Wyoming Game and Fish Department

Aquatic Invasive Species

Sampling and Monitoring Manual

LAST REVISED
JUNE 10, 2015

Table of Contents

Introduction

The Wyoming Game and Fish Department (WGFD) Aquatic Invasive Species (AIS) program was created in March 2010. Sampling and monitoring is an important component of the program and will focus on early detection of AIS to allow for rapid response plans to be implemented. Regular monitoring is necessary to determine when the introduction of an invasive species occurs. Early detection results in greater potential for containment or eradication, ultimately minimizing the negative impacts and financial burden from AIS. Post-detection sampling and monitoring provides insight into population persistence and impacts on the ecosystem, infrastructure, and human use of those waters. Monitoring provides an understanding of how environments are changing as a result of the invasion. Currently there are few, if any, effective control measures for most AIS. Monitoring efforts can contribute data to research on the success or failure of new control strategies. Sampling and monitoring are integral in understanding the size and nature of an infestation and can be important tools when planning the future of water management in Wyoming.

A water body risk assessment for zebra and quagga mussels was completed in 2009 for 52 waters in Wyoming (Bear 2009). The risk assessment identified 10 *High* risk waters, 13 *Moderate* risk waters, and 29 *Low* risk waters. In 2010, the WGFD sampled H*igh* and *Moderate* risk waters twice per season and *Low* risk waters once per season. Priority designation for waters was further modified to better reflect boater use and risk to water of AIS being transported through watercraft movement. All *High/Moderate* risk waters will be sampled twice, and all *Low* risk will be sampled once for invasive mussel veligers.

This manual describes standard procedures for sampling a variety of AIS, with emphasis on zebra and quagga mussel (ZQM) early detection surveys and water quality sampling. This manual combines protocols developed by Colorado Division of Wildlife (CDOW 2009), the U.S. Bureau of Reclamation (BOR 2009), and Montana Fish, Wildlife and Parks (MTFWP 2010). This manual will help ensure that all AIS sampling and monitoring performed in Wyoming is consistent and based on sound science. All WGFD employees should follow the protocols detailed in this manual for sampling, preservation, data documentation, identification, and equipment disinfection.

Aquatic Invasive Species Survey Protocols

Plankton Tow Sampling

Plankton tows are performed to find the initial microscopic life stage of mussels (veligers) while they are free floating in the water column. This allows for early detection to identify when the mussels are introduced into a water.

In the field:

Sampling should occur after water temperatures are above 60°F and preferably during times when turbidity is low. Samples should be strictly from the water column and should not contain large amounts of sediment unless the system has high amounts of suspended sediment regularly. Avoid dragging net along bottom of waterbody. If net is accidently drug across the bottom, dump out contents and redeploy net.

Determine the appropriate place at every water to conduct plankton tows. A summary table is included with the number and general location of tows at every water (Appendix A). There should be a minimum of three locations at every water. Typical sampling locations include areas where mussels are likely to be introduced (e.g. marinas, docks, boat launches, inlets, outlets, etc.), open water locations, and near dams. It is important to spread out tow sites to increase the likelihood of collecting veligers and focus on areas that are downwind or downstream. Conduct tows in both open water and near shore. Two of the tow locations should be in the same location as where substrate sampling is conducted.

Each tow location should consist of at least six individual tows. The first individual tow at a sample location will be used as a replicate copy to be stored until the end of the season. The remaining five individual tows at the same location are combined in a single sample bottle. As a result of the number of locations at each water body and the number of tows at each location, there will be a minimum of 15 tows (3 sample jars) per water that will be sent to a lab for analysis.

- *High*/*Moderate* risk waters must be visited twice per season. The first sampling should be conducted once surface water temperatures are above 60°F (June/July). If surface water temperature does not regularly exceed 60°F, the first sampling should be conducted when temperatures are at their warmest. The next sampling should occur during the fall in late September or early October.

- *Low* risk water bodies will be sampled once per season in the summer (June or July).

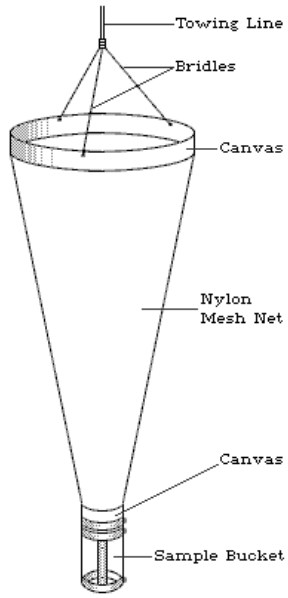


Figure 1. Simple conical plankton-tow net.

Vertical Plankton Tow:

1. Anchor the boat at the sampling site and make sure the boat is not drifting. Examine the tow net to ensure cod end (sample bucket) is secure and towing line is ready to be deployed.

2. Take a GPS point at the plankton tow sampling location every time you sample there. Record all data in the GPS unit and on a Plankton Tow datasheet (Appendix B) while in the field. *Ensure GPS settings are collecting coordinates using NAD27.*

3. Determine the appropriate tow length:
    a. Take a depth reading (in feet).
    b. Subtract 3 ft. from the depth. This prevents you from hitting the bottom with the tow net and kicking up sediment.
    c. Subtract the length of the tow net (3 ft.) to calculate how much tow line you will need to deploy.

    Example: At a location that is 50 feet deep, you would deploy 44 feet of line attached to the net to collect the sample.

4. Lower the tow net to the calculated depth (TIP: If air pockets occur, lower the net slowly with the opening tilted to the side). Be careful to not hit the bottom with the net– this will kick up sediment and you will have to retake the tow. Keep the net submerged at this depth for 30 seconds.

5. Slowly pull up the net using a hand-over-hand technique. As the net reaches the surface, pulse the net up and down to insure plankton are collected in the cod end.

6. Carefully unscrew the cod end from the net. Pour the plankton sample into the sample bottle. Thoroughly rinse the cod end several times with a spray bottle filled with distilled water. Pour the residual rinse water into the sample bottle. Be sure to rinse out the cod end after each tow. If algae collect in the net, after collecting the sample clean the net before conducting another tow.

7. There should be a total of <u>six</u> individual tows at each sample location.
    a. The <u>first</u> individual tow at a sample location should be collected in a separate bottle to be used as a replicate copy.
    b. Complete <u>five</u> additional tows at the sampling location and combine the contents of each of these tows into a single sample bottle.

8. Properly label both sample bottles from the sample location on the outside of the bottle using the labels found in Appendix B. *Do not place a label inside the plankton tow sample jar.* Store bottles in a cooler with ice pack until the sample can be placed in a refrigerator at the office prior to shipping.

9. Make sure all data has been entered accurately into the GPS unit and the Plankton Tow data sheet.

<u>Horizontal Tow:</u>

1. Horizontal tows are done in moving water or in areas that are not deep enough to conduct a thorough vertical tow. A general guideline is any area that is less than 20 ft deep should be sampled using a horizontal tow.

2. Examine the tow net to ensure cod end is secure and towing line is ready to be deployed.

3. Hold the ring of the net (the metal loop that holds the net mouth open) using thumb and forefinger. Make large loops with the line and hold loosely in the same hand holding the net.

4. Firmly hold the other end of the line with your free hand.

5. Throw the net using a sidearm-style, opening your hand upon release to allow line to feed out with the net.

6. Allow the opening of the net to sink into the water. If an air bubble gets trapped in the net, retrieve the net and start again.

7. Pull the net along horizontally for at least 20 ft. or longer if possible. Keep the net off the bottom to avoid both snagging and collecting debris. Slowly pull up the net using a

hand-over-hand technique. As the net reaches the surface, pulse the net up and down to insure plankton are collected in the cod end.

8. Carefully unscrew the cod end from the net. Pour the plankton sample into the sample bottle. Thoroughly rinse the cod end several times with a spray bottle filled with distilled water. Pour the residual rinse water into the sample bottle. Be sure to rinse out the cod end after each tow. If algae collect in the net, after collecting the sample clean the net before conducting another tow.

9. There should be a total of <u>six</u> individual tows at each sample location.
    a. The <u>first</u> individual tow at a sample location should be set aside as a replicate copy.
    b. Complete <u>five</u> additional tows at the sampling location and combine the contents of each of these tows into a single sample bottle. The composited length of the five individual tows at the same site should be a minimum of 100 ft.

10. Properly label both sample bottles from the sample location on the outside of the bottle using the labels found in Appendix B. *Do not place a label inside the plankton tow sample jar.* Store bottles in a cooler with ice pack until the sample can be placed in a refrigerator at the office prior to shipping.

11. Make sure all data is entered into the GPS unit and the Plankton Tow datasheet.

<u>Plankton Sample Collection and Storage:</u>

1. When you have completed all individual tows at a location, mark the water level on the sample bottles with permanent ink.

2. Add an appropriate volume of ethanol to the sample bottles.
    a. The **<u>replicate copy</u>** will need to have a **<u>75% ethanol</u>** (EtOH) final solution. This can be achieved by adding 3 parts EtOH (100%) to 1 part sample water.
    b. The <u>composited sample</u> bottle to be sent to the lab (**if sent to a BOR lab-see Appendix A)** will need to have a **<u>25% EtOH</u>** final solution. This can be achieved by adding 1 part EtOH (100%) to 3 parts sample water.
    c. The <u>composited sample</u> bottle to be sent to the lab (**if sent to MTFWP lab -see Appendix A)** will need to have a **<u>75% EtOH</u>** final solution. This can be achieved by adding 1 part EtOH (100%) to 3 parts sample water.

