Aquatic Invasive Species Early Detection and Monitoring Program

Monitoring Report 2018

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Abstract:
Annual report of aquatic invasive species sampling effort by Montana Fish, Wildlife, & Parks and partners for the 2018 sampling season.

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Appendices

The Montana Fish, Wildlife & Parks (FWP) Aquatic Invasive Species (AIS) Bureau implements the AIS Management Plan through coordination and collaboration, prevention of new AIS introductions, early detection and monitoring, control and eradication, and outreach and education. 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.

I. Early Detection and Monitoring – Background

Early detection and monitoring are essential aspects of any effective aquatic invasive species program. Montana's Aquatic Invasive Species (AIS) Early Detection and Monitoring Program has been in place since 2004. Early detection allows Montana Fish, Wildlife & Parks (FWP) biologists to locate small or source AIS populations, while monitoring allows FWP to study existing population trends and investigate suspect findings. FWP monitors for all aquatic invasive species, including zebra/quagga mussels (ZM/QM), Asian clams (AC), New Zealand mudsnails (NZMS), faucet snails (FS), Eurasian watermilfoil (EWM), flowering rush (FR), curlyleaf pondweed (CLPW), fragrant waterlily (FWL), and other species not known to occur in Montana. Plankton sampling for ZM, QM, and AC veligers (microscopic larvae) has increased each year within FWP's early detection program in addition to an increase in volunteer and partner sampling efforts. Overall monitoring and early detection efforts have increased steadily over the years but nearly tripled in recent years with the positive detection of mussel veligers in Tiber Reservoir and a suspect veliger detection in Canyon Ferry Reservoir.

II. New for 2018

Mobile Data Collection

Beginning in 2018, the AIS Early Detection and Monitoring Program used Survey123, an ArcGIS Online -based mobile collection system (used Panasonic Toughpads), to collect their early detection and monitoring data. ArcGIS Online is a cloud-based geographical information system mapping and analysis platform. The survey form was prebuilt by FWP's software developers and Geographical Data Services staff (Figure 1). It is downloaded by members that are part of FWP's sampling group, which included FWP AIS crews and our pilot partner, Whitefish Lake Institute. The survey data can be stored locally until collectors can use cellular data or Wi-Fi to upload their data. The data are then stored in the ArcGIS online cloud until program staff download them into the Aquatic Invasive Species database housed within FWP's servers. Program staff review them for potential errors and reports of AIS then these data are added into FWP's GIS data that are available to the public to view and download.

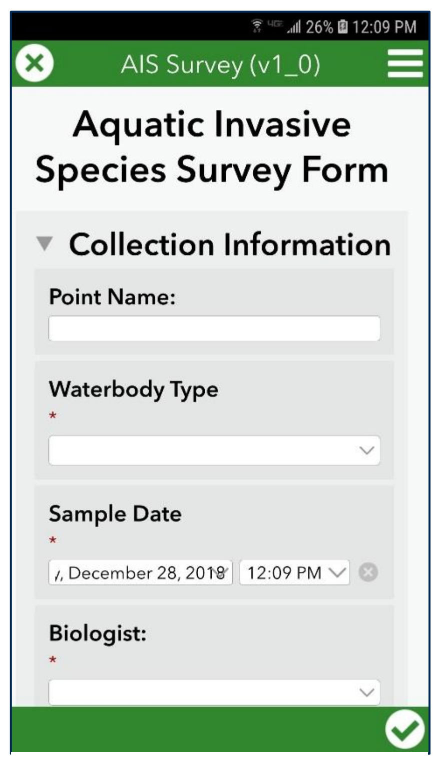

Figure 1: Screenshot of FWP's mobile data collection form on Survey123.

In addition, the MT Natural Heritage Program then takes these datasets and adds them to its database to be housed permanently.

This workflow allows crews to eliminate paper forms and data entry later, which results in faster turnaround time of data. After FWP crews or partners upload their data, it becomes useable almost instantaneously by the program, which is not the case with paper forms. This helps the program to be more efficient in reporting and responding to aquatic invasive species detections. In addition, it reduces transcription errors that occurs during the data entry process.

The application is now available for partners for use in 2019. As more partners begin to use the app, it will help streamline statewide AIS sampling and prevention efforts. The app also helps data coordination and reduces the number of disparate data sets that exist. If interested in using this application, please contact Craig McLane at cmclane@mt.gov.

New FWP AIS Lab Space

The new FWP AIS laboratory began operation in a new updated facility on August 15th, 2018 (Figure 2). The new space allows for expanded lab processing capacity. The lab is now located in the same building as other FWP AIS bureau staff and allows for closer communication and support.

Figure 2: The new FWP aquatic invasive species lab in Helena, MT.

III. Monitoring Methods

FWP assesses risk for AIS introductions to waterbodies annually. Annual plans are dynamic due to constantly evolving variables used in determining risk. Sites are prioritized based upon the previous years' work conducted by FWP, available calcium, water quality data and information collected by FWP including, angler/boater pressure, boater movement data from watercraft inspection stations, monitoring conducted by other state and federal agencies, surface-water hydrology, and other assorted variables. For improved effectiveness, at the end of 2016, Montana FWP began refining a newly developed matrix to prioritize all waters in Montana for monitoring, which was used to prioritize sampling efforts during the 2017-2018 field seasons. This matrix incorporated new data into the risk assessment including both habitat suitability (pH, Ca, hardness, conductivity, substrate composition, dissolved oxygen, and water velocity) and social pressure (angling pressure, non-native, warm to cool water fish presence, proximity to source of invasive mussels, non-angling boating use, position in watershed, and waterbody type (lentic vs. lotic). A high rank in either category resulted in a high invasion potential risk score regardless of the other category ranking. The outcome from this analysis is shown in Figure 3 and the criteria metrics in Appendix G.

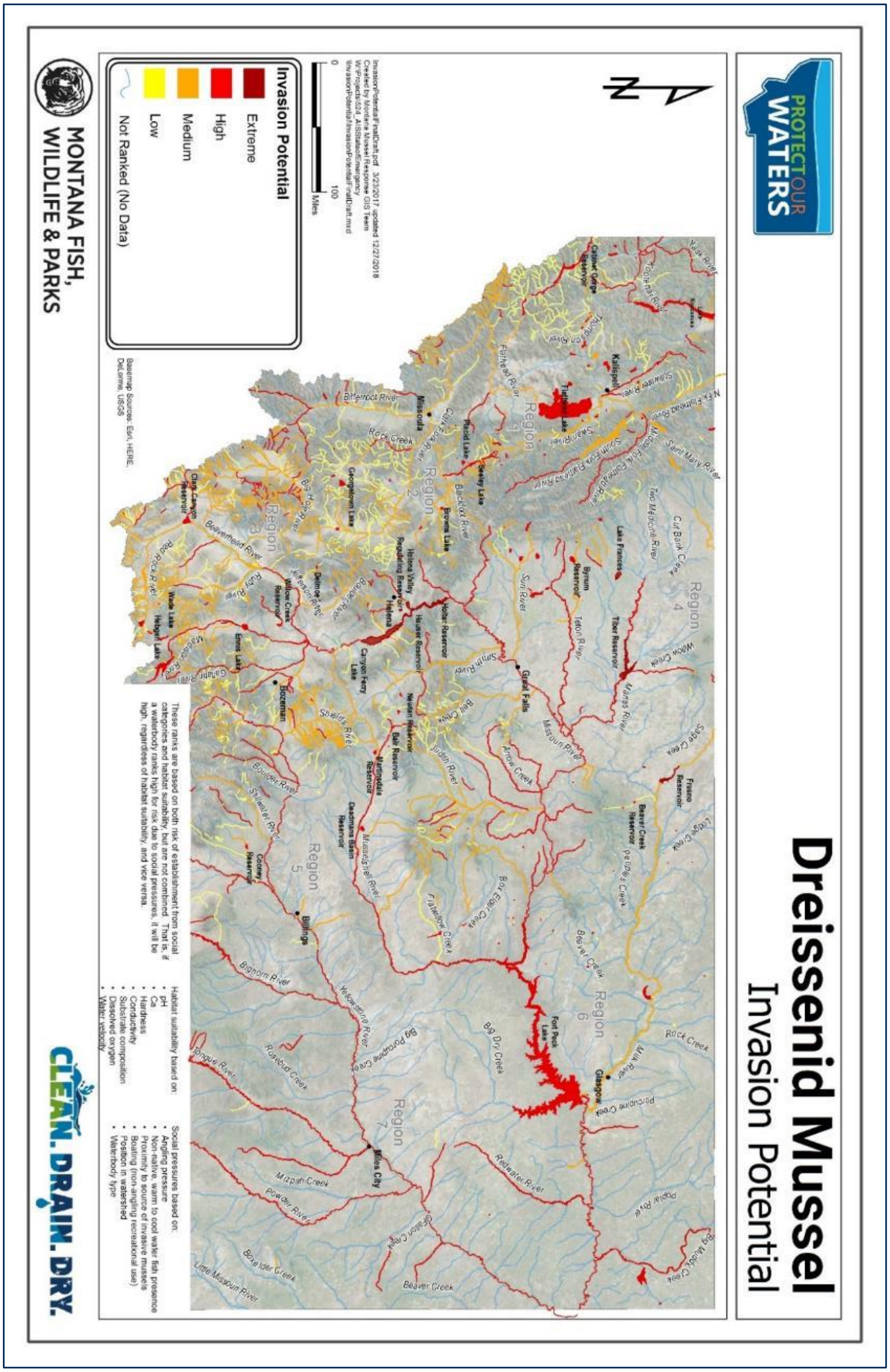

Figure 3: Map of Dreissenid Mussel Invasion Potential used to rank waters in Montana for overall AIS introduction threat and help determine frequency and quantity of sampling events.

Investigate Public Reporting of AIS

FWP also investigates reports of invasive species. FWP offices often receive calls when a member of the public or other sampling entity finds an unusual or unknown organism. Samples are often brought in to offices where regional staff will either identify them or send them to the AIS staff in Helena. If an organism can't be identified or verified, FWP staff will travel to the location to investigate the report.

In May of 2018, a fisherman was trolling in Lake Frances and picked up two clams and a snail on some vegetation. He submitted the samples to FWP fisheries staff in Great Falls who then sent the samples to FWP's AIS laboratory in Helena for identification. The clams were determined to be native pea clams from the family *Sphaeriidae* (often confused with the invasive Asian clams). FWP experts determined that the snail was an invasive Faucet Snail (also known as a Mud Bithynia - *Bithynia tentaculata*) (Figure 4). Photographs of the snail were sent to an independent expert who confirmed the AIS Lab's identification. Faucet snails had not been previously detected in Lake Frances or west of the Continental Divide in MT though they have been found in other parts of the state east of the divide. This faucet snail detection is an example of a success story where an angler assisted with the detection of a new invasive species location. No faucet snails were found in routine sampling locations near boat ramps, but they were found in deep water where local fishermen frequented. FWP relies on the public to be on the lookout for invasive species and report suspected sightings. Sightings can be reported by either calling the FWP fisheries office at 406-444-2440 or using the online reporting form at: http://fwp.mt.gov/doingBusiness/contactUs/aisSighting.html

Figure 4: Side by side faucet snails collected from Lake Frances in 2018. Snail on the left was collected by angler and snail on the right was collected by FWP to confirm presence at the location where the first snail was reported.

FWP Monitoring Staff

In 2018, FWP's AIS program had three permanent staff (one in Kalispell and two in Helena) conducting early detection and monitoring surveys in addition to their other duties. All three permanent staff worked together to hire and train three seasonal teams of two employees each based in Kalispell, Helena and Bozeman. These locations were based on the applicant pool of candidates received and vicinity to waters to be surveyed. The permanent Kalispell position supervised the Kalispell crew and the plant specialist in Helena supervised the other two crews. Improvements in 2018 included more extensive classroom and field sessions for seasonal staff. Crews were required to collect voucher samples of both invertebrate and plant specimens observed to improve overall sampling quality. Crews began in May instead of June where feasible, which allowed more statewide sampling efforts.

Both seasonal, part-time laboratory staff hired in 2017 returned for the 2018 season at the laboratory in Helena. This resulted in less time spent on training lab staff and more time to process samples.

Sampling Methods

Montana utilizes a variety of techniques in monitoring for AIS populations. All of Montana's monitoring standard operating procedures (Montana Fish, Widlife, & Parks, 2018) have been scientifically reviewed, are updated annually as needed, and are coordinated with neighboring states. Since there are a variety of aquatic invasive species, different sampling techniques are used to increase the likelihood of early detection of each of these species. 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.

Mussel Larvae (Veliger) Sampling

Plankton sampling involves the collection of microscopic organisms in the water column using specialized, fine mesh nets during the warmer spring, summer and fall months when water temperatures are above 48°F (9°C). Analysis of those samples occurs at the FWP Aquatic Invasive Species Laboratories. Plankton tow sampling tests a massive amount of water (compared to other methods) and is widely accepted as the most reliable and cost-effective method of detecting invasive mussel larvae. Cross-polarized light microscopy (CPLM) is the primary accepted method utilized by the laboratories to detect the larvae (veligers) of invasive bivalves such as Dreissenid mussels and Asian clams in plankton tow net samples. CPLM analysis is conducted at the main FWP AIS lab in Helena, MT, or at the FWP AIS satellite lab in Kalispell, MT. If a suspected dreissenid veliger is identified through CPLM, confirmation will be conducted through polymerase chain reaction (PCR) analysis by a contracted, independent laboratory.

