confirm
- Optimal conditions for eDNA detection is during dreissenid spawning
- Use eDNA to contribute to the weight of evidence to determine presence of dreissenids
    - Develop/utilize a decision tree that incorporates monitoring results from different methods, likelihood of invasion, etc.

Suggested parameters of a standard method

- Grab samples are thought to be better than plankton tows, but further evaluation is needed
- Surface water collection is preferred and is less problematic
- Bleach best for decontamination (50% solution)
- Minimize contamination with on-site processing
- Best to canvas waterbody with smaller samples
- Standardize assays using markers from different regions of the genome that are suited to answer question of study
- Use controls in the field and take replicate samples
- Use qPCR vs. conventional PCR

Conclusion and Next Steps

The MISC eDNA Science Advisory Panel was a successful step in better understanding the role for eDNA in management of aquatic invasive species for the future. The management of invasive species, specifically dreissenids, presents unique management and political challenges. Clear acknowledgement of gaps and recommendations from the advisory panel provides a path forward for developing this technology into an operational tool that manages are comfortable using for dreissenid monitoring. Action on this issue will require international effort and include both managers and researchers to address gaps and needs in the development of this technology as an early detection tool. This is an issue that affects aquatic invasive species prevention and management beyond the boundaries of Montana, and steps forward will benefit agencies and stakeholders across jurisdictions. MISC will encourage action on these issues, but interested partners nation-wide will need to help push this effort forward.

MISC has identified the following steps to utilize the information from the panel:

- Make all information generated from the scientific advisory panel available to all interested parties
- Encourage the development of open dialog among eDNA dreissenid scientific community to promote further standardization of this tool
- Encourage the completion of a laboratory round-robin project among appropriate partners to promote further standardization of this tool
- Engage the Western Regional Panel on ANS and/or the Western Governors Association to assist in the promotion/implementation of the next steps identified by the panelists
- Continue the discussion regarding the use of eDNA and promote coordination and cooperation as the development of this method moves forward

(Council, 2018)

Appendix J – Program Contact Information

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