3. Visual estimate does not have to be exact. Mark the level of added alcohol on the sample bottle with permanent ink.

4. All samples need to stay above a pH of 7.5. To accomplish this, add a minimum of 0.2ml (3 drops) of baking soda or tris-base buffer ($NaHCO_3$) to every sample bottle. If there is

heavy algae in the sample bottle double the amount of baking soda to 0.4ml (6 drops). Secure the lid and gently shake to dissolve baking soda.

5. Complete the appropriate label (Appendix B) for the sample bottles in pencil and adhere to the outside of the sample bottle. *Do not place a label inside the plankton tow sample jar.*

Date: 06/28/10
Water name: Granite Reservoir
Water code: GRR
Sampling Location: Main boat ramp
UTM: E 479499
N 4558282
Zone: 13T
Number of tows: 4
Length of tows: 40 ft each
Collectors: BB, SR
Agency: WGFD
Sample ID: 1 - GRR - 062810 - 479499
method code - water code - date - UTM E
Preservative: 75% ETOH
LAB SAMPLE

Figure 2. Example of completed sample label. Blank labels can be found in Appendix B.

The sample ID used on the label consists of the method code, water code, date, and UTM easting for that location. The method codes are as follows:
1 = Plankton tow
2 = Substrate survey
3 = Water quality
4 = Stream/Shoreline survey
5 = Plant survey

The water code is the AIS water code used in the watercraft inspection and decontamination manual. These codes can be found in Appendix F.

6. Tightly close the bottle lid and secure the cap with electrical tape to help prevent leakage. Place the bottle in a re-sealable plastic bag. Package all sample bottles in a cooler with ice packs and keep cool. Once at the office, place LAB samples in the refrigerator and ship samples to the lab the next morning. Keep REPLICATE samples at Regional Office until all analysis has been completed for the year (this sample acts as a duplicate in case additional testing is needed on any of the samples). Due to higher EtOH replicate samples do not need to be refrigerated.

All plankton tow samples processed for ZQM identification will follow a three-tier identification process. Samples will be first analyzed by visual identification using cross-polarized light microscopy. If positive or suspected positive results occur from microscopy, the samples will then be sent to an independent lab for DNA analysis via PCR and gene sequencing.

Samples being sent to the Bureau of Reclamation (BOR) must be split into two equal parts with one set sent to the Montana Fish, Wildlife and Parks Lab (MTFWP) and the other duplicate sent to BOR. *Unlike samples destined for the BOR, samples being shipped to MTFWP do NOT have to be shipped in Styrofoam coolers or with ice packs and should contain 75% EtOH.*

*Shipping Instructions-**BOR samples (preserved in 25% ethanol)**:*

1. Place samples and coolant (blue ice packs) in an airtight container (large freezer bag).

2. Place container in Styrofoam cooler and add cushioning material such as plastic grocery bags, scrap paper, etc.

3. Fill out Specimen Collection Report for lab (Appendix B) and include with shipment.

4. Ship samples being sent to the BOR via FedEx Standard Overnight Monday – Thursday to:

Bureau of Reclamation Lab, Attn: Denise M. Hosler
Environmental Applications and Research Group
Bureau of Reclamation, Denver Federal Center, Bldg 56, Rm 2010
West 6th Avenue & South Kipling Street
Denver, Colorado 80225-0007

*Shipping Instructions-**MTFWP samples (preserved in 75% ethanol)**:*

1. Place samples in an airtight container (large freezer bag).

2. Place container in box and add cushioning material such as plastic grocery bags, scrap paper, etc.

3. Fill out Specimen Collection Report for lab (Appendix B) and include with shipment.

4. Samples being sent to MTFWP can be send ground via FedEx to:
Montana Fish, Wildlife and Parks Lab
Attn: Stacy Schmidt
1420 East 6th Ave
Helena, MT 59620

***NOTE: CONSULT THE TABLE IN APPENDIX A TO DETERMINE THE APPROPRIATE LAB TO SEND THE SAMPLE TO FOR EACH INDIVIDUAL WATER.**

Make sure all samples are properly labeled, sealed, and packed with cushioning material before shipment. Keep the "Senders Copy" of the shipping label for reference and tracking purposes.

Notify Beth.Bear@wyo.gov when shipments have been sent to the lab.

Existing Surface Sampling

Existing surfaces or substrates can be sampled for the presence of ZQM or other AIS with relative ease. Preferred locations for surveys are underside of docks, shaded side of permanent structures, underside of buoys, inside crevices of anchors or other surfaces, and natural materials such as rocks, wood, or aquatic plants/animals.

Existing surface sampling will be conducted at *High/Moderate* risk waters in June/July and again in Sept/Oct, and at *Low* risk waters in June/July only.

1. Survey accessible, existing substrate for a distance of 100 to 200 feet, if possible. Keep in mind that if water levels fluctuate, mussels will take time to colonize newly submerged substrate. Additionally, mussels will generally not be located within a foot of the surface as wave action is deleterious.

2. Visually examine all surfaces for attached mussels. Inspect all types of surfaces, focusing on the shaded portion of the substrate.

3. Run a bare hand along the length of the surface (use caution since mussels and other organisms can be sharp). Newly settled mussels ("settlers") will feel like small, individual bumps the size of grains of sand and will be irregularly spaced. It may also feel like sandpaper. Larger juvenile and adult mussels will also be irregularly spaced and vary in size from a sunflower seed to an almond. Mussels can be distinguished from other organisms by the way they rotate and remain attached when lightly pushed.

4. Conduct existing surface surveys concurrent with artificial substrate checks.

5. Document survey results using data sheet provided (Appendix B).

6. If attached mussels or other AIS are found, preserve the specimen according to the specimen collection protocol (Appendix D).

Water Quality Testing

Water quality testing is important for several reasons. Water quality sampling can help to determine whether a water body has the parameters needed to support a mussel population to further assess risk to that water. It may also help to determine optimal locations within a water for populations to colonize. Lastly, sampling prior to and post an introduction can help provide information on changes to water quality as a result of the introduction. Temperature, pH, dissolved oxygen and calcium are the most important parameters to consider when testing

water quality for ZQM. Three forms of water quality testing (water quality meter, titration and secchi disc) will be used to determine these parameters.
Water quality testing will be conducted at *High/Moderate* risk waters in June/July and again in Sept/Oct, and at *Low* risk waters in June/July only.

Water Quality Meter:
Meters use water quality probes to assess water quality parameters such as temperature, pH and dissolved oxygen. Each crew may have a slightly different meter (or meters) for us to collect this information. Please follow the instruction manual for the meter when calibrating and operating the meter.

In the field:

1. Collect water quality information at each location after completing a plankton tow.

2. Using the water quality meter, collect surface pH and temperature at the water surface at each location. Record the results from surface tests on the plankton tow data sheet.

3. When you are able, conduct a water quality profile at the deepest point in the lake (often near the dam at a reservoir). Use the water quality data sheet to collect the information from the profile. Take measurements for Temp, Conductivity (SpC mS/m), Dissolved Oxygen (DO), and pH starting at the surface (1 ft) and collecting results every five feet (5 ft) until you reach the thermocline. The thermocline is the point where water temperatures stabilize. You will typically see a decline in water temperatures (often drastically) until you hit the thermocline, at which point there is little variation in temperature.

Some waters exhibit no thermocline because they are too shallow. In this case you will likely take readings until you hit bottom.

*Be sure meters have been calibrated according to the manual provided with the meter prior to use in the field.

Titration Kit:
This section includes the procedures for determining the following nutrient data at a site using Titration:

Calcium: Mussels need calcium to build their shells. Therefore, water bodies with higher concentrations of calcium are of a much higher risk of becoming infested.

Hardness: Hardness is a measure of calcium and magnesium. Aquatic ecosystems with hard water generally have more biological productivity and produce more biomass.

Alkalinity: The balance of carbon dioxide in the water body. Alkalinity is the amount of biocarbonates and carbonates present. Alkalinity assists with determining elevated metals in a water.

In the field:

1. Collect nutrient information at each location after completing a plankton tow. Record this information on the plankton tow datasheet.

2. Process the sample in the field using the instructions provided with the titration kit. If you're unable to process the sample in the field collect the water sample and process at the office or in the lab. *Be sure to perform the test within 24 hours of when the sample was collected.

Secchi Disc

Secchi Discs are used to determine clarity of the water; the higher the reading, the clearer the water. If time allows, perform a secchi disc reading when you conduct a water quality profile at the deepest point in the lake.

1. Try to plan your sampling so that you can take the Secchi reading mid-day under full sunlight. Be sure measurements in feet are marked on the secchi disc rope (use a permanent marker to mark depth intervals on the rope or on tape attached to the rope).

2. Slowly lower the disc into the water until it disappears from sight and note the depth (ft) (**L**owering Depth) on the water quality datasheet.

3. Lower the disc down another several feet or until it is well out of sight then slowly raise the disc until it is visible again and note this depth (ft) (**R**aising Depth) on the water quality datasheet.

4. The Secchi disc reading (Final Depth) is the average of the two recorded depths (Lowering Depth and Raising Depth). Record the Final Depth (ft) on the water quality datasheet.

The depth at which the Secchi disc disappears or appears may vary from observer to observer and from day to day due to light conditions.

Shoreline Surveys

The following procedures outline sampling and monitoring lentic (shorelines of lakes, reservoirs, and ponds) aquatic environments. The intent is to qualitatively locate and document populations of AIS including mussels, snails, crayfish, and plants.

Shoreline surveys will be conducted:

- Every year at all priority waters (High, Medium, Low) when doing water sampling. Efforts should focus on surveying a new survey location at each water.
- At locations of known AIS. Crews should "resurvey" every year to assess current distribution and abundance of known AIS (quantitative sampling).
- At other waters as time allows (priorities set regionally).