Montana FWP utilizes enhanced sampling methods at Tiber and Canyon Ferry to confirm the presence of a reproducing dreissenid mussel population. These methods include scuba diving, snorkeling, placing artificial substrates, environmental DNA (eDNA) and mussel-sniffing dogs. No evidence of dreissenid larvae, adults or DNA were detected in 2018.

eDNA is an emerging science for dreissenid mussel early detection. FWP is working with researchers in Montana and throughout the region to help develop eDNA into a tool that can enhance Montana's dreissenid early detection effort. In April of 2018, a panel of experts was assembled at the Flathead Lake Biological Station in Polson Montana to discuss gaps and needs for the development of eDNA as a tool for mussel early detection. See Appendix G for the results of this panel.

Invertebrate Sampling

Invertebrate sampling involves the use of many tools and techniques to observe and collect species living in the water. Most freshwater invertebrates avoid predation by living in hidden areas and are not easily noticed by the casual observer (they are often camouflaged and can swim away quickly to escape capture.) FWP uses a suite of sampling methods in their capture, collection and identification since they cannot always be collected with any one method, which includes rock picking, kick nets (Figure 5), shorelines and structure surveys.

Figure 5: Crewmember learning to use a kick net to sample for macroinvertebrates in Canyon Ferry Lake.

For seasonal crews, FWP added a specimen voucher collection protocol to seasonal sampling crew training. This would allow for crosscheck of suspect samples as well as verification when needed.

Fish Pathogen Testing

Fish pathogens, such as whirling disease, are considered AIS, and therefore, FWP conducts pathogen testing in fish in conjunction with other AIS monitoring in coordination with the FWP Fish Health Laboratory in Great Falls. This testing involves collecting tissue samples from fish (such as heads, kidneys, and spleens), and sending samples to the Bozeman Fish Health Center operated by the U.S. FWS (Figure 6). This lab provides services for bacteriology, histology, virology, parasitology, and wild fish health surveys. The three major areas of responsibility include:

- Inspection testing services for hatchery facilities to facilitate annual health certifications.
- Diagnostic assistance for chronic or acute health problems in cultured and wild stock.
- National Wild Fish Health Survey to determine the distribution of fish pathogens in free-ranging fish populations.

For more information on the Bozeman Fish Health Center see their website at: https://www.fws.gov/mountain-prairie/fisheries/fhc.php

FWP trained FWS staff in Bozeman to conduct hatchery inspections on out-of-state facilities in 2018. This was to aid in requests for fish importation on out-of-state facilities into Montana so that those facilities could be properly inspected prior to any fish movement. This training included a day-long classroom course as well as ongoing field training.

AIS Sampling Prior to Wild Fish Transfers

The movement of fish can 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 Fish Health Laboratory and the Aquatic Invasive Species Laboratory work closely together to inspect all federal, state and commercial hatcheries annually as well as waterbodies that fish biologists use for wild fish stock

Figure 6: Pathogen testing in 2018 at Holter Lake during a fish spawning event.

transfers. AIS inspections include both on-site AIS surveys and disease/pathogen testing in fish as discussed above. AIS program protocols include monitoring for all aquatic invasive species taxa whenever possible. The FWP Fish Health Staff in Great Falls, as part of the AIS Bureau, increased the number of hatchery AIS inspections due to the time constraints of other AIS bureau staff in 2017 and 2018.

Plant Sampling

FWP samples for macrophytes at high-risk sites as part of the departments all-taxa AIS sampling effort. In 2013, FWP integrated Montana Department of Agriculture’s plant specialist into its AIS program and began performing comprehensive aquatic plant sampling in select waterbodies throughout the state to locate or confirm aquatic invasive plant populations. In conjunction with other AIS sampling, macrophyte sampling occurs from early summer until plants begin to die off with colder water temperatures. Sampling occurs typically from June to October though sampling dates fluctuate with temperatures and spring runoff. 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 plant fragments would settle and establish.

IV. 2018 AIS Sampling Results

In 2018, a total of 238 waterbodies were sampled in Montana. Appendix A provides a listing of all water surveyed during the 2018 field season. It also shows the extent of the effort at each of these locations (type of survey conducted, how many times it was conducted at that waterbody, and by whom). More sampling details for Tiber Reservoir and Canyon Ferry Reservoir are available in Appendix C and Appendix D, respectively. For more specific information on individual waters or areas, send a specific information request to Craig McLane (cmclane@mt.gov) or download the survey data through FWP's GIS data page at http://gis-mtfwp.opendata.arcgis.com/datasets?q=AIS.

In 2018, new detections of AIS included the following locations:

- Missouri River at Riverside campground (Hauser Lake), New Zealand Mudsnails
- Lake Frances, Faucet snails
- Kootenai River, Curlyleaf pondweed
- Holland Lake, Fragrant waterlily

The following tables show the results from monitoring of waters with known AIS. Locations details of AIS in MT can be found in Appendix E and Appendix F.

Dreissenid Mussels (Zebra mussels (Dreissena polymorpha) and Quagga Mussels (Dreissena bugensis)) and Asian Clam (Corbicula fluminea)

	Adult Dreissenid Mussels	Dreissenid Mussel Larvae (Plankton Sampling)	Dreissenid mussel eDNA	Adult Asian Clam	Asian Clam Larvae (Plankton Sampling)
Tiber Reservoir	NO	NO	NO	NO	NO
Canyon Ferry Reservoir	NO	NO	NO	NO	NO
Other Statewide Locations	NO	NO	NO	NO	NO

New Zealand mudsnails (Potamopyrgus antipodarum)

Water body where historically found	Results of 2018 Sampling
Beaverhead River	NO
Bighorn River	NO
Bluewater Creek	YES
Darlington Ditch 1	YES
Gardner River	YES
Hauser Reservoir	YES
Jefferson River	NO
Madison River	YES

Water body where historically found	Results of 2018 Sampling
Missouri River (below Holter)	YES
Nelson Spring Cr	Not Sampled
Odell Creek	Not Sampled
Poindexter Slough	Not Sampled
Quake Lake	NO
Ruby River	NO
Upper Holter Lake	YES
Yellowstone River	YES

Faucet snail (Bithynia tentaculata)

Water body where historically found	Results of 2018 Sampling	Water body where historically found	Results of 2018 Sampling
Georgetown Lake	NO	McWennegar Slough	Not Sampled
Lake Frances (New in 2018)	YES	Upsata Lake	YES (Partner)

Eurasian watermilfoil (Myriophyllum spicatum)

Water body where historically found	Results of 2018 Sampling	Water body where historically found	Results of 2018 Sampling
Beaver Lake	NO	Jefferson River	YES
Cabinet Gorge Reservoir	YES	Jefferson Slough	YES
Clark Fork River (below Thompson Falls Reservoir)	YES	Missouri River (upstream of Canyon Ferry Res.)	YES
Fort Peck Dredge Cuts	YES	Noxon Rapids Reservoir	YES
Fort Peck Lake	YES	Pond 4 - Canyon Ferry	NO

Curlyleaf Pondweed (Potamogeton crispus).

Water body where historically found	Results of 2018 Sampling	Water body where historically found	Results of 2018 Sampling
Beaverhead River	NO	Holter Reservoir	YES
Bitterroot River	YES	Jefferson River	YES
Blackfoot River	NO	Kicking Horse Reservoir	YES
Cabinet Gorge Reservoir	YES	Kootenai River (New in 2018)	YES
Canyon Ferry Lake	YES	Lake Helena	NO
Clark Canyon Reservoir	NO	Madison River	YES
Clark Fork River	YES	Marias River	NO
East Gallatin River	NO	Missouri River	YES
Ennis Lake	YES	Newlan Reservoir	YES
Flathead Lake	YES	Noxon Rapids Reservoir	YES
Flathead River	YES	Pablo Reservoir	Not Sampled
Fort Peck Lake	NO	Pond 4 - Canyon Ferry	NO
Gallatin River	NO	Post Creek	YES
Hauser Reservoir	YES	Quake Lake	YES
Hebgen Lake	YES	Rainbow Dam Reservoir	YES
Helena Valley Regulating Reservoir	YES	Smith River	YES

Flowering Rush (Butomus umbellatus)

Water body where historically found	Results of 2018 Sampling	Water body where historically found	Results of 2018 Sampling
Cabinet Gorge Reservoir	YES	Flathead River	YES
Clark Fork River	YES	Noxon Rapids Reservoir	YES
Flathead Lake	YES	Thompson Falls Reservoir	NO

Fragrant waterlily (Nymphaea odorata)

Water body where historically found	Results of 2018 Sampling	Water body where historically found	Results of 2018 Sampling
Beaver Lake	NO	Loon Lake	YES
Browns Lake	NO	Placid Lake	YES
Duck Lake (in NW MT)	YES	Salmon Lake	YES
Elbow Lake	Yes	Savage Lake	YES
Holland (reported in 2018)	YES	Seeley Lake	YES
Lake Inez	YES	Swan Lake	YES
Lake Mary Ronan	NO	Upsata Lake	YES

Statewide Sampling Efforts

Figure 7 illustrates the statewide emphasis placed on AIS monitoring, which includes AIS monitoring sites for 2018 with focus on plankton sampling sites (though most sites included all-taxa surveys as well). Montana FWP surveys all high risk sites annually at a minimum and may survey lower risk sites less frequently. Figure 8 shows the mussel veliger sampling effort by FWP and partners for each waterbody sampled in 2018. The program goal is to comprehensively monitor the state every year, which includes all types of waterbodies (lakes, reservoirs, ponds, creeks, rivers, etc.) and for all taxa.

With the new detection of Dreissenid mussel larvae within the state in 2016, the agency has nearly tripled its efforts in 2017 and increased again in 2018. Partners are increasing efforts in invasive species detection as well. Figure 9 illustrates how many MT samples the FWP lab received and processed in 2018 from FWP (AIS staff, fisheries staff) as well as outside entities. FWP is dedicated to working closely with existing partners and to creating new partnerships to encourage AIS sampling on a local level.