Site selection – Sampling should occur at sites where initial introductions of AIS are likely to occur. These are typically areas of high recreation use such as docks, marinas, launch sites, and river access points. Only wadeable portions of shorelines and streams should be sampled. When selecting a sampling site, consider land ownership, accessibility, and safety.

Adult mussel, clam, or snail surveys:

1. Search for mussels, clams, and snails on large substrates (rocks, logs, plants, roots) in or adjacent to the stream or along the shoreline for 100 ft above and 100 ft below access point, boat dock, launch site, etc. (200 ft. total surveyed). Use the AquaScope underwater viewer to view deep waters and to limit disturbance.

2. Remove any specimens with forceps and place in small vial or brush off specimen from substrate with nylon brush into collection pan with water. Any specimens can then be picked from the pan and placed in a small vial.

3. Look, scrape, or feel on undercut banks and emergent vegetation for snails, clams, or mussels.

4. Sample fine substrate by using a kitchen strainer or kick net to collect substrate and transfer to a collection pan. Pick specimens from sediment with forceps and place in vial. Attempt to collect 15 samples per site.

5. Use one sample bottle per site and fill with 25% water and 75% ETOH. Fill out appropriate label (Appendix B). Complete the label for the sample bottles in pencil on waterproof paper and insert label into the sample bottle. In addition, complete another label in permanent marker and adhere to the outside of the sample bottle. Fill out data sheet regardless of whether any specimens are found. If specimens are found fill out the results section on the sampling datasheet for each species you collect (see species abundance survey below).

6. Send any specimens to: Beth Bear, 528 S. Adams, Laramie, WY 82070

Crayfish surveys:

Crayfish surveys will be conducted at *High/Moderate* risk waters in June/July and again in Sept/Oct, and at *Low* risk waters in June/July only.

1. Visually search for crayfish in slow water habitat. Identify locations and plan on sampling with crayfish traps.

2. Sample each location where crayfish were visually identified, 500 ft above and below the point. If no crayfish are identified, install traps 500 ft above and below areas of likely introduction (access point, boat ramp, etc.).

3. Install at least five crayfish traps along the 1000 ft stretch of shoreline.
    a. Install the traps where there is cover available. Any heavy rock areas, overgrown banks with grass cover make for good placement.
    b. Leave bait (hot dogs, fresh fish and fish guts, cat food, etc.) in each of the traps.

4. Leave the traps overnight for 24 hours but no longer.

5. Use one jar per site and fill with 75% water and 25% ETOH. Use a large jar so that you can fill the vial with multiple specimens if needed. Fill out appropriate label (Appendix B). Complete the label for the sample bottles <u>in pencil</u> on waterproof paper and insert label into the sample bottle. In addition, complete another label <u>in permanent marker</u> and adhere to the outside of the sample bottle. Fill out a data sheet regardless of whether any specimens are found. If specimens are found fill out the results section on the sampling datasheet for each species you collect (see species abundance survey below).

6. Send any specimens to: Beth Bear, 528 S. Adams, Laramie, WY 82070

<u>*Aquatic plants:*</u>
<u>Aquatic plant surveys will be conducted at *High/Moderate* risk waters in June/July and again in Sept/Oct, and at *Low* risk waters in June/July only.</u>

An aquatic vegetation rake can be used when there is a population off shore and it is not feasible to collect a specimen by hand. The rake can be used from the shore or from a boat.

1. Examine the rake line to ensure it is ready to be deployed. Ensure the rake is securely fastened to the tow line. Remove all knots and tangles from the line.

2. Throw the rake by thrusting it from your body, allow extra line to feed out with the net.

3. The rake has float material and will settle out on the surface after it lands just below the waterline. Begin pulling the rake slowly back to you using a hand-over-hand technique.

4. Pull the rake up onto the shore or into the boat.
    Note: Because many aquatic plants can begin new populations with only small fragments of a mature plant, try to limit the number of times you sample a specific

population and the amount of loose plant material that sheds off into the water while you are pulling the rake back toward you.

5. There may be a considerable amount of aquatic vegetation on the rake. Pull enough vegetation off to ensure a satisfactory sample. Be sure to include complete leaves and flowers (if present) and as much of the stem as possible in the sample. Place the sample into a bucket with water from the water body you are sampling until you are able to preserve it.

6. If there is excess vegetation, place the excess vegetation in a location that is high and dry. Do not throw excess plant material back into the water.

7. Fill out a data sheet regardless of whether any specimens are found. If specimens are found fill out the results section on the sampling datasheet for each species you collect (see species abundance survey below).

8. To preserve specimens of known or suspect aquatic invasive plants, place a section of the plant (which should include a section of the stem, leaves (leaflets) and flower if possible) into a plastic bag with a wet paper towel. Fill out the appropriate label (Appendix B). Complete the label for the sample in pencil or pen on waterproof paper taped to the outside of the sample bag. Plant samples should be kept cool either in a cooler with lake water or on ice while in the field.

9. Refrigerate all specimens until they are ready to be shipped.

10. Place individually wrapped and label plant specimens in a padded envelope for shipment. If you suspect Eurasian watermilfoil , keep a portion (if possible) of the plant sample in the office fridge, in case it needs to be sent in for DNA confirmation. Prior to shipment, ensure the samples are properly labeled and the plant specimens are not dry and the paper towel in the Ziploc bag is wet to keep samples moist during shipping. To avoid decomposition in transit, ship samples standard via FedEx on a **Monday** or **Tuesday** to:

    Montana Fish, Wildlife and Parks Lab:
    Attn: Craig McLane
    1420 East 6th Ave
    Helena, MT 59620

Flowing Water Surveys

The following procedures outline sampling and monitoring lotic (streams, rivers, and ditches) aquatic environments. The intent is to locate and document populations of AIS. Surveys should be conducted every year in Sept/Oct with water priorities being set regionally.

Qualitative Survey (locations with no documented AIS)

The objective of the following sampling procedures is to locate and document unknown AIS populations (mussels, snails, clams, crayfish, and vegetation).

Site selection

Sampling should occur at sites where AIS introductions are likely (river access points, launch ramps, areas of disturbance or high recreational use). Only wadeable portions of streams and rivers should be sampled. When selecting a site, consider land ownership, accessibility, and safety.

Reach delineation and sampling procedures

Sampling should occur 100ft above and 100ft below the point of likely AIS introduction (200ft sample reach). Record UTM coordinates using NAD27 and fill out the appropriate data sheet (see Appendix B).

1. Visually search for mussels, claims, and snails on existing substrates (rocks, logs, plants, etc.) within the designated sample reach. Remove any detected specimens with quips or tweezers and place them into a sample vial containing water, when completed rinse specimens and replace water with a 75% EtOH solution.
2. Using kick nets, sample the substrate in at least fifteen (15) locations within the sample reach. For best results place the mouth of the kick net facing into and perpendicular to the flow. Stir up the substrate directly in front of the net with your feet, dislodge rocks and logs etc. Sample multiple habitat types, including undercut banks.
3. Place net contents in a bucket, add water and stir collected material with your hand or the handle of the kick net. Pour the suspended material back through the kick net or sieve (Figure 1). Empty the contents from the net or sieve into a collection pan with a small amount of water. Any specimens (mussels, clams, and snails) can then be picked from the pan, rinsed and placed into the vial containing 75% EtOH (Figure 2). If large amounts of materials are collected, repeat the above process until no additional organic material remains (a magnifying glass may aid in detecting specimens).
4. Use one vial per sample reach, construct a label from rite in the rain paper and in pencil record the location name, date and time, UTM coordinates and insert into the vial. Before shipment, fill out the appropriate label (Appendix B) and attach to the outside of vial.
5. If visible signs of crayfish are present and an adequate crayfish sample is not obtained via kick nets, crayfish traps may be used. Place crayfish specimens in a separate sample jar or vial in a 25% EtOH solution. Fill out the appropriate labels (Appendix B) and attach to outside of sample jar or vial.

6. Mail any specimens collected to Beth Bear, 528 S. Adams, Laramie, WY 82070.
7. Search for any aquatic vegetation within the sample reach. Use the AquaScope to survey deep sections of the sample reach. Collect any detected vegetation by hand or if you cannot safely remove vegetation by hand, use the vegetation rake to collect a sample. See the Aquatic Plant sections for sample preparation, storage and shipping instructions.
8. If the sample reach contains deep pools, perform a horizontal plankton tow in a pool. See the Plankton Tow section for sample preparation, storage and shipping instructions.

Quantitative Survey (locations with a known AIS population)

The objective of the following sampling procedures is to estimate species abundance of known or newly detected AIS populations (mussels, snails, and clams).

Site selection

Quantitative sampling should occur at sites where AIS populations are known or recently detected. Only wadeable portions of streams and rivers should be sampled. When selecting a site, consider land ownership, accessibility, and safety.

Reach delineation

Select a location where AIS were likely introduced. To incorporate habitat variation in meandering stream channels and to account for the potential patchy distribution of targeted AIS, sample reaches should be scaled proportionate to stream size encompassing forty (40) times base flow width or a minimum of 500ft. A minimum of two (2) different fast-water habitats (riffles) and two (2) back-water habitats (eddies) should be sampled if possible. Record UTM coordinates using NAD27 and fill out the appropriate data sheet (Appendix B).

Placement of Surber Sampler (1ft^2 sample area)

After delineation of the sample reach has been completed and habitat types identified, a minimum of six (6) individual surber samples should be collected. To avoid bias and to ensure each square foot of habitat has an equal probability of be selected, select surber locations using random four digit numbers between 0000 and 9999, where the first two numbers represent the percentage of length upstream from the bottom of the sample reach and the second two numbers represent the percentage of stream width from the left bank (Appendix G). Surber samples should be taken at the point where the length and width intersect.