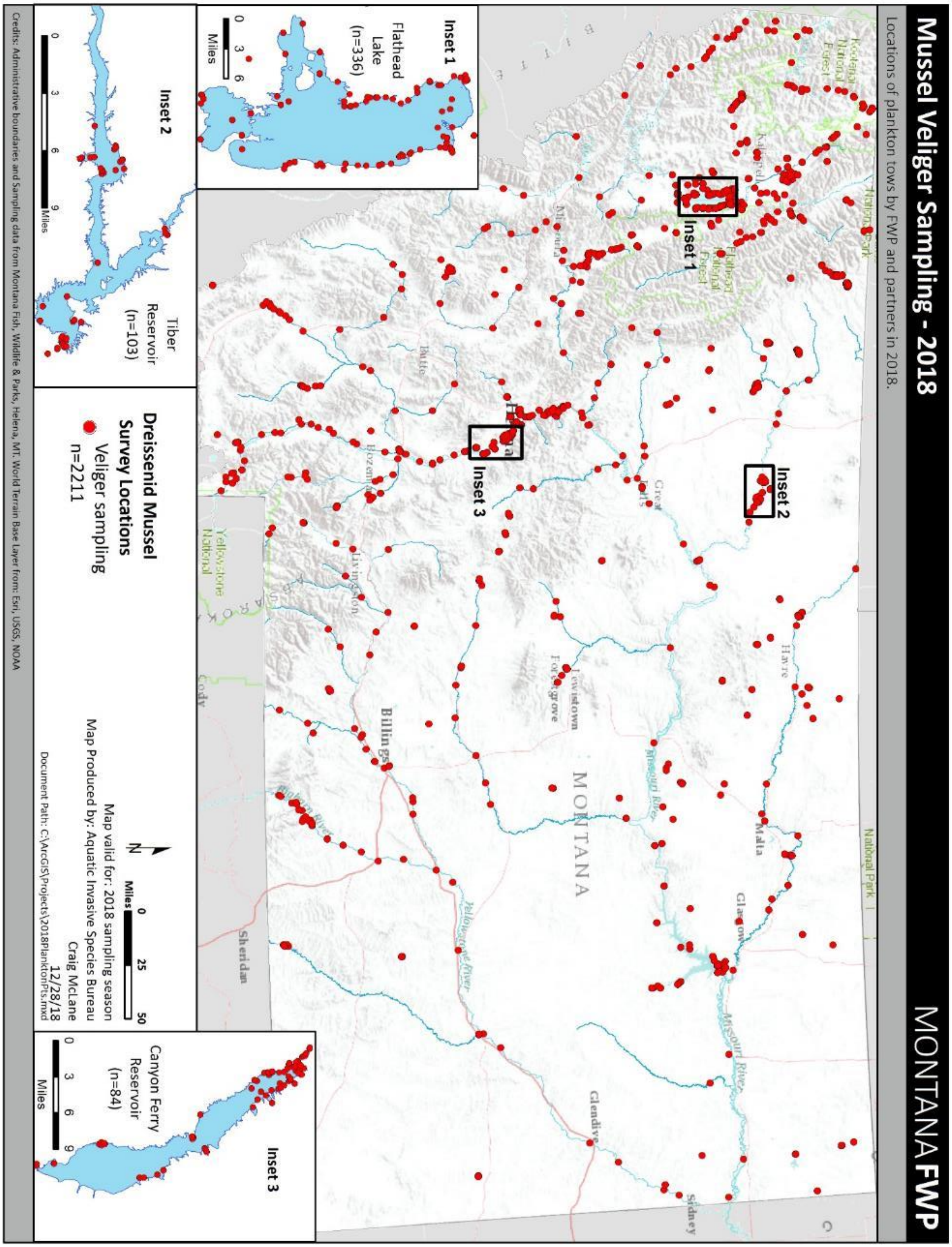

Figure 7: Map of AIS plankton sampling locations, 2018

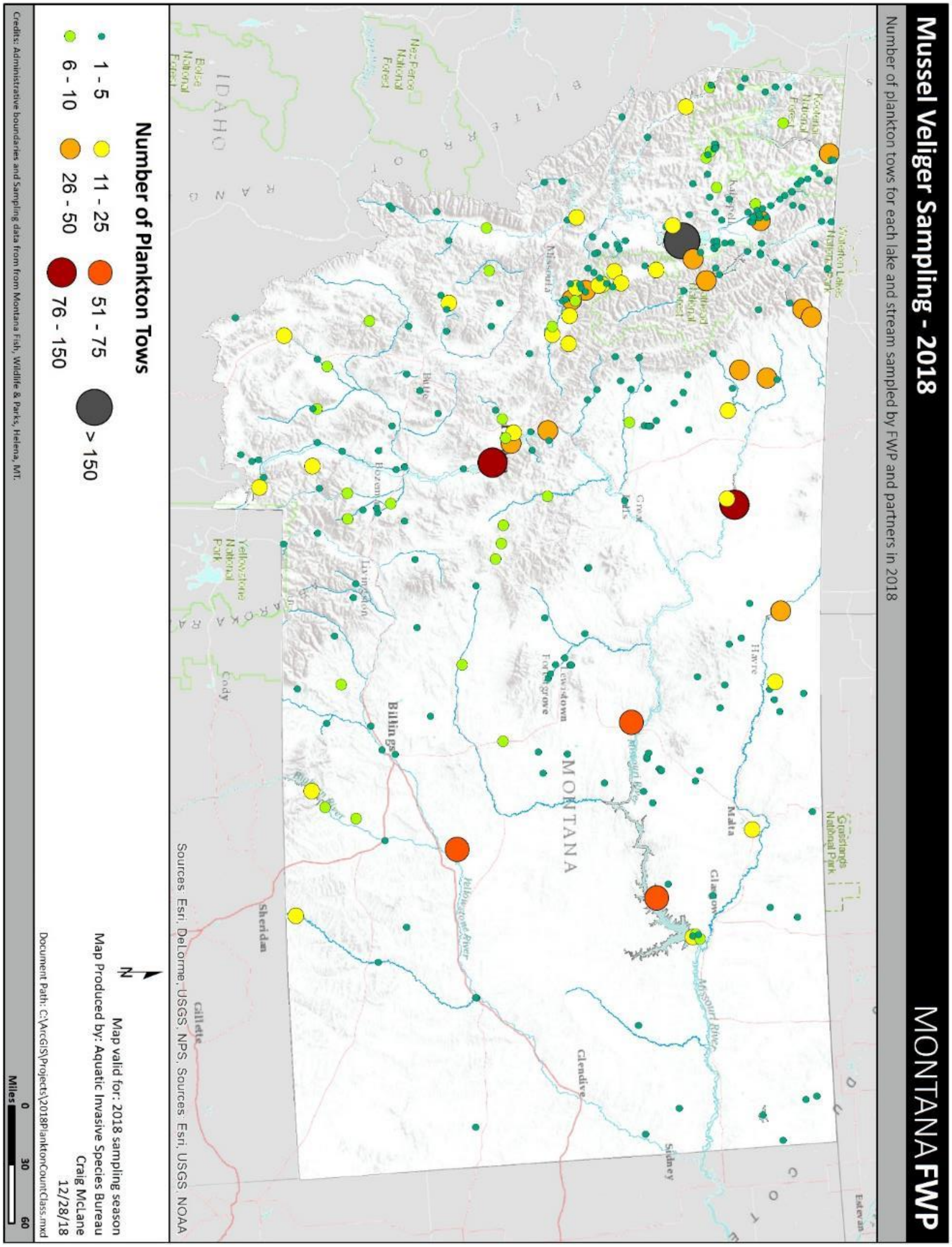

Figure 8: Mussel veliger sampling effort by FWP and partners in 2018 for each waterbody sampled. See Appendix A for sampling details for each waterbody.

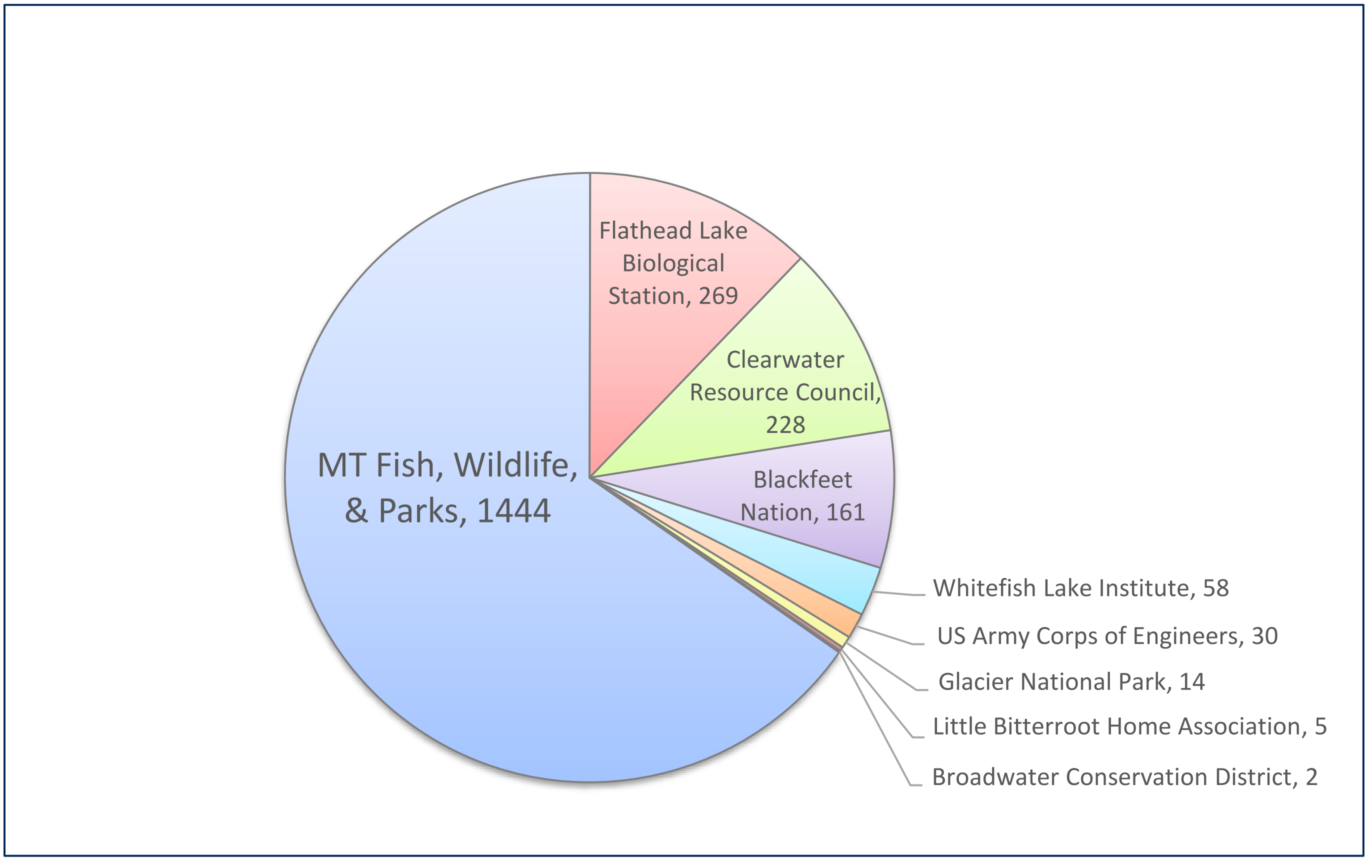

Figure 9: 2018 Aquatic Invasive Species Plankton Sampling Effort by All Reported Entities

Aquatic Plant Sampling Results

FWP surveyed waterbodies that were high-risk, suspect to contain AIS, or locations needing confirmation of AIS. In addition, several locations were resurveyed to examine the dynamics and abundance of established AIS populations, as well as to support invasive management activities in Noxon Rapids Reservoir, Cabinet Gorge Reservoir, and canals within Canyon Ferry Wildlife Management Area. In all, FWP crews surveyed 8 waterbodies. Table 1 shows the locations of FWP comprehensive sampling for aquatic invasive plants in 2018. More detailed results for each water sampled are available in Appendix B as well as in reports to the Blackfeet Nation (McLane, Crete, Monroe, & Trombley, 2018) and reservoir-wide plant surveys (McLane, Reservoir-wide Report, 2018) and post-treatment surveys (McLane, Sanders County 6-weeks Post-treatment Report, 2018) on Noxon Rapids Reservoir and Cabinet Gorge Reservoir for the Sanders County Invasive Plants Task Force. Results for a 6-week post-treatment survey on canals within Canyon Ferry Wildlife Management area in 2018 is also available (McLane, CFWMA 6-week Report, 2018).

Table 1. 2018 Aquatic plant sampling locations

Water Body	County	Sampling Type	Sampling Days	Sampling Points	Findings
Bull Lake	Lincoln	Point-Intercept	2	172	No AIS found
Cabinet Gorge Reservoir	Sanders	Point-Intercept	4	336	Existing Eurasian watermilfoil, curlyleaf pondweed, & flowering rush
Canals within Canyon Ferry WMA	Broadwater	Whole Canal	2	30	Existing Eurasian watermilfoil & curlyleaf pondweed
Duck Lake	Glacier	Point-Intercept	2	102	No AIS found
Four Horns Lake	Glacier	Point-Intercept	1	117	No AIS found
Jefferson River	Jefferson/ Madison/ Gallatin/ Broadwater	Whole Reach Survey	3	35 miles	Existing curlyleaf pondweed & Eurasian watermilfoil
Lower Saint Mary Lake	Glacier	Point-Intercept	2	175	No AIS found
Mission Lake	Glacier	Point-Intercept	1	110	No AIS found
Noxon Rapids Reservoir	Sanders	Point-Intercept	10	649	Existing Eurasian watermilfoil, curlyleaf pondweed, & flowering rush

V. Aquatic Invasive Species Laboratory

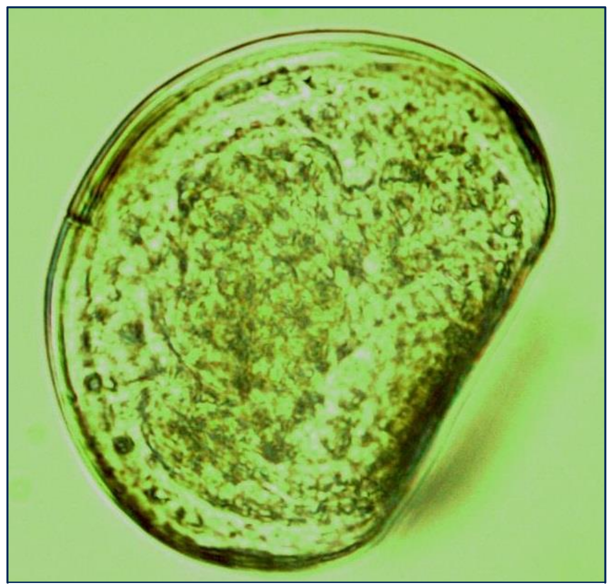

Figure 10: Photograph of Zebra mussel veliger found in an out-of-state sample processed in 2017 by FWP AIS Laboratory in Helena. Length of veliger = 111 µm.

The primary FWP Aquatic Invasive Species Laboratory is in Helena, MT. It was established in coordination with the Missouri River Basin Panel and the U.S. Fish and Wildlife Service to provide the service of early detection of Dreissenid mussels for those states. It currently processes plankton samples to look for larval mussels (veligers) (Figure 10) for New Mexico and the Missouri River Basin (MRB), including Colorado, Kansas, Nebraska, North Dakota, Wyoming, and Montana. Support from USFWS allows the AIS lab to process samples for partner states within the MRB as an in-kind service. The lab also offers to process samples from outside the basin as a confirmatory service for other labs. The base funding for this lab is provided by the U.S. Fish and Wildlife Service as well as other state and federal funding sources. Figure 11 and Figure 12 illustrate the volume of samples handled by the lab each year. The lab has discovered new populations of *Dreissena spp.* veligers as well as *Corbicula sp.* (Asian clam) veligers for multiple downstream states. The lab undergoes routine quality control testing by other states and has participated in a community double-blind round robin study on the reliability of early detection methods (Fischer, Nierzwicki-Bauer, & Kelly, 2011). FWP staff are also participating in workgroups organized by the Western Regional Panel to standardize both laboratory and sampling techniques across western states.

In 2018, no Dreissenid veligers were found in any samples collected in MT, including from Tiber Reservoir and Canyon Ferry Reservoir by either FWP or BOR.