If no fast-water habitats are present divide the sample reach into six (6) equally spaced transects. Carefully delineate and sample a 1ft^2 area using a kick net in each transect alternating left side, center, and right side, with the first transect being randomly selected.

Sampling procedures

1. In randomly selected areas, place the surber sampler with the mouth facing into and perpendicular to the flow. Insure the sampler is firmly embedded into the substrate to minimize lost material which could result in inaccurate population estimations.
2. If it is not possible to collect a sample in the randomly selected area (e.g if it's too deep or there is an obstacle in the way etc.) draw additional random numbers until you selected an area that can be sampled.
3. Working from the upstream edge within the surber frame, carefully dislodge, rub and clean large substrate (rocks, logs etc) directly in front of the net opening. Inspect each object to insure all invertabrates have been cleaned off, and then discard. If a rock is lodged in the stream bed, rub exposed surfaces concentrating on any cracks or indentations (initial disturbance). After all large objects are dislodged and removed, disturb small substrates (sand or gravel) to a depth of approximately two inches by raking and stirring with your hands or a brush. Continue this process until you can no longer detect benthic organisms or organic material being washed into the net (final disturbance).
4. Place surber contents into a bucket add water and stir collected material with your hand or the handle of a kick net. Pour the suspended material back through a kick net or 250 µm sieve. Repeat this process until no additional organic material remains. Transfer material from the sieve to a sample jar with a spoon and wash any remaining material from the sieve into the jar with a spray bottle. Move to the next randomly selected sample location and repeat the outlined procedure
5. Fill out the appropriate label (Appendix B) and store samples in a 75% EtOH solution until the samples are ready to be processed.

Figure 1. AIS techs stirring and pouring collected material into a 250 µm sieve

Figure 2. AIS techs picking specimens from sieve and collection pan for a qualitative assessment

Figure 3. AIS techs using kick nets to qualitatively sample for invasive mussels, clams, snails and plants in the Bear River

Documenting Species Results

Enter results from a shoreline or species abundance survey in the species results section of the shoreline/stream survey datasheet. All species results link to a survey whether it is a historic survey location or new. For each species sampled determine:

- Species surveyed (common name)
- Survey Method: Circle from the following list; visual, plants, surber sample, trapping, or fishing.
- Dimensions of the survey in feet. Dimensions N/S (north south) and Dimensions E/W (east west). Surber sample is a square foot or 1ft x 1ft.
- Number of Individuals: Quantity of individuals within the sample area (based on dimensions above). It may be too difficult to count quantity of individuals at the field site. It may be more effective for you to transfer the sample material into jars and process at the office later. If quantifying individuals at the office, transfer the material from the sampler into jars and preserve at 75% EtOH.
- Density: Estimate density low, medium, high.
  Mollusks: High (H)=>20 shells, Medium (M)= 9-20 shells, Low (L)= 4-8 shells and Scattered (S)= 1-3 shells.
- Depth: Depth of the area where the majority of the species was collected. Average the depth if the survey area covers a large area with multiple depths.
- Life Stage: assess the life stage of the majority of the specimens.
  - Animals – Egg, Larvae, Juvenile, Adult, etc.
  - Plants – Seedling, Fragment, Flowering, Seed Set, etc.
- Controlled: This will likely be edited in the database later. This is related to whether the population has been controlled and when.

Equipment Disinfection

Equipment disinfection (decontamination) is critical to avoid potentially transporting AIS between sampling sites. Decontamination should occur at least 200 feet away from a water body and avoid discarding decontamination solution in the field. Decontamination solution should be discarded into a drain that goes to a wastewater treatment facility or in a gravel area where discharge water will evaporate.

Plankton Net and Cod End Decontamination:

1. Thoroughly rinse net and cod end with clean water. Remove all debris, mud, plant material, etc.

2. Fully submerge the net and cod end in 5% vinegar solution. Ideal soak time is overnight and minimum soak time is one hour.

3. After decontamination, rinse net and cod end with clean water.

Water Quality Meter Decontamination:

1. Thoroughly rinse cord, guard, probe, and storage cup with clean water. Remove all debris, mud, plant material, etc.

2. Submerge cord, guard, and storage cup in decontamination solution for 15 minutes (DO NOT submerge sensors/probes or transmitter. Probes should only be cleaned with water).

3. After decontamination, rinse cord, guard, and storage cup with fresh water. Fill storage cup with 0.5 inch of fresh DI or tap water to prevent the meter probe from drying out.

Other Gear Decontamination:

1. Thoroughly rinse gear with clean water. Remove all debris, mud, plant material, etc.

2. Submerge gear in decontamination solution for at least 15 minutes.

3. After decontamination, rinse gear with fresh water.

Watercraft Decontamination:

Inspect boats after use in the water after each visit. Drain all water from the boat (pull plugs), clean all mud, debris, and plants from the boat, and allow to dry thoroughly before using in another water. If possible, use the decontamination unit at the Regional Office to decontaminate the boat after each use.

References

Barbour, M.T., J. Gerritsen, B.D. Snyder, and J.B. Stribling. 1999. Rapid Bioassessment Protocols for use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish, Second Edition. EPA 841-B-99-002. U.S. Environmental Protection Agency; Office of Water; Washington D.C.

Bear, E. A. 2009. Assessing the risk of a mussel invasion in Wyoming waters. Wyoming Game and Fish Department Administrative Report. Cheyenne, WY. Available at: http://gfi.state.wy.us/Fish/Management/Reports/2009_Bear_Assessing%20the%20Risk%20of%20a%20Mussel%20Invasion%20in%20Wyoming%20Waters.pdf

Bureau of Reclamation (BOR). 2009. Reclamation plankton sample collection protocols for Dreissenid veliger early detection monitoring. U.S. Department of the Interior report.

Colorado Division of Wildlife (CDOW). 2009. State Aquatic Nuisance Species Program Sampling and Monitoring Procedures Manual. Colorado Department of Natural Resources report.

Montana Fish, Wildlife and Parks (MTFWP). 2010. Dreissenid veliger lab protocol.

Appendix A: Mussel sampling locations for each water by region.

CASPER			***Sampling Locations***				***Sampling Frequency***			
Water Name	**Priority/Risk**	**Lab**	**Ramps**	**Inlets**	**Outlets/Dam**	**Total Locations**	**Times sample/yr**	**Early samples (July)**	**Late samples (Sept)**	**Total samples**
Alcova Reservoir	High/Mod	BOR & MTFWP	3	1	1	5	**2**	5	5	10
Glendo Reservoir	High/Mod	BOR & MTFWP	3	1	1	5	**2**	5	5	10
Guernsey Reservoir	High/Mod	BOR & MTFWP	3	1	1	5	**2**	5	5	10
Pathfinder Reservoir	High/Mod	BOR & MTFWP	3	1	1	5	**2**	5	5	10
Seminoe Reservoir	High/Mod	BOR & MTFWP	3	1	1	5	**2**	5	5	10

CODY/LANDER			***Sampling Locations***				***Sampling Frequency***			
Water Name	**Priority/Risk**	**Lab**	**Ramps**	**Inlets**	**Outlets/Dam**	**Total Locations**	**Times sample/yr**	**Early samples (July)**	**Late samples (Sept)**	**Total samples**
Beartooth Lake	No Rank	MTFWP	1	1	1	3	1	3	0	3
Beck Lake	Low	MTFWP	1	1	1	3	1	3	0	3
Big Horn Lake	High/Mod	BOR & MTFWP	3	1	1	5	**2**	5	5	10
Buffalo Bill Reservoir	High/Mod	BOR & MTFWP	3	1	1	5	**2**	5	5	10
Deaver Reservoir	Low	MTFWP	1	1	1	3	1	3	0	3
East Newton Lake	Low	MTFWP	1	1	1	3	1	3	0	3
Harrington Reservoir	Low	MTFWP	1	1	1	3	1	3	0	3
Island Lake	No Rank	MTFWP	1	1	1	3	1	3	0	3
Lewis Lake (YNP)	No Rank	MTFWP	2	1	1	3	**2**	4	4	8
Meadowlark Lake	Low	MTFWP	1	1	1	3	1	3	0	3
Upper Sunshine Res.	Low	MTFWP	1	1	1	3	1	3	0	3
Wardell Reservoir	Low	MTFWP	1	1	1	3	1	3	0	3
West Newton Lake	Low	MTFWP	1	1	1	3	1	3	0	3
Boysen Reservoir	High/Mod	BOR & MTFWP	1	1	1	5	**2**	5	5	10
Ocean Lake	Low	MTFWP	3	1	1	5	1	5	0	5
Pilot Butte Reservoir	Low	MTFWP	1	1	1	3	1	3	0	3
Yellowstone Lake	No Rank	MTFWP	3	1	1	5	**2**	5	5	10