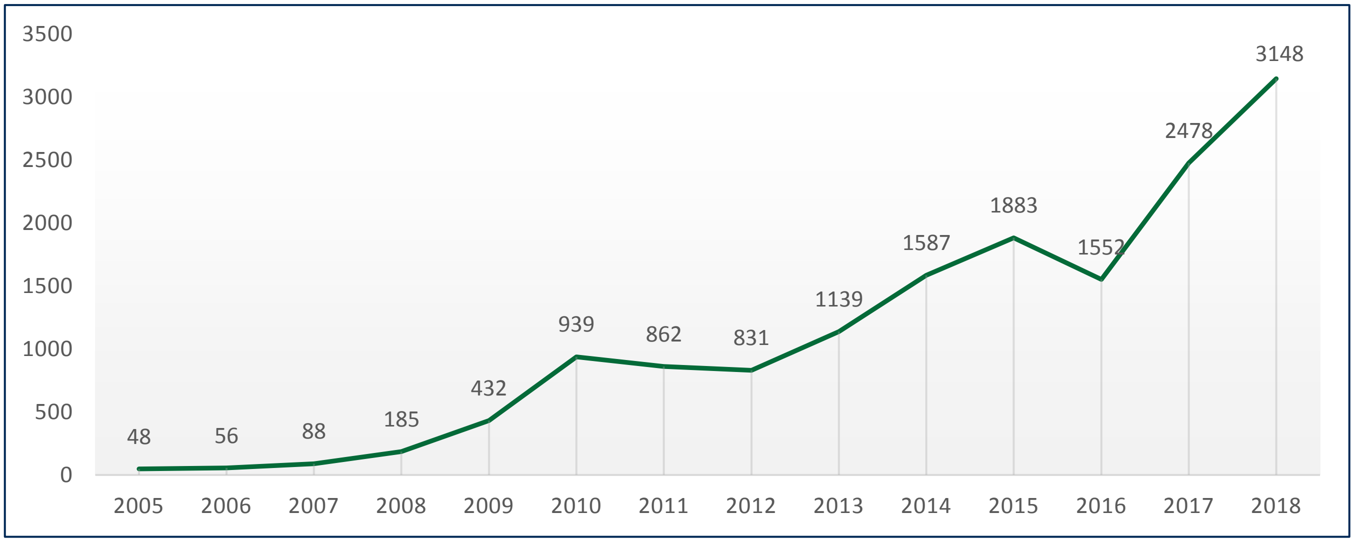

Figure 11: Number of samples processed by FWP AIS lab each year

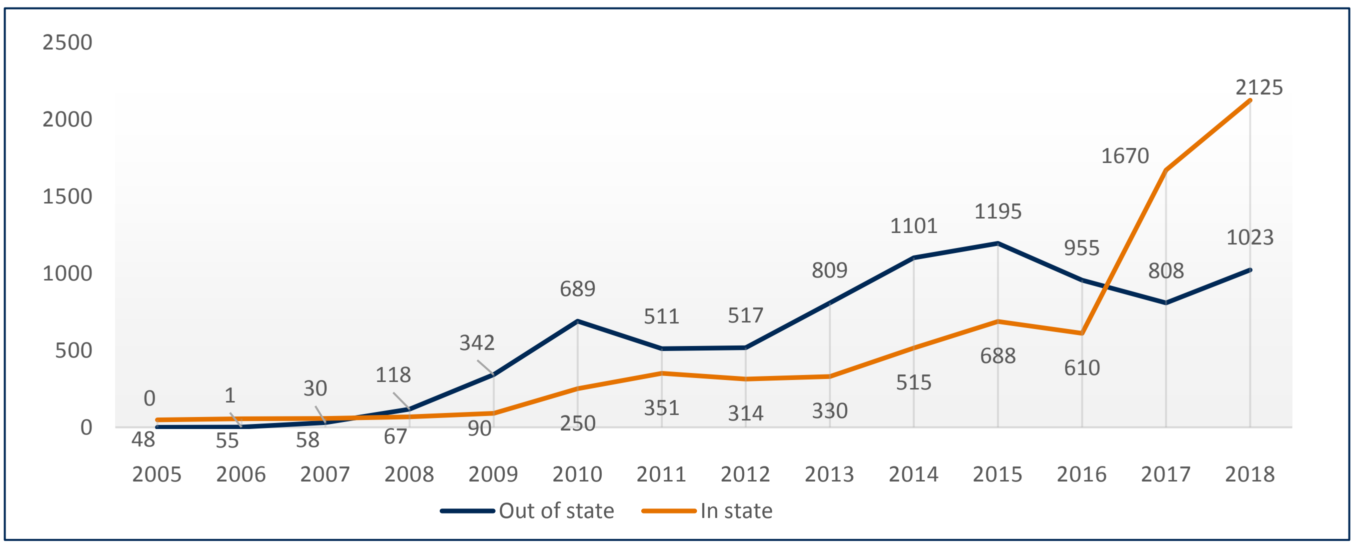

Figure 12: Number of plankton samples processed by year: in-state (FWP and partners) vs. out-of-state.

Lab Process Turn-around Time

All Montana samples were completed by Dec 13, 2018. High priority Montana samples were processed within an average of 8 days. Lower priority Montana samples had an average turnaround of 31 days and out-of-state samples took longer to process. Out-of-state samples will be completed in January 2019. In 2018, samples were processed in a shorter timeframe than prior years. The FWP AIS laboratories are continuing to work on methods to improve sample processing time with annual increases in quantity of samples.

Out-State-Sample Results

Results for out-of-state samples (n=1023) include *Corbicula* veligers found in 8 out-of-state waterbodies and Dreissenid veligers found in 3 samples from 2 states. Two of these detections were quality assurance/quality control (QA/QC) samples sent to the lab as double-blind samples. Both the Helena and Kalispell lab undergo QA/QC testing to ensure lab technicians detect target organisms. All QA/QC samples received in 2018 and previous years have been detected. Methods to improve turnaround time for out-of-state samples are continually being evaluated and reassessed.

In 2018, the lab's detection of veligers in a plankton sample from other states led to a new detection of adult mussels. In 2018, samples from Nebraska collected at Glen Cunningham Lake were sent to the lab, and zebra mussel veligers of varying age classes were found. Nebraska officials were able to then confirm an adult population of mussels. The state of Nebraska is now working with the US Army Corps of Engineers to draw the lake down to control mussel population and Asian carp population. (Nebraska Game and Parks, 2018)

FWP AIS Lab Improvements

FWP AIS laboratory staff receive additional training during the winter months to improve microscopy and photography skills. Additional training is also provided in the identification of freshwater zooplankton, phytoplankton, macroinvertebrates and aquatic plants.

In 2017, additional measures were taken to accommodate the higher sample load to get samples processed more efficiently. Two permanent/part-time, seasonal staff were hired and trained to assist in the Helena lab. Those employees returned in 2018 and began processing samples helping to maintaining lab turn-around times.

Figure 13: VFW boat ramp on Tiber Reservoir - 2018.

VI. Mussel Response at Tiber and Canyon Ferry Reservoirs

After the declaration of a statewide natural resource emergency in the fall of 2016 following the first detection of larval Dreissenid mussels within the state, Montana stepped up its fight against invasive species significantly in 2017, which continued in 2018.

Figure 14: Marker buoy above artificial substrate sampler at VFW boat ramp near Tiber Dam.

FWP increased focus on Tiber and Canyon Ferry Reservoirs due to the detection of invasive mussel larvae in Tiber and a suspect detection in Canyon Ferry in November 2016. As the only two waterbodies in Montana where mussels were detected or suspected, many efforts were made to detect any further presence of mussels. These efforts included plankton tow sampling, artificial substrate sampling (Figure 14), underwater inspections using scuba divers (Figure 15) and snorkelers, mussel detecting dogs (Figure 16) and the use of environmental DNA (eDNA) sampling. This year, 103 plankton tow samples taken at Tiber and 84 samples taken at Canyon Ferry were analyzed through microscopy for the presence of invasive mussel larvae. No adult mussels or larvae were found throughout all sampling efforts. July eDNA samples from Tiber were processed by Pisces Molecular LLC. All samples results came back negative for Dreissenid mussel DNA in 2018 (Pisces Molecular LLC, 2018).

The use of eDNA as a sampling method for early detection of invasive mussels is an emerging technology and research into the method is ongoing. eDNA as an early detection tool is in the research phase and was discussed during the incident command and implementation periods of the mussel response (See Appendix H and Appendix I). Due to questions surrounding this method, FWP and the Montana Invasive Species Council formed a scientific advisory panel to provide guidance on the use of DNA methods for early detection of invasive mussels. The panel convened in April 2018, and key findings of that panel can be found in Appendix J or in the panel report (Montana Invasive Species Council, 2018). Based upon on the advice of the scientific advisory panel

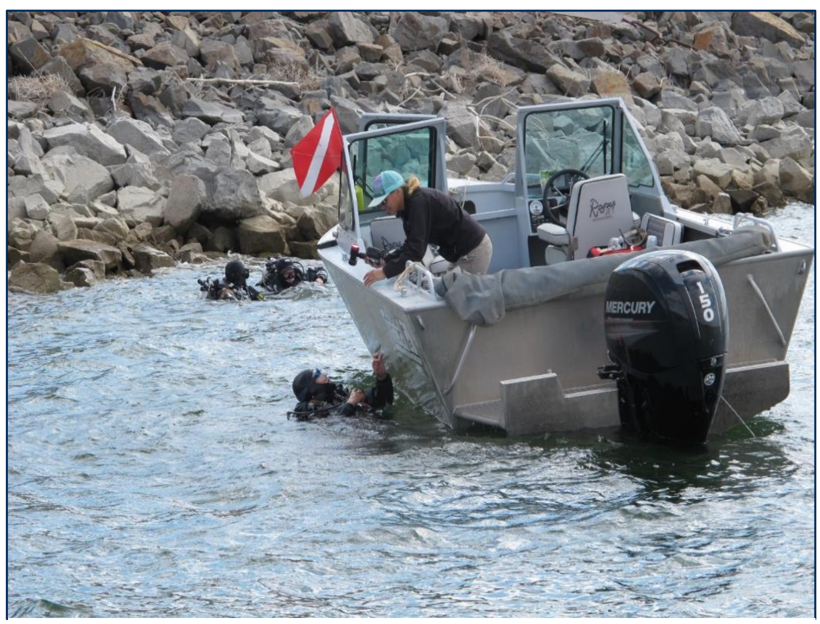

Figure 15: US Fish and Wildlife Service divers preparing for a dive at Tiber Dam to search for adult Dreissenid mussels. August

eDNA sampling was used during the 2018 sampling season on Tiber Reservoir to continue to develop the science.

Continued Efforts

FWP is continually evaluating and adapting the early detection and monitoring program based on best available science. FWP will continue to improve coordination of sampling efforts with partners while also encouraging recruitment of others. Three sampling crews of two people will be hired to sample most of the waters in the state with assistance from permanent AIS staff and partners. All crews will continue FWP's all-taxa survey approach with an emphasis on invasive mussels.

Figure 16: Alberta team Cindy Sawchuk and Hilo searching the shoreline at South Bootlegger Boat Ramp, Tiber Reservoir, October 2017. Mussel-sniffing dogs were used again in 2018 at Tiber and Canyon Ferry Reservoirs.

No adult mussels or veliger detections occurred in 2017 or 2018. Consistent with regional standards, if no adults or veligers are found after three years on Canyon Ferry Reservoir, its "Suspect Detection" classification will be removed (potential delisting in 2020). Similarly, after five years of sampling on Tiber Reservoir without any adults or additional veligers, its "Positive Detection" classification will be dropped (potential delisting in 2022). Sampling on Canyon Ferry Reservoir and Tiber Reservoir for the 2019 season will continue like 2017 and 2018 levels.

VII. Looking Forward

Fish, Wildlife, & Parks is evaluating the AIS Early Detection and Monitoring Program to identify opportunities to improve efficacy and efficiency. These improvements will lead to more reliable sampling efforts, data collection, and sampling handling. Plans to improve FWP's monitoring program include:

- Re-establish regional meetings to better loop-in partners and interested entities in planning and end of season debriefing
- Help partners implement mobile data collection with the use of FWP's Survey123 form.
- Review annual monitoring plan to ensure adequate frequency and intensity at highest priority waterbodies sampling occurs.
- Help partners improve their all-taxa AIS monitoring efforts through trainings and technical expertise.

- Develop an FWP AIS dive team to help with early detection and rapid response. The team will be formed in 2019, with training beginning summer 2019.
- Continue to consolidate statewide sampling data into Montana Heritage Program's database.
- Survey for AIS in known geothermal areas.

Statewide monitoring efforts by FWP and partners continues to become more effective and expand capacity. These efforts are critical to the early detection and monitoring of invasive species and are an important aspect of the AIS program and the statewide AIS Management Plan. While these efforts do not guarantee discovery of all AIS species as they are introduced, they do significantly increase the potential to discover new populations before they become established or spread beyond their current boundaries. Limiting the establishment or spread of AIS allows time for new research in control and eradication methods emerge and allows for greater efficiency in monitoring and early detection methods. These advances will ultimately save the state time and money protecting its aquatic resources and infrastructure.

VI. Literature Cited

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Appendix A. 2017 AIS Monitoring Locations,Types, & Numbers

Only includes FWP and data that we received from partners (typically those that send plankton samples to FWP's AIS laboratory.

* Indicates waters where comprehensive macrophyte surveys were conducted. See Appendix B.

LOCATION	AGENCY	Plankton Net - Microscopy	Hand Grab	Kick Net	Rock Picking	Rake Toss	Scuba	Snorkel	Substrate	Mussel-sniffing dogs	Hand grab - eDNA water
Abbot Lake	MT Fish, Wildlife, & Parks	2									
Abbot Lake	Whitefish Lake Institute	1									
Ackley Lake	MT Fish, Wildlife, & Parks	2	1	2	1	1					
Alvord Lake	MT Fish, Wildlife, & Parks	2		2		2					
Anderson Reservoir	MT Fish, Wildlife, & Parks	1		1		1					
Arapooish Pond	MT Fish, Wildlife, & Parks	1		1		1					
Ashley Lake	MT Fish, Wildlife, & Parks	4		4		4					
Ashley Lake	Whitefish Lake Institute	1									
Bailey Lake	Whitefish Lake Institute	1									
Bailey Reservoir	MT Fish, Wildlife, & Parks	4		4		4					
Bair Reservoir	MT Fish, Wildlife, & Parks	6	2	6	5	4					
Banana Lake	MT Fish, Wildlife, & Parks	2		2		2					
Basin Creek	MT Fish, Wildlife, & Parks	1	1	1	1						
Bean Lake	MT Fish, Wildlife, & Parks	2		2		2					
Bear Creek	MT Fish, Wildlife, & Parks	1	1		1						
Bearpaw Lake	MT Fish, Wildlife, & Parks	2		2		2					
Beaver Creek	MT Fish, Wildlife, & Parks	1		1							
Beaver Creek Reservoir	MT Fish, Wildlife, & Parks	4		4		4					
Beaver Lake	MT Fish, Wildlife, & Parks	2		1		2					
Beaver Lake	Whitefish Lake Institute	2									
Beaverhead River	MT Fish, Wildlife, & Parks	6	6	6	5						
Big Casino Creek Reservoir	MT Fish, Wildlife, & Parks	2		2	2	2					
Big Hole River	MT Fish, Wildlife, & Parks	6	6	6	5						
Big Spring Creek	MT Fish, Wildlife, & Parks	4	4	2	2						
Big Therriault Lake	MT Fish, Wildlife, & Parks	1	1	1							
Big Therriault Lake	Whitefish Lake Institute	1									
Bighorn Lake	MT Fish, Wildlife, & Parks	18		4	4						
Bighorn River	MT Fish, Wildlife, & Parks	8	3	8	7	4					
Birch Creek	MT Fish, Wildlife, & Parks	1			1						
Bison Bone Reservoir	MT Fish, Wildlife, & Parks	1		1		1					
Bitterroot River	MT Fish, Wildlife, & Parks	8	6	8		2					
Blackfoot River	MT Fish, Wildlife, & Parks	6	6	6							
Blacktail Meadows Kids Pond	MT Fish, Wildlife, & Parks	1		1	1	1					
Blaine Spring Creek	MT Fish, Wildlife, & Parks	1	1		1						
Blanchard Lake	MT Fish, Wildlife, & Parks	2	2	2							
Blanchard Lake	Whitefish Lake Institute	1									
Bluewater Creek	MT Fish, Wildlife, & Parks	1	1		1						
Bootjack Lake	MT Fish, Wildlife, & Parks	2		2		2					
Bootjack Lake	Whitefish Lake Institute	1									

LOCATION	AGENCY	Plankton Net - Microscopy	Hand Grab	Kick Net	Rock Picking	Rake Toss	Scuba	Snorkel	Substrate	Mussel-sniffing dogs	Hand grab - eDNA water
Boulder River	MT Fish, Wildlife, & Parks	7	5	6	6	1					
Bowman Lake	Glacier National Park	4									
Boxelder Lake	MT Fish, Wildlife, & Parks	3		3		3					
Br 047 Reservoir	MT Fish, Wildlife, & Parks	1		1		1					
Broadview Pond	MT Fish, Wildlife, & Parks	1		1							
Browns Lake	Clearwater Resource Council	15									
Browns Lake	MT Fish, Wildlife, & Parks	2		2		2					
Brush Lake	MT Fish, Wildlife, & Parks	2		2		2					
Bull Lake*	MT Fish, Wildlife, & Parks	6	1	6		269					
Bynum Reservoir	MT Fish, Wildlife, & Parks	4		4		3					
Cabinet Gorge Reservoir*	MT Fish, Wildlife, & Parks	9	2	9		336					
Canyon Ferry Lake	MT Fish, Wildlife, & Parks	84	5	9	5	46		6	8	4	
Carters Pond	MT Fish, Wildlife, & Parks	1	1	1							
Castle Rock Reservoir	MT Fish, Wildlife, & Parks	4		4	4	4					
Cibid Lake	MT Fish, Wildlife, & Parks	2		2		2					
Clark Canyon Reservoir	MT Fish, Wildlife, & Parks	11	7	11	7	4					
Clark Fork River	MT Fish, Wildlife, & Parks	11	9	11		2					
Clarks Fork Yellowstone River	MT Fish, Wildlife, & Parks	5	5	6	4						
Clearwater Lake	MT Fish, Wildlife, & Parks	1	1	1							
Clearwater River	MT Fish, Wildlife, & Parks	2	2	2							
Cliff Lake	MT Fish, Wildlife, & Parks	1		1	1	1					
Coe Campsite Pond #2	MT Fish, Wildlife, & Parks	1		1		1					
Compton Reservoir	MT Fish, Wildlife, & Parks	1		1		1					
Cooney Reservoir	MT Fish, Wildlife, & Parks	6	3	6	5	3					
Coopers Lake	Clearwater Resource Council	12									
Coopers Lake	MT Fish, Wildlife, & Parks	1		1		1					
Cottonwood Creek	MT Fish, Wildlife, & Parks	3	2	1	2	1					
Cow Creek Reservoir	MT Fish, Wildlife, & Parks	3		3		3					
Crystal Lake	MT Fish, Wildlife, & Parks	2		1		1					
Cut Bank Creek	MT Fish, Wildlife, & Parks	4	1		4	3					
Dailey Lake	MT Fish, Wildlife, & Parks	4	2	4	4	2					
Darlington Ditch 1	MT Fish, Wildlife, & Parks	2	1	3	2						
Deadmans Basin Reservoir	MT Fish, Wildlife, & Parks	10	3	10	8	6					
Dearborn River	MT Fish, Wildlife, & Parks	2		2		2					
Delmoe Lake	MT Fish, Wildlife, & Parks	1		1		1					
Dickey Lake	MT Fish, Wildlife, & Parks	3	1	3		2					
Dickey Lake	Whitefish Lake Institute	1									
Dollar Lake	Whitefish Lake Institute	1									
Don Reservoir	MT Fish, Wildlife, & Parks	1									
Drag Creek Reservoir	MT Fish, Wildlife, & Parks	1		1	1	1					
Dry Fork Reservoir	MT Fish, Wildlife, & Parks	3		3		3					
Duck Lake	Blackfeet Nation	40									
Duck Lake*	MT Fish, Wildlife, & Parks	1		1		102					
East Fork Reservoir	MT Fish, Wildlife, & Parks	6	3	6	2	3					
East Gallatin River	MT Fish, Wildlife, & Parks	7	5	7	6						
Echo Lake	MT Fish, Wildlife, & Parks	4		4		4					
Echo Lake	Whitefish Lake Institute	1									
Elbow Lake	MT Fish, Wildlife, & Parks	1	1	1							
Elk Lake	MT Fish, Wildlife, & Parks	1		1	1	1					

LOCATION	AGENCY	Plankton Net - Microscopy	Hand Grab	Kick Net	Rock Picking	Rake Toss	Scuba	Snorkel	Substrate	Mussel-sniffing dogs	Hand grab - eDNA water
Elmo, Lake	MT Fish, Wildlife, & Parks	4		4	5	3					
Ennis Lake	MT Fish, Wildlife, & Parks	4	2	4	4	2					
Ester Lake	MT Fish, Wildlife, & Parks	1		1		1					
Eureka Reservoir	MT Fish, Wildlife, & Parks	1		1		1					
Eyraud Lake, lower	MT Fish, Wildlife, & Parks	2		2		2					
Fairy Lake	MT Fish, Wildlife, & Parks	1	1	1	1						
Fish Creek	MT Fish, Wildlife, & Parks	1	1	1							
Fish Lake	Clearwater Resource Council	9									
Fish Lake	MT Fish, Wildlife, & Parks	1		1		1					
Fish Lake	Whitefish Lake Institute	1									
Flathead Lake	Flathead Lake Biological Station	257									
Flathead Lake	MT Fish, Wildlife, & Parks	76	17	33		16					
Flathead Lake	Whitefish Lake Institute	6									
Flathead River	Flathead Lake Biological Station	6									
Flathead River	MT Fish, Wildlife, & Parks	17	14	15		1					
Flinstone Reservoir	MT Fish, Wildlife, & Parks	1		1	1	1			1		
Flynn Pond	MT Fish, Wildlife, & Parks	1		1		1					
Forsman Reservoir	MT Fish, Wildlife, & Parks	1		1		1					
Fort Peck Dredge Cuts	MT Fish, Wildlife, & Parks	8		8		8					
Fort Peck Lake	MT Fish, Wildlife, & Parks	46	1	47	1	46					
Fort Peck Lake	US Army Corps of Engineers	19									
Fort Peck Powerhouse Tailrace	MT Fish, Wildlife, & Parks	10		9		10					
Fort Peck Powerhouse Tailrace	US Army Corps of Engineers	8									
Fort Peck Trout Pond	MT Fish, Wildlife, & Parks	5		5		5					
Four Horns Lake	Blackfeet Nation	40									
Four Horns Lake*	MT Fish, Wildlife, & Parks					117					
Foy Lake	MT Fish, Wildlife, & Parks	2		2		2					
Foy Lake	Whitefish Lake Institute	1									
Freezeout Lake	MT Fish, Wildlife, & Parks	1				1					
Frenchtown Pond	MT Fish, Wildlife, & Parks	1		1		1					
Fresno Reservoir	MT Fish, Wildlife, & Parks	30		30		30					
Gallatin River	MT Fish, Wildlife, & Parks	8	8	9	5						
Gardner River	MT Fish, Wildlife, & Parks	1	1	1	1						
Gartside Reservoir	MT Fish, Wildlife, & Parks	1		1		1					
Georgetown Lake	MT Fish, Wildlife, & Parks	14		14		14					
Gibson Reservoir	MT Fish, Wildlife, & Parks	4		4		4					
Glen Lake	MT Fish, Wildlife, & Parks	2		2		2					
Glen Lake	Whitefish Lake Institute	1									
Goldberg Reservoir	MT Fish, Wildlife, & Parks	1		1		1					
Green Timber Lake	MT Fish, Wildlife, & Parks					1					
Gullwing Reservoir	MT Fish, Wildlife, & Parks	1		1		1					
Halfmoon Lake	Whitefish Lake Institute	1									
Handkerchief Lake	MT Fish, Wildlife, & Parks	1	1	1							
Hansen Reservoir	MT Fish, Wildlife, & Parks	1		1		1					
Hanson-doyle Lake	Whitefish Lake Institute	1									
Harpers Lake	MT Fish, Wildlife, & Parks	2		2		2					
Hauser Reservoir	MT Fish, Wildlife, & Parks	28	8	16	1	7					

LOCATION	AGENCY	Plankton Net - Microscopy	Hand Grab	Kick Net	Rock Picking	Rake Toss	Scuba	Snorkel	Substrate	Mussel-sniffing dogs	Hand grab - eDNA water
Hebgen Lake	MT Fish, Wildlife, & Parks	10	4	9	8	5					
Helena Valley Regulating Reservoir	MT Fish, Wildlife, & Parks	6	1	4		3					
Hidden Lake	MT Fish, Wildlife, & Parks	1		1		1					
Holland Lake	Clearwater Resource Council	10									
Holland Lake	MT Fish, Wildlife, & Parks	4	3	4		1					
Holland Lake	Whitefish Lake Institute	6									
Holter Reservoir	MT Fish, Wildlife, & Parks	28	2	10	2	10					
Homestead Reservoir	MT Fish, Wildlife, & Parks	1		1		1					
Horseshoe Lake	MT Fish, Wildlife, & Parks	2	1	2		1					
Hubbart Reservoir	MT Fish, Wildlife, & Parks	1	1	1							
Hump Lake	MT Fish, Wildlife, & Parks	1		1	1	1			1		
Hungry Horse Reservoir	MT Fish, Wildlife, & Parks	34	25	34		9					
Hyalite Creek	MT Fish, Wildlife, & Parks	1	1	1	1						
Hyalite Reservoir	MT Fish, Wildlife, & Parks	6		6	5	6					
Indian Creek	MT Fish, Wildlife, & Parks		1								
Indian Road Pond	MT Fish, Wildlife, & Parks	1	1	1	1						
Jefferson River	MT Fish, Wildlife, & Parks	2	51	2	2						
Jessup Mill Pond	MT Fish, Wildlife, & Parks	1				2					
Jette Lake	Whitefish Lake Institute	1									
Jocko River	MT Fish, Wildlife, & Parks	1									
Johnson Reservoir	MT Fish, Wildlife, & Parks	1		1		1					
Judith River	MT Fish, Wildlife, & Parks	3	1	3		2					
Karsten Coulee Reservoir	MT Fish, Wildlife, & Parks	1		1		1					
Kicking Horse Reservoir	MT Fish, Wildlife, & Parks	1		1		1					
Kilbrennan Lake	MT Fish, Wildlife, & Parks	1	1	1							
Kolar Reservoir 1	MT Fish, Wildlife, & Parks	1		1	1	1					
Kolar Reservoir 2	MT Fish, Wildlife, & Parks	1		1		1					
Kootenai River	MT Fish, Wildlife, & Parks	9	3	10		6					
Lake Alva	Clearwater Resource Council	12									
Lake Alva	MT Fish, Wildlife, & Parks	4		4		4					
Lake Blaine	MT Fish, Wildlife, & Parks	1		1		1					
Lake Blaine	Whitefish Lake Institute	1									
Lake Como	MT Fish, Wildlife, & Parks	4	4	4							
Lake Elsina	MT Fish, Wildlife, & Parks	1	1	1							
Lake Five	MT Fish, Wildlife, & Parks	2		2		2					
Lake Five	Whitefish Lake Institute	1									
Lake Frances	MT Fish, Wildlife, & Parks	14		7	1	10					
Lake Helena	MT Fish, Wildlife, & Parks	12	2	8	1	7					
Lake Inez	Clearwater Resource Council	12									
Lake Inez	MT Fish, Wildlife, & Parks	2		2		2					
Lake Josephine	MT Fish, Wildlife, & Parks	2		2	1	2					
Lake Koocanusa	MT Fish, Wildlife, & Parks	47	21	30		6					
Lake Marshall	MT Fish, Wildlife, & Parks	1	1	1							
Lake Mary Ronan	MT Fish, Wildlife, & Parks	3		3		3					
Lake Mary Ronan	Whitefish Lake Institute	1									
Lake Sutherlin	MT Fish, Wildlife, & Parks	6	2	6	5	4					
Laurel Pond	MT Fish, Wildlife, & Parks	1		1	1	1					
Lindbergh Lake	Clearwater Resource Council	18									