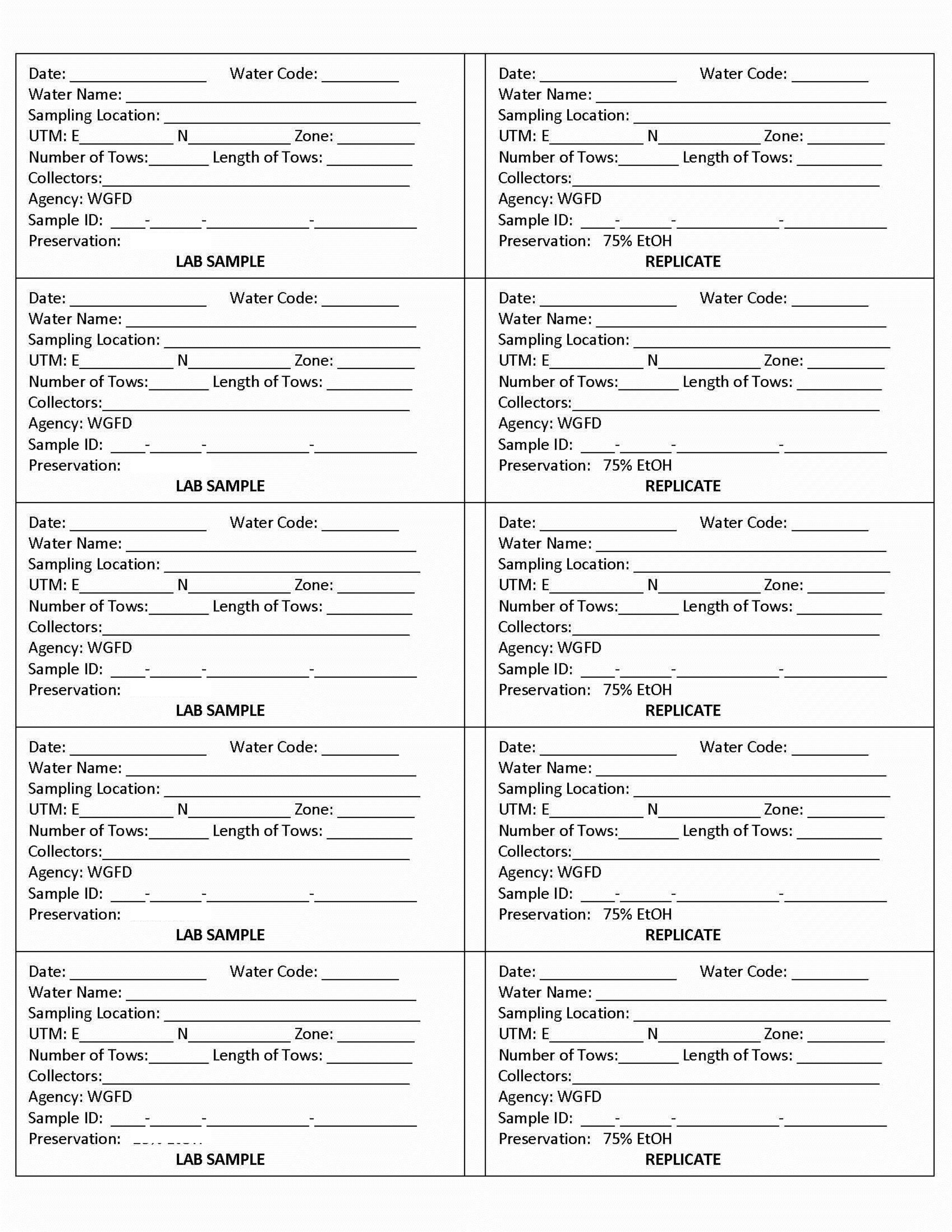
GREEN RIVER			*Sampling Locations*				*Sampling Frequency*			
Water Name	**Priority/Risk**	**Lab**	**Ramps**	**Inlets**	**Outlets/Dam**	**Total Locations**	**Times sample/yr**	**Early samples (July)**	**Late samples (Sept)**	**Total samples**
Big Sandy Res.	Low	MTFWP	1	1	1	3	1	3	0	3
Flaming Gorge Res.	High/Mod	BOR & MTFWP	3	1	1	5	**2**	5	5	10
Fontenelle Res.	High/Mod	BOR & MTFWP	3	1	1	5	**2**	5	5	10
High Savery Res.	Low	MTFWP	1	1	1	3	1	3	0	3
Jim Bridger Pond	Low	MTFWP	1	1	1	3	1	3	0	3
Meeks Cabin Res.	Low	MTFWP	1	1	1	3	1	3	0	3
Naughton Plant Pond	Low	MTFWP	1	1	1	3	1	3	0	3
Sulphur Creek Res.	High/Mod	MTFWP	1	1	1	3	**2**	3	3	6
Viva Naughton Res.	High/Mod	MTFWP	2	1	1	4	**2**	4	4	8
Woodruff Narrows	Low	MTFWP	1	1	1	3	1	3	0	3

JACKSON/PINEDALE			*Sampling Locations*				*Sampling Frequency*			
Water Name	**Priority/Risk**	**Lab**	**Ramps**	**Inlets**	**Outlets/Dam**	**Total Locations**	**Times sample/yr**	**Early samples (July)**	**Late samples (Sept)**	**Total samples**
Jackson Lake	High/Mod	BOR & MTFWP	3	1	1	5	**2**	5	5	10
Lower Slide Lake	Low	MTFWP	1	1	1	3	1	3	0	3
Palisades Res.	High/Mod	MTFWP	2	1	1	4	**2**	4	4	8
Boulder Lake	Low	MTFWP	1	1	1	3	1	3	0	3
Burnt Lake	Low	MTFWP	1	1	1	3	1	3	0	3
Fremont Lake	High/Mod	MTFWP	2	1	1	4	**2**	4	4	8
Halfmoon Lake	Low	MTFWP	1	1	1	3	1	3	0	3
Jenny Lake	No Rank	MTFWP	1	1	1	3	1	3	0	3
Lower Green River Lake	Low	MTFWP	1	1	1	3	1	3	0	3
Middle Piney Lake	Low	MTFWP	1	1	1	3	1	3	0	3
New Fork Lake	Low	MTFWP	1	1	1	3	1	3	0	3
Soda Lake	Low	MTFWP	1	1	1	3	1	3	0	3
String Lake	No Rank	MTFWP	1	1	1	3	1	3	0	3
Willow Lake	Low	MTFWP	1	1	1	3	1	3	0	3

LARAMIE			*Sampling Locations*				*Sampling Frequency*			
Water Name	**Priority/Risk**	**Lab**	**Ramps**	**Inlets**	**Outlets/Dam**	**Total Locations**	**Times sample/yr**	**Early samples (July)**	**Late samples (Sept)**	**Total samples**
Crystal Res.	Low	MTFWP	1	1	1	3	1	3	0	3
Gelatt Lake	No Rank	MTFWP	1	1	1	3	1	3	0	3
Granite Res.	High/Mod	MTFWP	1	1	1	3	**2**	3	3	6
Grayrocks Res.	High/Mod	MTFWP	2	1	1	4	**2**	4	4	8
Hawk Springs Res.	High/Mod	MTFWP	1	1	1	3	**2**	3	3	6
Hog Park Res.	Low	MTFWP	1	1	1	3	1	3	0	3
Lake Hattie	No Rank	MTFWP	1	1	1	3	1	3	0	3
Lake Owen	Low	MTFWP	1	1	1	3	1	3	0	3
North Crow Res.	No Rank	MTFWP	0	2	1	3	1	3	0	3
Rob Roy Res.	Low	MTFWP	1	1	1	3	1	3	0	3
Saratoga Lake	Low	MTFWP	1	1	1	3	1	3	0	3
Wheatland #1 Res.	Low	MTFWP	2	1	1	4	1	4	0	4

SHERIDAN			*Sampling Locations*				*Sampling Frequency*			
Water Name	**Priority/Risk**	**Lab**	**Ramps**	**Inlets**	**Outlets/Dam**	**Total Locations**	**Times sample/yr**	**Early samples (July)**	**Late samples (Sept)**	**Total samples**
Keyhole Res.	High/Mod	BOR & MTFWP	5	1	1	5	**2**	5	5	10
Lake DeSmet	High/Mod	MTFWP	2	1	1	4	**2**	4	4	8

Appendix B: Labels and datasheets for plankton tow sampling.



Date: ____________ Water Code: ________
Water Name: ______________________________
Sampling Location: ____________________________
UTM: E__________ N__________ Zone: ________
Number of Tows:______ Length of Tows: _________
Collectors:________________________________
Agency: WGFD
Sample ID: ____-______-___________-__________
Preservation:
LAB SAMPLE

Date: ____________ Water Code: ________
Water Name: ______________________________
Sampling Location: ____________________________
UTM: E__________ N__________ Zone: ________
Number of Tows:______ Length of Tows: _________
Collectors:________________________________
Agency: WGFD
Sample ID: ____-______-___________-__________
Preservation: 75% EtOH
REPLICATE

Date: ____________ Water Code: ________
Water Name: ______________________________
Sampling Location: ____________________________
UTM: E__________ N__________ Zone: ________
Number of Tows:______ Length of Tows: _________
Collectors:________________________________
Agency: WGFD
Sample ID: ____-______-___________-__________
Preservation:
LAB SAMPLE

Date: ____________ Water Code: ________
Water Name: ______________________________
Sampling Location: ____________________________
UTM: E__________ N__________ Zone: ________
Number of Tows:______ Length of Tows: _________
Collectors:________________________________
Agency: WGFD
Sample ID: ____-______-___________-__________
Preservation: 75% EtOH
REPLICATE

Date: ____________ Water Code: ________
Water Name: ______________________________
Sampling Location: ____________________________
UTM: E__________ N__________ Zone: ________
Number of Tows:______ Length of Tows: _________
Collectors:________________________________
Agency: WGFD
Sample ID: ____-______-___________-__________
Preservation:
LAB SAMPLE

Date: ____________ Water Code: ________
Water Name: ______________________________
Sampling Location: ____________________________
UTM: E__________ N__________ Zone: ________
Number of Tows:______ Length of Tows: _________
Collectors:________________________________
Agency: WGFD
Sample ID: ____-______-___________-__________
Preservation: 75% EtOH
REPLICATE