LOCATION	AGENCY	Plankton Net - Microscopy	Hand Grab	Kick Net	Rock Picking	Rake Toss	Scuba	Snorkel	Substrate	Mussel-sniffing dogs	Hand grab - eDNA water
Lindbergh Lake	MT Fish, Wildlife, & Parks	2		2		2					
Lindbergh Lake	Whitefish Lake Institute	1									
Lion Lake	MT Fish, Wildlife, & Parks	2	1	2		1					
Little Bitterroot Lake	Little Bitterroot Lake Association	5									
Little Bitterroot Lake	MT Fish, Wildlife, & Parks	3		3		3					
Little Bitterroot Lake	Whitefish Lake Institute	1									
Little Loon Lake	MT Fish, Wildlife, & Parks	2	1	2		1					
Little Therriault Lake	MT Fish, Wildlife, & Parks	1		1							
Little Warm Reservoir	MT Fish, Wildlife, & Parks	1		1		1					
Loon Lake	MT Fish, Wildlife, & Parks	3	2	3		1					
Lost Coon Lake	Whitefish Lake Institute	1									
Lower Glaston Lake	MT Fish, Wildlife, & Parks	2		2	1	2					
Lower Jocko Lake	MT Fish, Wildlife, & Parks	1	1	1							
Lower Saint Mary Lake	Blackfeet Nation	40									
Lower Saint Mary Lake*	MT Fish, Wildlife, & Parks					175					
Lower Stillwater Lake	MT Fish, Wildlife, & Parks	2	1	2		1					
Lower Stillwater Lake	Whitefish Lake Institute	1									
Lower Willow Creek Reservoir	MT Fish, Wildlife, & Parks	1		1		1					
Madison River	MT Fish, Wildlife, & Parks	17	15	16	15	1					
Marias River	MT Fish, Wildlife, & Parks	17		12	2	14					
Martinsdale Reservoir	MT Fish, Wildlife, & Parks	4		4	2	4					
McDonald Creek	Glacier National Park	1									
Mcdonald Lake	Glacier National Park	3									
Mcdonald Lake	MT Fish, Wildlife, & Parks	1	1	1							
Mcgilvray Lake	Whitefish Lake Institute	1									
Mcgregor Lake	MT Fish, Wildlife, & Parks	2		1		2					
Medicine Lake	MT Fish, Wildlife, & Parks	4		4		4					
Miles City Hatchery Pond	MT Fish, Wildlife, & Parks	1	1	2	2						
Milk River	MT Fish, Wildlife, & Parks	24		24		24					
Mission Lake	Blackfeet Nation	40									
Mission Lake*	MT Fish, Wildlife, & Parks					110					
Mission Reservoir, In Part	MT Fish, Wildlife, & Parks	1	1	1							
Missouri River	Broadwater Conservation District	2									
Missouri River	MT Fish, Wildlife, & Parks	61	25	48	19	34					
Morrison Lake	MT Fish, Wildlife, & Parks	1	1	1	1						
Murphy Lake	MT Fish, Wildlife, & Parks	1		1		1					
Murphy Lake	Whitefish Lake Institute	1									
Murray Lake	Whitefish Lake Institute	1									
Musselshell River	MT Fish, Wildlife, & Parks	10	8	10	9	1					
Nelson Dredge	MT Fish, Wildlife, & Parks	4		4		4					
Nelson Dredge	US Army Corps of Engineers	3									
Nelson Reservoir	MT Fish, Wildlife, & Parks	15		15		15					
Nevada Reservoir	MT Fish, Wildlife, & Parks	4		4		4					
Newlan Reservoir	MT Fish, Wildlife, & Parks	6		6	6	6					
Nilan Reservoir	MT Fish, Wildlife, & Parks	1		1		1					
North Fork Flathead River	MT Fish, Wildlife, & Parks	4	4	4							
North Polly Reservoir	MT Fish, Wildlife, & Parks	1									
Noxon Rapids Reservoir*	MT Fish, Wildlife, & Parks	14		14		649					

LOCATION	AGENCY	Plankton Net - Microscopy	Hand Grab	Kick Net	Rock Picking	Rake Toss	Scuba	Snorkel	Substrate	Mussel-sniffing dogs	Hand grab - eDNA water
Number One, Pond	MT Fish, Wildlife, & Parks	1	1	1							
Number Three, Pond	MT Fish, Wildlife, & Parks	1		2		1					
Number Two, Pond	MT Fish, Wildlife, & Parks	1		1		1					
O'juel Lake	MT Fish, Wildlife, & Parks	1		1		1					
Ostle Reservoir	MT Fish, Wildlife, & Parks	1		1		1					
Painted Rocks Reservior	MT Fish, Wildlife, & Parks	4	1	4		3					
Paulo Reservoir	MT Fish, Wildlife, & Parks	1		1		1					
Payola Reservoir	MT Fish, Wildlife, & Parks	1		1	1	1					
Peck Lake	MT Fish, Wildlife, & Parks	1	1	1							
Peterson Lake	MT Fish, Wildlife, & Parks	2									
Peterson Lake	Whitefish Lake Institute	1									
Petrolia Reservoir	MT Fish, Wildlife, & Parks	3		3	1	3					
Pishkun Reservoir	MT Fish, Wildlife, & Parks	2		2		2					
Placid Lake	Clearwater Resource Council	14									
Placid Lake	MT Fish, Wildlife, & Parks	11		10		11					
Post Creek	MT Fish, Wildlife, & Parks	1	1		1						
Priest Butte Lake	MT Fish, Wildlife, & Parks	1		1		1					
Quake Lake	MT Fish, Wildlife, & Parks	1		1	1	1					
Rainy Lake	MT Fish, Wildlife, & Parks	3	1	3		2					
Raymond Dam	MT Fish, Wildlife, & Parks	1		1		1					
Red Meadow Lake	MT Fish, Wildlife, & Parks	1	1	1							
Redwater River	MT Fish, Wildlife, & Parks	1		1		1					
Regional Parks Ponds	MT Fish, Wildlife, & Parks	1		1	1	1					
Rock Creek	MT Fish, Wildlife, & Parks	8	8	8							
Rock Lake	MT Fish, Wildlife, & Parks	1	1	1							
Roe River	MT Fish, Wildlife, & Parks	1	1	1							
Rogers Lake	MT Fish, Wildlife, & Parks	1		1		1					
Rogers Lake	Whitefish Lake Institute	1									
Rose Creek	MT Fish, Wildlife, & Parks	1									
Rose Creek Slough	MT Fish, Wildlife, & Parks	1									
Ross Lake	MT Fish, Wildlife, & Parks	1		1	1	1			1		
Ruby River	MT Fish, Wildlife, & Parks	5	5	5	5						
Ruby River Reservoir	MT Fish, Wildlife, & Parks	7	3	7	6	4					
Sagebrush Reservoir	MT Fish, Wildlife, & Parks	1		1		1					
Saint Regis River	MT Fish, Wildlife, & Parks	4	4	4							
Salmon Lake	Clearwater Resource Council	27									
Salmon Lake	MT Fish, Wildlife, & Parks	11	6	11		5					
Savage Lake	MT Fish, Wildlife, & Parks	2	2	1		1					
Seeley Lake	Clearwater Resource Council	27									
Seeley Lake	MT Fish, Wildlife, & Parks	14		14		14					
Shields River	MT Fish, Wildlife, & Parks	4	4	4	4						
Silver Lake	MT Fish, Wildlife, & Parks	1	1	1							
Skalkaho Creek	MT Fish, Wildlife, & Parks	1	1	1							
Skyles Lake	Whitefish Lake Institute	1									
Smith Lake	MT Fish, Wildlife, & Parks	1		1		1					
Smith Lake	Whitefish Lake Institute	1									
Smith River	MT Fish, Wildlife, & Parks	6	5	6	5	1					
Sophie Lake	MT Fish, Wildlife, & Parks	1		1		1					
Sophie Lake	Whitefish Lake Institute	1									

LOCATION	AGENCY	Plankton Net - Microscopy	Hand Grab	Kick Net	Rock Picking	Rake Toss	Scuba	Snorkel	Substrate	Mussel-sniffing dogs	Hand grab - eDNA water
South Fork Fish Creek	MT Fish, Wildlife, & Parks	1	1	1							
South Fork Flathead River	MT Fish, Wildlife, & Parks	1	1	1							
South Sandstone Reservoir	MT Fish, Wildlife, & Parks	4		4	4	4					
Spar Lake	MT Fish, Wildlife, & Parks	2	1	2		1					
Spencer Lake	Whitefish Lake Institute	1									
Spook Lake	MT Fish, Wildlife, & Parks	1		1		1					
Spotted Eagle Lake	MT Fish, Wildlife, & Parks	4	1	4	4	3					
Spring Branch	MT Fish, Wildlife, & Parks				1						
Spring Meadow Lake	MT Fish, Wildlife, & Parks	6		3		3					
St Marys Lake	MT Fish, Wildlife, & Parks	1	1	1							
St. Mary Lake	Glacier National Park	4									
Stillwater River	MT Fish, Wildlife, & Parks	5	4	5	4	1					
Sun River	MT Fish, Wildlife, & Parks	6		6		6					
Swan Lake	Clearwater Resource Council	30									
Swan Lake	MT Fish, Wildlife, & Parks	5	1	5		4					
Swan Lake	Whitefish Lake Institute	2									
Swift Reservoir	MT Fish, Wildlife, & Parks	2		2		2					
Taint Reservoir	MT Fish, Wildlife, & Parks	1		1		1					
Tally Lake	MT Fish, Wildlife, & Parks	4	2	4		2					
Tally Lake	Whitefish Lake Institute	3									
Tenmile Creek	MT Fish, Wildlife, & Parks	1	2	1							
Tepee Lake	MT Fish, Wildlife, & Parks	1		1		1					
Tetrault Lake	MT Fish, Wildlife, & Parks	1		1		1					
Tetrault Lake	Whitefish Lake Institute	1									
Thompson Falls Reservoir	MT Fish, Wildlife, & Parks	2	2	2							
Thompson Lake, Lower	MT Fish, Wildlife, & Parks	6	2	6		4					
Thompson Lake, Middle	MT Fish, Wildlife, & Parks	4		4		4					
Thompson Lake, Upper	MT Fish, Wildlife, & Parks	6	1	6		5					
Thompson Park Pond	MT Fish, Wildlife, & Parks	1		1		2					
Three Forks Pond	MT Fish, Wildlife, & Parks	2		1	1	1					
Three Forks Pond East	MT Fish, Wildlife, & Parks	1		1	1	1					
Three Forks Pond West	MT Fish, Wildlife, & Parks			1		1					
Tiber Reservoir	MT Fish, Wildlife, & Parks	103	1	2	1	1	4	10	14	14	26
Tongue River	MT Fish, Wildlife, & Parks	1		1	1	1					
Tongue River Reservoir	MT Fish, Wildlife, & Parks	14	3	14	11	9					
Topless Lake	MT Fish, Wildlife, & Parks	2		2		2					
Triangle Pond	MT Fish, Wildlife, & Parks	2	1	2		1					
Trout Lake	MT Fish, Wildlife, & Parks	1	1	1							
Tuppers Lake	MT Fish, Wildlife, & Parks	1	1	1							
Twin Lakes	MT Fish, Wildlife, & Parks	1		1		1					
Two Medicine Lake	Glacier National Park	3									
Unnamed	MT Fish, Wildlife, & Parks				2						
Upper Carters Pond	MT Fish, Wildlife, & Parks	1		1		1					
Upper Holter Lake	MT Fish, Wildlife, & Parks	2	2	2							
Upper Stillwater Lake	MT Fish, Wildlife, & Parks	2		2		2					
Upper Stillwater Lake	Whitefish Lake Institute	1									
Upper Whitefish Lake	MT Fish, Wildlife, & Parks	1		1		1					
Upper Whitefish Lake	Whitefish Lake Institute	1									
Upsata Lake	Clearwater Resource Council	15									