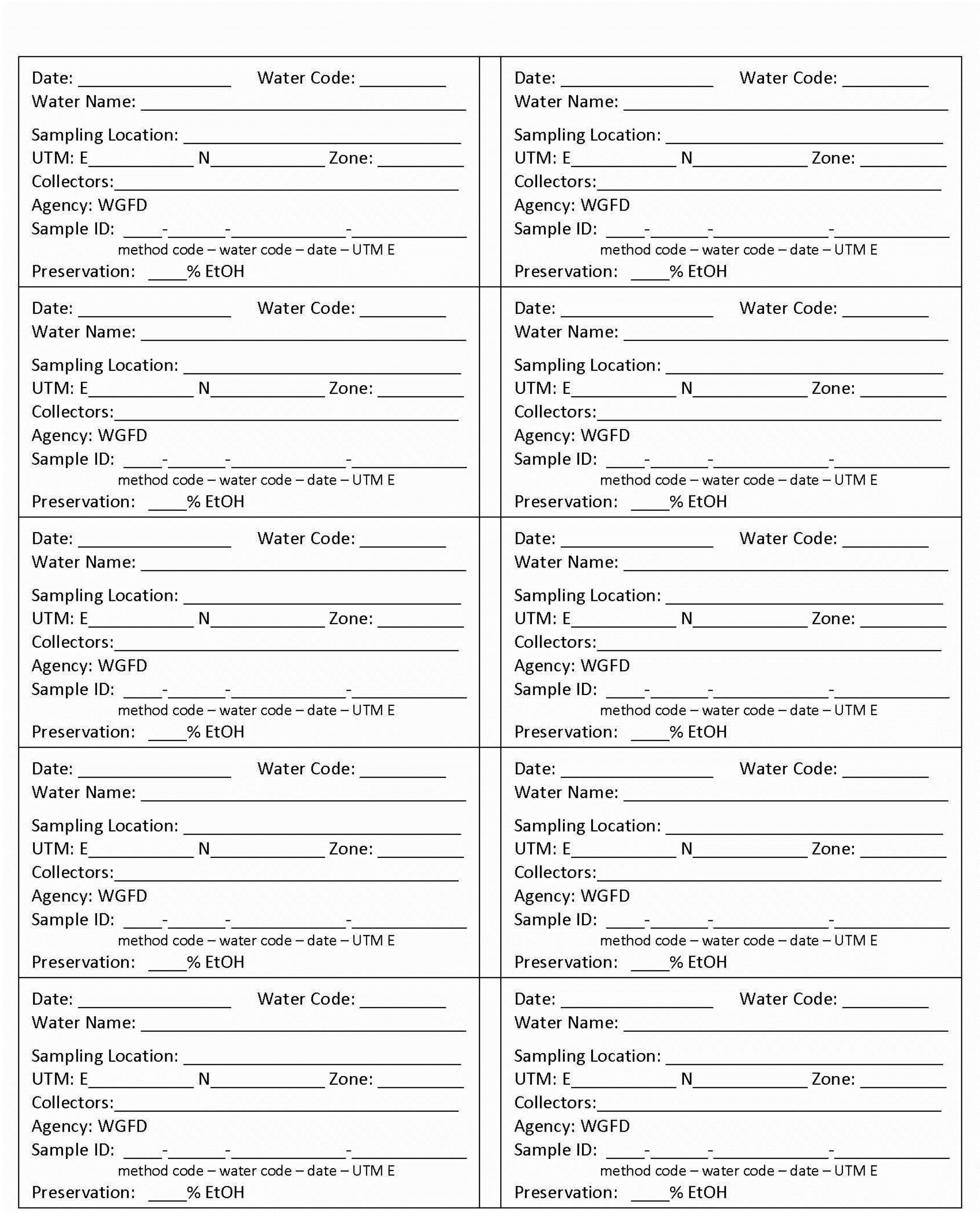
Date: ____________ Water Code: ________
Water Name: ______________________________
Sampling Location: ____________________________
UTM: E__________ N__________ Zone: ________
Number of Tows:______ Length of Tows: _________
Collectors:________________________________
Agency: WGFD
Sample ID: ____-______-___________-__________
Preservation:
LAB SAMPLE

Date: ____________ Water Code: ________
Water Name: ______________________________
Sampling Location: ____________________________
UTM: E__________ N__________ Zone: ________
Number of Tows:______ Length of Tows: _________
Collectors:________________________________
Agency: WGFD
Sample ID: ____-______-___________-__________
Preservation: 75% EtOH
REPLICATE

Date: ____________ Water Code: ________
Water Name: ______________________________
Sampling Location: ____________________________
UTM: E__________ N__________ Zone: ________
Number of Tows:______ Length of Tows: _________
Collectors:________________________________
Agency: WGFD
Sample ID: ____-______-___________-__________
Preservation:
LAB SAMPLE

Date: ____________ Water Code: ________
Water Name: ______________________________
Sampling Location: ____________________________
UTM: E__________ N__________ Zone: ________
Number of Tows:______ Length of Tows: _________
Collectors:________________________________
Agency: WGFD
Sample ID: ____-______-___________-__________
Preservation: 75% EtOH
REPLICATE

Labels to include inside sample bottles (or bags) with ethanol and taped to outside of sample bottles (or bags) when collecting samples from <u>existing substrate, mussel or snail, crayfish, or plant surveys</u>.



Date: ____________ Water Code: _______
Water Name: __________________________
Sampling Location: ______________________
UTM: E________ N_________ Zone: _______
Collectors:___________________________
Agency: WGFD
Sample ID: ___-_____-_________-_________
method code – water code – date – UTM E
Preservation: ___% EtOH

Date: ____________ Water Code: _______
Water Name: __________________________
Sampling Location: ______________________
UTM: E________ N_________ Zone: _______
Collectors:___________________________
Agency: WGFD
Sample ID: ___-_____-_________-_________
method code – water code – date – UTM E
Preservation: ___% EtOH

Date: ____________ Water Code: _______
Water Name: __________________________
Sampling Location: ______________________
UTM: E________ N_________ Zone: _______
Collectors:___________________________
Agency: WGFD
Sample ID: ___-_____-_________-_________
method code – water code – date – UTM E
Preservation: ___% EtOH

Date: ____________ Water Code: _______
Water Name: __________________________
Sampling Location: ______________________
UTM: E________ N_________ Zone: _______
Collectors:___________________________
Agency: WGFD
Sample ID: ___-_____-_________-_________
method code – water code – date – UTM E
Preservation: ___% EtOH

Date: ____________ Water Code: _______
Water Name: __________________________
Sampling Location: ______________________
UTM: E________ N_________ Zone: _______
Collectors:___________________________
Agency: WGFD
Sample ID: ___-_____-_________-_________
method code – water code – date – UTM E
Preservation: ___% EtOH

Date: ____________ Water Code: _______
Water Name: __________________________
Sampling Location: ______________________
UTM: E________ N_________ Zone: _______
Collectors:___________________________
Agency: WGFD
Sample ID: ___-_____-_________-_________
method code – water code – date – UTM E
Preservation: ___% EtOH

Date: ____________ Water Code: _______
Water Name: __________________________
Sampling Location: ______________________
UTM: E________ N_________ Zone: _______
Collectors:___________________________
Agency: WGFD
Sample ID: ___-_____-_________-_________
method code – water code – date – UTM E
Preservation: ___% EtOH

Date: ____________ Water Code: _______
Water Name: __________________________
Sampling Location: ______________________
UTM: E________ N_________ Zone: _______
Collectors:___________________________
Agency: WGFD
Sample ID: ___-_____-_________-_________
method code – water code – date – UTM E
Preservation: ___% EtOH

Date: ____________ Water Code: _______
Water Name: __________________________
Sampling Location: ______________________
UTM: E________ N_________ Zone: _______
Collectors:___________________________
Agency: WGFD
Sample ID: ___-_____-_________-_________
method code – water code – date – UTM E
Preservation: ___% EtOH

Date: ____________ Water Code: _______
Water Name: __________________________
Sampling Location: ______________________
UTM: E________ N_________ Zone: _______
Collectors:___________________________
Agency: WGFD
Sample ID: ___-_____-_________-_________
method code – water code – date – UTM E
Preservation: ___% EtOH

WGF Aquatic Invasive Species--Plankton Tow Data Sheet

Water Name:______________________ Water Code:___________ GF Region:______________________

UTM coordinates should be collected using datum NAD27

Sampling Location:			UTM Coordinates			Unique ID				
			E _ _ _ _ _ _ _ _ N _ _ _ _ _ _ _ _ _ _			1 - _ _ _ _ - _ _ _ _ _ _ _ _ - _ _ _ _ _ _ _ _				
Location Description:							Collectors:			Comments:
Date & Time	Tow Type	# Tows	Depth/Length (ft)	Tow Total	Net Opening Area	Temp °F	Calcium	Hardness	pH	
	V H									
Sampling Location:			UTM Coordinates			Unique ID				
			E _ _ _ _ _ _ _ _ N _ _ _ _ _ _ _ _ _ _			1 - _ _ _ _ - _ _ _ _ _ _ _ _ - _ _ _ _ _ _ _ _				
Location Description:							Collectors:			Comments:
Date & Time	Tow Type	# Tows	Depth/Length (ft)	Tow Total	Net Opening Area	Temp °F	Calcium	Hardness	pH	
	V H									
Sampling Location:			UTM Coordinates			Unique ID				
			E _ _ _ _ _ _ _ _ N _ _ _ _ _ _ _ _ _ _			1 - _ _ _ _ - _ _ _ _ _ _ _ _ - _ _ _ _ _ _ _ _				
Location Description:							Collectors:			Comments:
Date & Time	Tow Type	# Tows	Depth/Length (ft)	Tow Total	Net Opening Area	Temp °F	Calcium	Hardness	pH	
	V H									
Sampling Location:			UTM Coordinates			Unique ID				
			E _ _ _ _ _ _ _ _ N _ _ _ _ _ _ _ _ _ _			1 - _ _ _ _ - _ _ _ _ _ _ _ _ - _ _ _ _ _ _ _ _				
Location Description:							Collectors:			Comments:
Date & Time	Tow Type	# Tows	Depth/Length (ft)	Tow Total	Net Opening Area	Temp °F	Calcium	Hardness	pH	
	V H									
Sampling Location:			UTM Coordinates			Unique ID				
			E _ _ _ _ _ _ _ _ N _ _ _ _ _ _ _ _ _ _			1 - _ _ _ _ - _ _ _ _ _ _ _ _ - _ _ _ _ _ _ _ _				
Location Description:							Collectors:			Comments:
Date & Time	Tow Type	# Tows	Depth/Length (ft)	Tow Total	Net Opening Area	Temp °F	Calcium	Hardness	pH	
	V H									