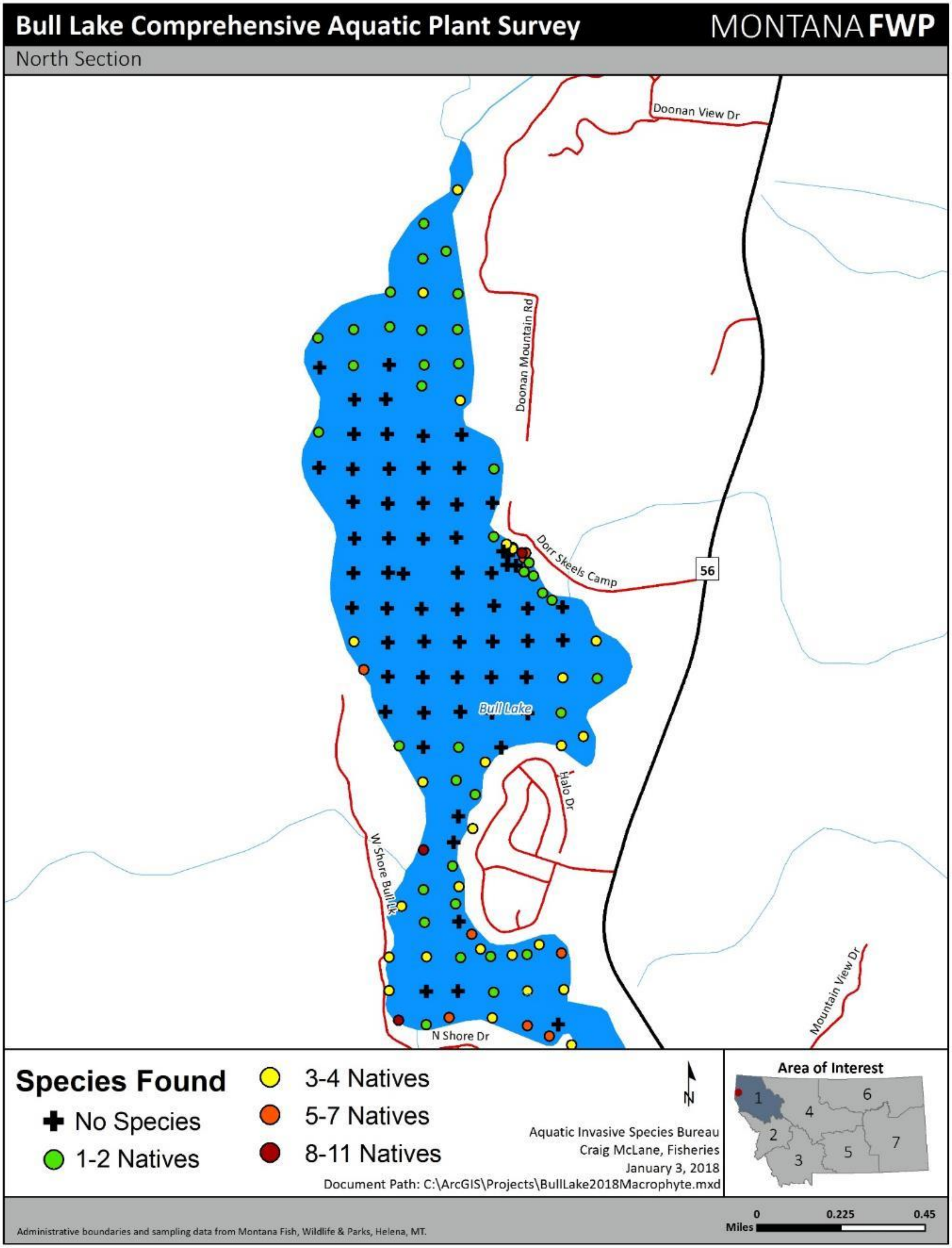
LOCATION	AGENCY	Plankton Net - Microscopy	Hand Grab	Kick Net	Rock Picking	Rake Toss	Scuba	Snorkel	Substrate	Mussel-sniffing dogs	Hand grab - eDNA water
Upsata Lake	MT Fish, Wildlife, & Parks	2		2		2					
Valley Reservoir	MT Fish, Wildlife, & Parks	1		1		1					
Van Lake	Clearwater Resource Council	12									
Van Lake	MT Fish, Wildlife, & Parks	2	2	2							
Wade Lake	MT Fish, Wildlife, & Parks	3	3	1	1	2					
Wapiti Reservoir	MT Fish, Wildlife, & Parks	1		1		1					
Warm Springs Creek	MT Fish, Wildlife, & Parks	1	1		1						
Waterton Lake	Glacier National Park	3									
Wayne Edsall Pond	MT Fish, Wildlife, & Parks	1		1		1					
West Boulder River	MT Fish, Wildlife, & Parks	1	1	1	1						
West Fork Bitterroot River	MT Fish, Wildlife, & Parks	1	1	1							
West Fork Gallatin River	MT Fish, Wildlife, & Parks	2	1	2	1						
Whitefish Lake	MT Fish, Wildlife, & Parks	11	1	12		10					
Whitefish Lake	Whitefish Lake Institute	23									
Willow Creek Reservoir	MT Fish, Wildlife, & Parks	4		4	1	3					
Wood Lake	MT Fish, Wildlife, & Parks	1		1							
Yellow Water Reservoir	MT Fish, Wildlife, & Parks	2		2	2	2					
Yellowstone River	MT Fish, Wildlife, & Parks	52	45	49	48	6					
Yellowtail Afterbay Reservoir	MT Fish, Wildlife, & Parks	6		6	6	6					

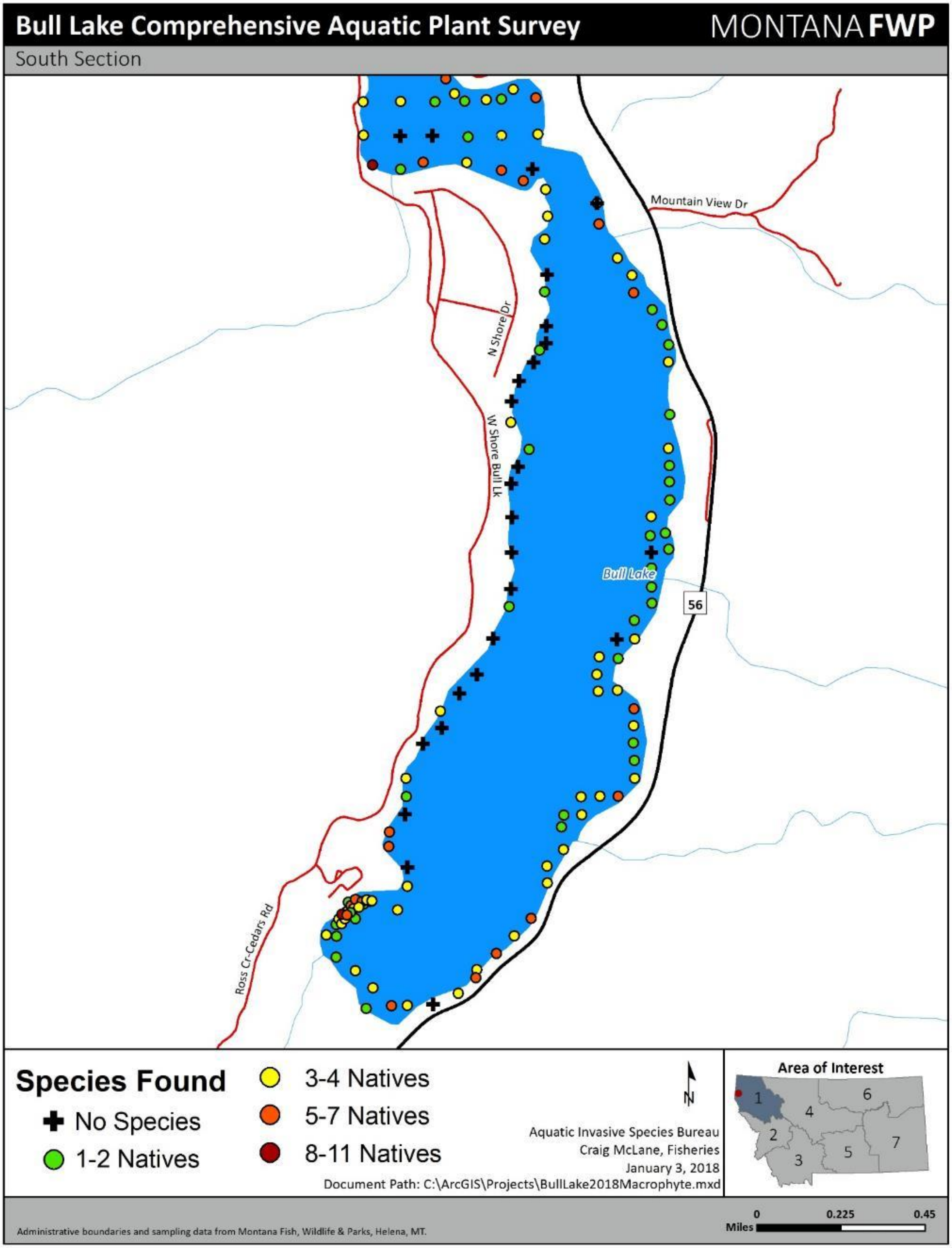
Appendix B. Results of Aquatic Plant Surveys

This appendix contains details of plant sampling within the listed waterbodies. Plant locations and species frequency (based on all sample points within the water body) are noted for each waterbody surveyed.

1. Bull Lake

Frequency of Species in Waterbody	Percent Occurrence of Points in Waterbody	Common Name	Scientific Name
89	33.1%	No Species	No Species
117	43.5%	Elodea	Elodea canadensis
81	30.1%	Stonewort	Nitella spp
49	18.2%	Northern Watermilfoil	Myriophyllum sibiricum
49	18.2%	Fern-leaved pondweed	Potamogeton robbinsii
37	13.8%	Common arrowhead	Sagittaria latifolia
37	13.8%	Bulrush spp	Scirpus spp
35	13.0%	Muskgrass	Chara spp
34	12.6%	White Stemmed Pondweed	Potamogeton praelongus
33	12.3%	Slender water-nymph	Najas flexilis
32	11.9%	Quillwort	Isoetes spp
15	5.6%	White Water Buttercup	Ranunculus aquatilis
5	1.9%	Beck's Water-marigold	Bidens beckii
5	1.9%	Pond water-starwort	Callitriche stagnalis
5	1.9%	Rush Spp	Juncus spp
3	1.1%	Water Star-wort (Unspecified)	Callitriche spp
3	1.1%	Horsetail	Equisetum spp
2	0.7%	Unidentified Pondweed	Potamogeton spp
1	0.4%	Narrowleaf water-plantain	Alisma gramineum
1	0.4%	Common wate-rnymph	Najas guadalupensis
1	0.4%	Spatterdock	Nuphar polysepala
1	0.4%	Water smartweed	Polygonum amphibium
1	0.4%	Large-leaf pondweed	Potamogeton amplifolius
1	0.4%	Floating-leaved pondweed	Potamogeton natans
1	0.4%	Northern arrowhead	Sagittaria cuneata


Bull Lake Comprehensive Aquatic Plant Survey
MONTANA FWP
North Section
Doonan View Dr
Doonan Mountain Rd
Dorr Skeels Camp
56
Bull Lake
Halo Dr
W Shore Bull Lk
N Shore Dr
Mountain View Dr
Species Found
No Species
1-2 Natives
3-4 Natives
5-7 Natives
8-11 Natives
N
Aquatic Invasive Species Bureau
Craig McLane, Fisheries
January 3, 2018
Document Path: C:\ArcGIS\Projects\BullLake2018Macrophyte.mxd
Area of Interest
1
2
3
4
5
6
7
Administrative boundaries and sampling data from Montana Fish, Wildlife & Parks, Helena, MT.
0
0.225
0.45
Miles



Bull Lake Comprehensive Aquatic Plant Survey
MONTANA FWP
South Section
Mountain View Dr
N Shore Dr
W Shore Bull Lk
Bull Lake
56
Ross Cr-Cedars Rd
Species Found
No Species
1-2 Natives
3-4 Natives
5-7 Natives
8-11 Natives
N
Aquatic Invasive Species Bureau
Craig McLane, Fisheries
January 3, 2018
Document Path: C:\ArcGIS\Projects\BullLake2018Macrophyte.mxd
Area of Interest
1
2
3
4
5
6
7
Administrative boundaries and sampling data from Montana Fish, Wildlife & Parks, Helena, MT.
0
0.225
0.45
Miles