Date entered into database: ____________________ DB Initials: _______________

WGF Aquatic Invasive Species--Shoreline/Stream Survey Data Sheet

Water Name:____________________ Water Code:____________ GF Region:______________________

UTM coordinates should be collected using datum NAD27

Sampling Location:	UTM Coordinates E __ __ __ __ __ __ __ N __ __ __ __ __ __ __ __	Unique ID 4 - __ __ __ - __ __ __ __ __ __ __ __ - __ __ __ __ __ __ __ __
Date & Time	Location Description:	Collectors:
Dominant Vegetation OR Substrate (Boulder, Cobble, Gravel, Sand, Silt):	Species Collected:	
Comments:		

Provide ***results*** *for any species listed under species collected above:*

Unique ID (if different from shoreline survey ID above): 4 - __ __ __ - __ __ __ __ __ __ __ __ - __ __ __ __ __ __ __ __				Date & Time (if different from above):	
Species Common Name:			Survey Method (circle): Visual, Plants, Surber Sample, Trapping, Fishing		
Dim E/W (ft)	Dim N/S (ft)	# of Individuals (if known):	Density (circle) H, M, L, S	Depth (ft)	Life Stage (of the majority)
Controlled (Y/N) Control Dates:			Collectors:	Comments:	

Unique ID (if different from shoreline survey ID above): 4 - __ __ __ - __ __ __ __ __ __ __ __ - __ __ __ __ __ __ __ __				Date & Time (if different from above):	
Species Common Name:			Survey Method (circle): Visual, Plants, Surber Sample, Trapping, Fishing		
Dim E/W (ft)	Dim N/S (ft)	# of Individuals (if known):	Density (circle) H, M, L, S	Depth (ft)	Life Stage (of the majority)
Controlled (Y/N) Control Dates:			Collectors:	Comments:	

Unique ID (if different from shoreline survey ID above): 4 - __ __ __ - __ __ __ __ __ __ __ __ - __ __ __ __ __ __ __ __				Date & Time (if different from above):	
Species Common Name:			Survey Method (circle): Visual, Plants, Surber Sample, Trapping, Fishing		
Dim E/W (ft)	Dim N/S (ft)	# of Individuals (if known):	Density (circle) H, M, L, S	Depth (ft)	Life Stage (of the majority)
Controlled (Y/N) Control Dates:			Collectors:	Comments:	

Add additional results pages if needed but be sure to note Survey Location Unique ID so datasheets can be matched to the correct location.

Date entered into database: ______________________ DB Initials: ________________

WGF AIS Water Quality Data Sheet (Hydrolab or Water Quality Meter)

Date/Time: Water Code: Location Description:

Water Name: GPS Coordinates E:_ _ _ _ _ _

Total Depth (F): N: _ _ _ _ _ _ _ _ Sample ID:_-_ _ _-_ _ _ _ _ _ _-_ _ _ _ _ _

Hydrolab

Depth (ft):	1	5	10	15	20	25	30	***35***	***40***	***45***	***50***	***55***	***60***	***65***	***70***	***75***	***80***	***Depth (ft):***
Temp °F																		Temp °F
SpC mS/m																		SpC mS/m
DO mg/L																		DO mg/L
pH																		pH
Depth (ft):	85	90	95	100	105	110	115	***120***	***125***	***130***	***135***	***140***	***145***	***150***	***155***	***160***	***165***	***Depth (ft):***
Temp °F																		Temp °F
SpC mS/m																		SpC mS/m
DO mg/L																		DO mg/L
pH																		pH
Depth (ft):																		***Depth (ft):***
Temp °F																		Temp °F
SpC mS/m																		SpC mS/m
DO mg/L																		DO mg/L
pH																		pH

Comments:

Secchi Disk

Lowering Depth (ft):

Raising Depth (ft):

Final Depth (ft):

Date entered into database _____ Initials:_____

Lab Collection Report Forms

MONTANA FISH, WILDLIFE & PARKS LAB				
Water Name:		Date of Collection:	Collector(s):	
Sample ID:	Location:	UTM E: UTM N:	Sample Depth	___%EtOH
Sample ID:	Location:	UTM E: UTM N:	Sample Depth	___%EtOH
Sample ID:	Location:	UTM E: UTM N:	Sample Depth	___%EtOH
Sample ID:	Location:	UTM E: UTM N:	Sample Depth	___%EtOH
Sample ID:	Location:	UTM E: UTM N:	Sample Depth	___%EtOH
Agency: Wyoming Game and Fish Dept.		Contact: Beth Bear Aquatic Invasive Species Coordinator 307-745-5180 x256		
Ship samples with collection report form to: Attn: Eileen Ryce/Stacy Schmidt 1420 East 6th Ave Helena, MT 59620				

MONTANA FISH, WILDLIFE & PARKS LAB				
Water Name:		Date of Collection:	Collector(s):	
Sample ID:	Location:	UTM E: UTM N:	Sample Depth	___%EtOH
Sample ID:	Location:	UTM E: UTM N:	Sample Depth	___%EtOH
Sample ID:	Location:	UTM E: UTM N:	Sample Depth	___%EtOH
Sample ID:	Location:	UTM E: UTM N:	Sample Depth	___%EtOH
Sample ID:	Location:	UTM E: UTM N:	Sample Depth	___%EtOH
Agency: Wyoming Game and Fish Dept.		Contact: Beth Bear Aquatic Invasive Species Coordinator 307-745-5180 x256		
Ship samples with collection report form to: Attn: Eileen Ryce/Stacy Schmidt 1420 East 6th Ave Helena, MT 59620				

BUREAU OF RECLAMATION LAB				
Water Name:		Date of Collection:	Collector(s):	
Sample ID:	Location:	UTM E: UTM N:	Sample Depth	___%EtOH
Sample ID:	Location:	UTM E: UTM N:	Sample Depth	___%EtOH
Sample ID:	Location:	UTM E: UTM N:	Sample Depth	___%EtOH
Sample ID:	Location:	UTM E: UTM N:	Sample Depth	___%EtOH
Sample ID:	Location:	UTM E: UTM N:	Sample Depth	___%EtOH
Agency: Wyoming Game and Fish Dept.		Contact: Beth Bear Aquatic Invasive Species Coordinator 307-745-5180 x256		
Ship samples with collection report form to: Attn: Denise M. Hosler Environmental Applications and Research Group Bureau of Reclamation, Denver Federal Center, Bldg 56, Rm 2010 West 6th Avenue & South Kipling Street Denver, Colorado 80225-0007				

BUREAU OF RECLAMATION LAB				
Water Name:		Date of Collection:	Collector(s):	
Sample ID:	Location:	UTM E: UTM N:	Sample Depth	___%EtOH
Sample ID:	Location:	UTM E: UTM N:	Sample Depth	___%EtOH
Sample ID:	Location:	UTM E: UTM N:	Sample Depth	___%EtOH
Sample ID:	Location:	UTM E: UTM N:	Sample Depth	___%EtOH
Sample ID:	Location:	UTM E: UTM N:	Sample Depth	___%EtOH
Agency: Wyoming Game and Fish Dept.		Contact: Beth Bear Aquatic Invasive Species Coordinator 307-745-5180 x256		
Ship samples with collection report form to: Attn: Denise M. Hosler Environmental Applications and Research Group Bureau of Reclamation, Denver Federal Center, Bldg 56, Rm 2010 West 6th Avenue & South Kipling Street Denver, Colorado 80225-0007				

Appendix C: Equipment list for AIS sampling

- Sampling and Monitoring Manual
- Clipboard with datasheets: Plankton Tow, Substrate, Water Quality, Stream/Shoreline
- Maps for each lake/reservoir
- Water code list
- Pencil bag with permanent markers, pencils, pens, highlighters
- GPS unit
- Digital camera
- 100% EtOH
- Plankton Tow Surveys
    - ☐ Plankton tow net with cod end and deployment line
    - ☐ Sample bottles and labels
    - ☐ Electrical tape (for sealing bottle lids)
    - ☐ Re-sealable plastic bags
    - ☐ Baking soda (for buffering plankton tow samples)
    - ☐ Cooler with blue ice packs
    - ☐ Styrofoam coolers (for shipping samples to lab)

☐ Artificial Substrates
    - ☐ Substrate plates
    - ☐ Hard plastic material (black CDs)
    - ☐ Dark polypropylene rope
    - ☐ Zip ties
    - ☐ Anchor weights (rocks, cement cylinders, hand weights, etc.)
    - ☐ Buoys
    - ☐ Labels

☐ Water Quality Testing
    - ☐ Water quality meter and accessories
    - ☐ Sample bottle
    - ☐ Titration kit

☐ Shoreline/Stream Surveys
    - ☐ Rubber-soled hip waders or knee-high rubber boots
    - ☐ Latex gloves
    - ☐ Sample bottles
    - ☐ Sorting tray
    - ☐ Kitchen strainer
    - ☐ Stiff nylon brush
    - ☐ Small garden trowel
    - ☐ Vegetation rake
    - ☐ Underwater viewer scope
    - ☐ Crayfish traps
    - ☐ Disposable wooden sticks / popsicle sticks (for scraping samples from rocks)

☐ Equipment Disinfection
    - ☐ De-ionized or Distilled water
    - ☐ Spray bottles (2)
    - ☐ Vinegar solution (5%)
    - ☐ 5 gallon bucket

Appendix D: Specimen collection protocol for adult or juvenile mussels, clams, and snails. Note: Specimen collection protocols for ZQM plankton tows and aquatic plants are described elsewhere in this manual.

Specimen Collection:

1. Take digital photos of known or suspect AIS specimens. Make sure the appropriate GPS information and field datasheet are complete.

2. Place specimens in an appropriately sized bottle. Preserve specimens with **75% EtOH.**

3. It is preferable to use pre-mixed 75% EtOH. If you need to mix the EtOH in the field, begin by filling the sample bottle with water so that all specimens are covered. Mark the water level on the sample bottle with permanent ink. Add an appropriate volume of ethanol to get a 75% final solution in the sample bottle. Mark the level of added alcohol on the sample bottle with permanent ink.

4. Complete the label for the sample bottles in pencil on waterproof paper and insert label into the sample bottle. In addition, complete another label in permanent marker and adhere to the outside of the sample bottle. Label information should include sample ID, date, location, UTM (easting and northing), collector, and water name and code.

7. Secure the specimen bottle lid with electrical tape to help prevent leakage. Place the bottle in a re-sealable plastic bag.

8. Specimens in 75% EtOH do not need to be refrigerated.

If suspected AIS are found, contact the WGF Aquatic Invasive Species Coordinator immediately:
Beth Bear, Aquatic Invasive Species Coordinator at Beth.Bear@wyo.gov
OR
ReportAIS@wyo.gov

Shipping Instructions:

1. Complete specimen collect sheet and send with sample package.
2. Ship specimens to:

Beth Bear
528 S. Adams
Laramie, WY 82070

Appendix E: Classification of Waters for Aquatic Invasive Species

Testing Protocol

Sampling of Wyoming waters is conducted annually in accordance with the "Wyoming Game and Fish Department Aquatic Invasive Species Sampling and Monitoring Manual". To determine whether Wyoming waters contain evidence of aquatic invasive species (AIS), specimens of adult or juvenile crayfish, snails, mollusks, plants, etc. are collected during routine sampling and any specimen suspected of being AIS must be positively identified by at least two independent experts.

For dreissenid (zebra and quagga) mussels, identification of an adult of juvenile specimen by two independent experts and DNA confirmation of the sample, or identification of the larval form and verification using cross-polarized light microscopy and confirmation by DNA analysis is required.

Verification Definitions

- **Verification** – the scientifically-based process to confirm the presence of AIS.
- **Detection, detect or detected** – the verified presence of AIS.
- **Minimum to verify detection:** Two independent results from the same sample, using scientifically accepted techniques.

Definitions of water status

- **Unknown/Not tested:** A water body that has not been sampled for aquatic invasive species.

- **Negative:** A water body at which sampling is ongoing and nothing has been detected (or nothing has been detected within the time frames for de-listing).

- **Inconclusive (temporary status):** A water body that has not met the minimum criteria for detection (i.e., evidence of mussel veligers is detected via microscopy but cannot be confirmed by DNA analysis)**Additional sampling of this water will be conducted to determine whether the water body is classified as negative or suspect.*

- **Suspect:** A water body that has met the minimum criteria for detection (i.e., one sample verified by visual confirmation and confirmed with DNA analysis).

- **Positive:** Multiple (2 or more) subsequent sampling events that meet the minimum criteria for detection (i.e., samples from two different sampling events are verified by both visual identification and DNA confirmation).

- **Infested:** A water body that has an established (recruiting or reproducing) population of AIS.

De-listing a Water Body for AIS:

- **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.

Appendix F: List of water codes for use when completing sampling and monitoring forms.

Water Name	Water Code
Alcova Reservoir	ACR
Beartooth Lake	BTL
Beck Lake	BKL
Big Horn Lake	BHL
Big Sandy Reservoir	BSR
Boulder Lake	BDL
Boysen Reservoir	BYR
Buffalo Bill Reservoir	BBR
Burnt Lake	BNL
Crystal Reservoir	CYR
Deaver Reservoir	DVR
East Newton Lake	ENL
Flaming Gorge Reservoir	FGR
Fontenelle Reservoir	FNR
Fremont Lake	FML
Gelatt Lake	GEL
Glendo Reservoir	GLR
Granite Reservoir	GRR
Grayrocks Reservoir	GYR
Green River Lake	LGR
Guernsey Reservoir	GUR
Halfmoon Lake	HML
Harrington Reservoir	HRR
Hawk Springs Reservoir	HWS
High Savery Reservoir	HSR
Hog Park Reservoir	HPR
Island Lake	ISL
Jackson Lake	JKL
Jenny Lake	JNY
Jim Bridger Pond	JBP
Keyhole Reservoir	KHR
Lake DeSmet	LDM
Lake Hattie	HAT

Water Name	Water Code
Lake Owen	LOW
Lower Shoshone	LSR
Lower Slide Lake	LSL
Meadowlark Lake	MWL
Meeks Cabin Reservoir	MCR
Middle Piney Lake	MPL
Naughton Plant Pond	NPP
New Fork Lake	LNF
North Crow Reservoir	NCR
Northfork Shoshone	NFS
Ocean Lake	OCL
Palisades Reservoir	PSR
Pathfinder Reservoir	PFR
Pilot Butte Reservoir	PBR
Rob Roy Reservoir	RRR
Saratoga Lake	STL
Seminoe Reservoir	SMR
Snake River Jackson	SKJ
Snake River Palisades	SKP
Soda Lake	SOL
String Lake	STR
Sulphur Creek Reservoir	SCR
Upper New Fork Lake	UNF
Upper Snake River	SKU
Upper Sunshine Reservoir	USR
Viva Naughton Reservoir	VNR
Wardell Reservoir	WDR
West Newton Lake	WNL
Wheatland #1 Reservoir	WLR
Willow Lake	WLL
Woodruff Narrows Reservoir	WNR
Other	OTR
NA	NA

Appendix G: Four digit random numbers generated by random.org

	01	02	03	04	05	06	07	08	09	10	11	12
01	6433	6861	4196	5869	2741	7336	3899	2733	0693	4160	1226	4703
02	8299	8784	9359	2441	3314	3270	5130	5859	4170	8898	5374	7271
03	5251	2125	0864	9354	2959	2079	6792	5151	1391	0341	9419	5784
04	1313	1215	8709	1169	6219	2895	6443	7859	3006	4467	4203	8532
05	6419	3830	5730	8488	1743	7431	4072	2661	6072	5949	3336	2347
06	8678	7020	3309	7909	0371	0878	4824	6460	7335	1209	8491	2437
07	3831	7124	3313	5026	6441	9381	9662	2176	5751	5320	6834	1219
08	8041	2285	7674	9027	2480	6123	7189	2135	9320	3046	7719	1110
09	6367	6157	6075	7638	3200	9282	8147	6485	7987	8187	8207	6415
10	4719	6289	1420	8882	4664	4927	3672	4357	5699	9514	7717	8611
11	6776	2180	9963	5575	0470	8097	0730	9173	0727	7002	4015	1192
12	2549	9311	9634	0254	0370	2546	8058	3367	2857	7285	1863	9115
13	7186	9680	4992	6104	9431	1677	0320	3152	4214	5317	7643	2286
14	7803	0777	7935	6634	7510	0569	8826	9075	2449	0074	9221	9582
15	6761	6449	7291	6200	4146	9500	8698	3310	0239	1802	5553	6030
16	2370	5509	7722	5594	4048	9948	0605	6050	9321	6798	0945	7505
17	8173	2062	4386	9234	5783	2556	5430	7262	5795	0714	8700	0668
18	5670	9063	4099	1776	9203	0884	6497	3138	3549	4332	1304	3485
19	1580	1962	3447	6337	2312	2202	5741	8484	4654	2938	2413	3957
20	9367	2955	0820	7140	4581	1164	1243	3915	4951	9093	6223	3567
21	9270	2402	6112	5471	4769	7875	9198	2946	4059	2408	1613	7596
22	6843	2456	2382	6410	7148	8656	4228	8270	5234	0287	5105	1350
23	7984	0069	7622	4096	4403	3358	3582	7582	9123	9807	1066	6486
24	6605	2534	0897	1032	5689	3274	6714	2208	2461	6745	1890	0652
25	6780	1158	0056	3911	2888	2423	6922	0114	7078	6294	4300	3902
26	6698	5133	3518	5040	0531	1596	7328	9127	989	9928	3194	2728
27	8099	6090	8194	0266	4800	9551	1957	1024	4994	3329	5386	9907
28	8268	3493	6967	1522	3547	1142	8361	0214	9193	9843	0345	2931
29	1089	7944	8883	9942	6868	4342	7095	5300	8881	5235	6549	9317
30	4827	0533	3294	4806	0065	4804	8236	8378	3799	6093	7011	7356
31	6514	6100	3511	4830	5330	2502	0123	9058	6570	1063	7437	0027