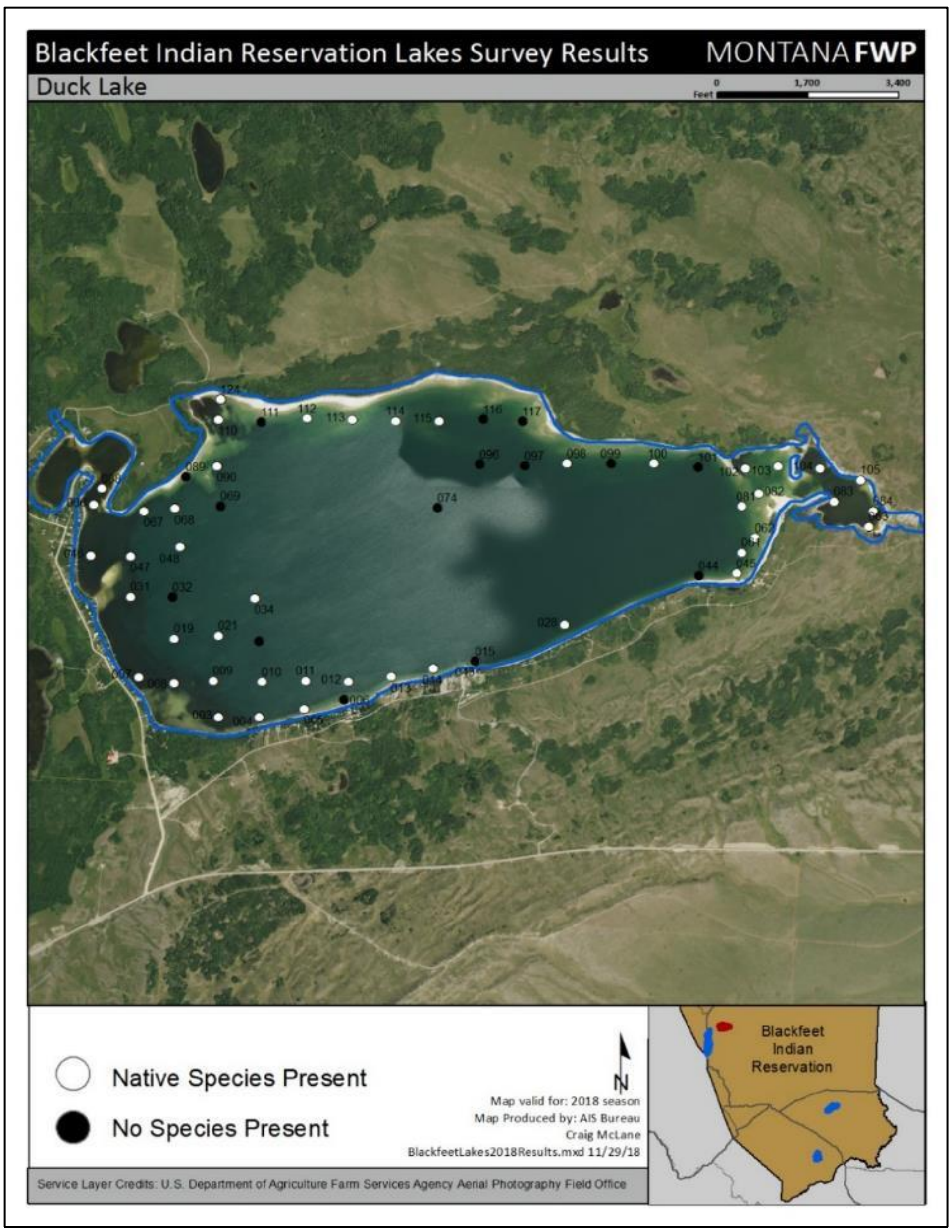
2. *Cabinet Gorge Reservoir*

Detailed maps of sampling results for the pre-treatment survey, post-treatment survey, and reservoir-wide surveys can be obtained by emailing Craig McLane cmclane@mt.gov. 336 sample points for reservoir wide survey.

Frequency of Species in Waterbody	Percent Occurrence of Points in Waterbody	Common Name	Scientific Name
72	21.4%	No Species	No Species
212	63.1%	Elodea	*Elodea canadensis*
165	49.1%	Eurasian Watermilfoil	*Myriophyllum spicatum*
158	47.0%	Coontail	*Ceratophyllum demersum*
42	12.5%	Richardson's Pondweed	*Potamogeton richardsonii*
39	11.6%	Curlyleaf Pondweed	*Potamogeton crispus*
24	7.1%	Leafy Pondweed	*Potamogeton foliosus*
23	6.8%	White Water Buttercup	*Ranunculus aquatilis*
21	6.3%	Muskgrass	Chara spp.
10	3.0%	Northern Watermilfoil	*Myriophyllum sibiricum*
6	1.8%	Sheathed Pondweed	*Potamogeton vaginatus*
5	1.5%	Flowering Rush	*Butomus umbellatus*
4	1.2%	Stonewort	Nitella spp.
4	1.2%	Sago Pondweed	*Stuckenia pectinata*
3	0.9%	Largeleaf Pondweed	*Potamogeton amplifolius*
2	0.6%	Quillwort	Isoetes spp.
2	0.6%	Unspecified Pondweed	Potamogeton spp.
1	0.3%	Water Stargrass	*Heteranthera dubia*
1	0.3%	Flatstem Pondweed	*Potamogeton zosteriformis*
1	0.3%	Arumleaf Arrowhead	*Sagitaria cunneata*

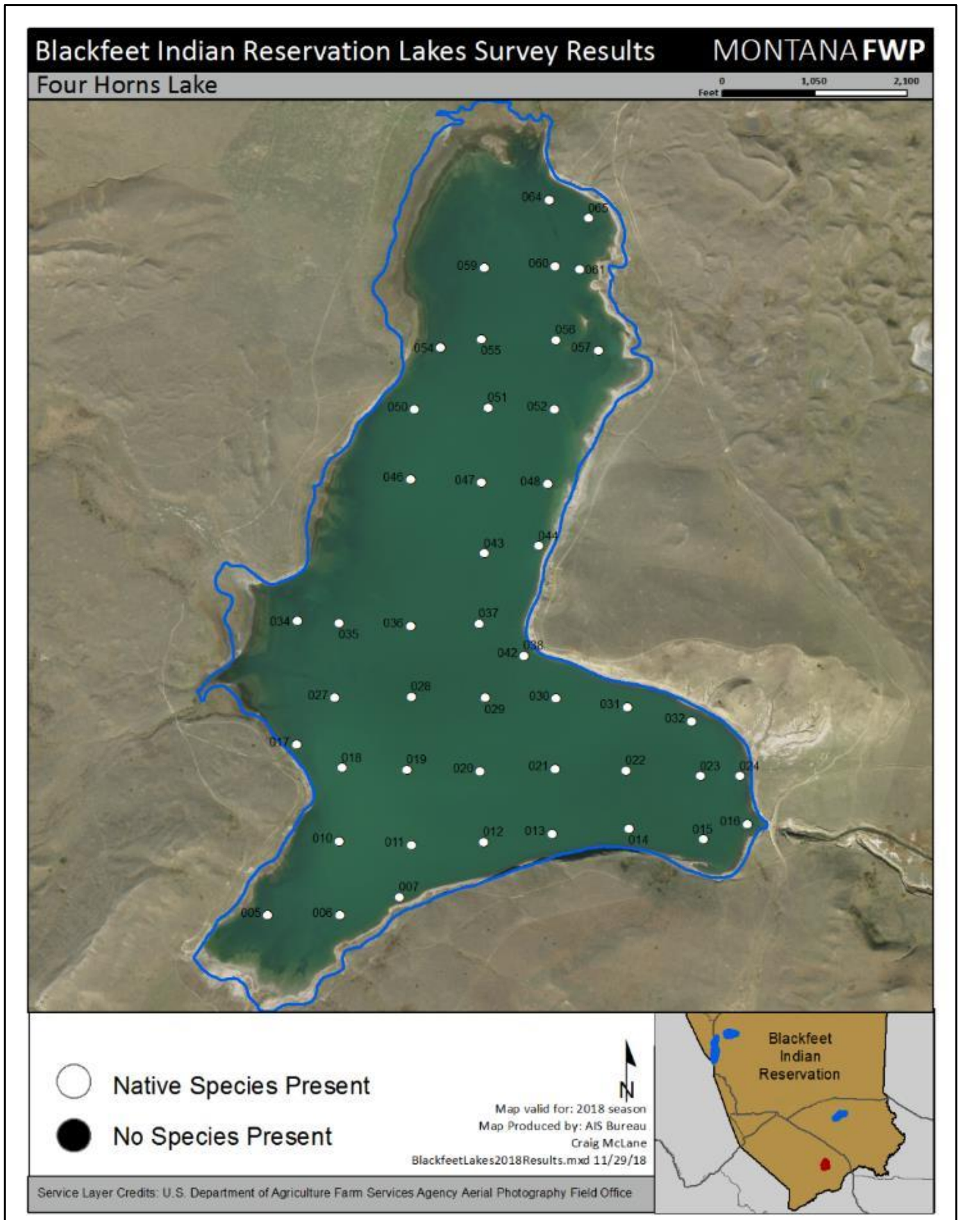
3. *Duck Lake*

Frequency of Species in Waterbody	Percent Occurrence of Points in Waterbody	Common Name	Scientific Name
15	14.7%	No Species	No species present
26	25.5%	Common Water Moss	*Fontinalis antipyretica*
18	17.6%	Northern Watermilfoil	*Myriophyllum sibiricum*
16	15.7%	Slender-leaved Pondweed	*Potamogeton filiformis*
13	12.7%	Muskgrass	Chara species
10	9.8%	Sago Pondweed	*Stuckenia pectinata*
1	1.0%	Quillwort	Isoetes species
1	1.0%	Unidentified Pondweed	Potamogeton species
1	1.0%	Leafy Pondweed	*Potamogeton foliosus*
1	1.0%	Sheathed Pondweed	*Potamogeton vaginatus*



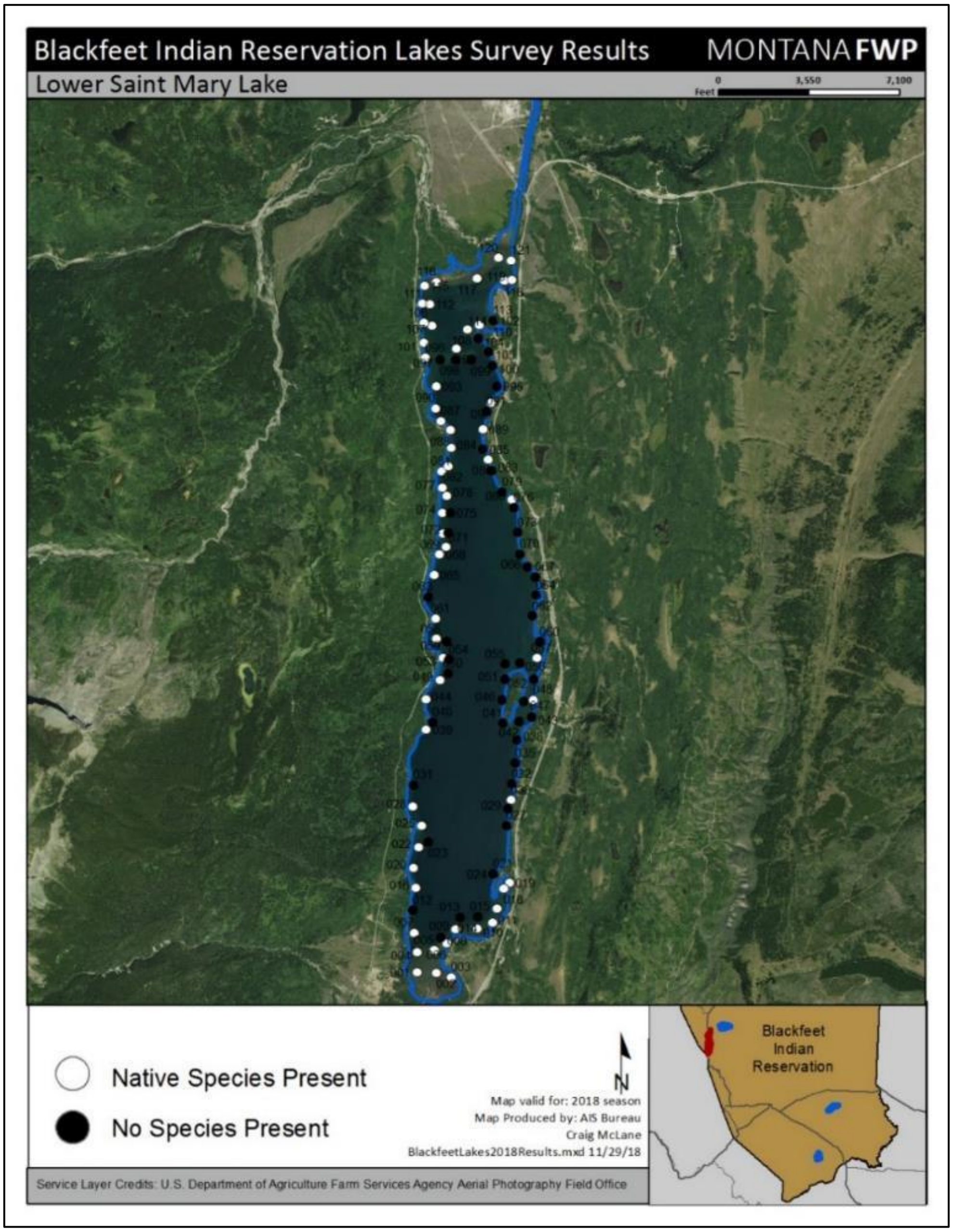
4. Four Horns Lake

Frequency of Species in Waterbody	Percent Occurrence of Points in Waterbody	Common Name	Scientific Name
32	27.4%	Muskgrass	Chara species
28	23.9%	Canada waterweed	*Elodea canadensis*
21	17.9%	Coontail	*Ceratophyllum demersum*
11	9.4%	Richardson's Pondweed	*Potamogeton richardsonii*
8	6.8%	Sago Pondweed	*Stuckenia pectinata*
5	4.3%	Northern Watermilfoil	*Myriophyllum sibiricum*
4	3.4%	White water buttercup	*Ranunculus aquatilis*
3	2.6%	Mare's Tail	*Hippuris vulgaris*
3	2.6%	Leafy Pondweed	*Potamogeton foliosus*
1	0.9%	Stonewort	*Nitella species*
1	0.9%	White-stemmed Pondweed	*Potamogeton praelongus*



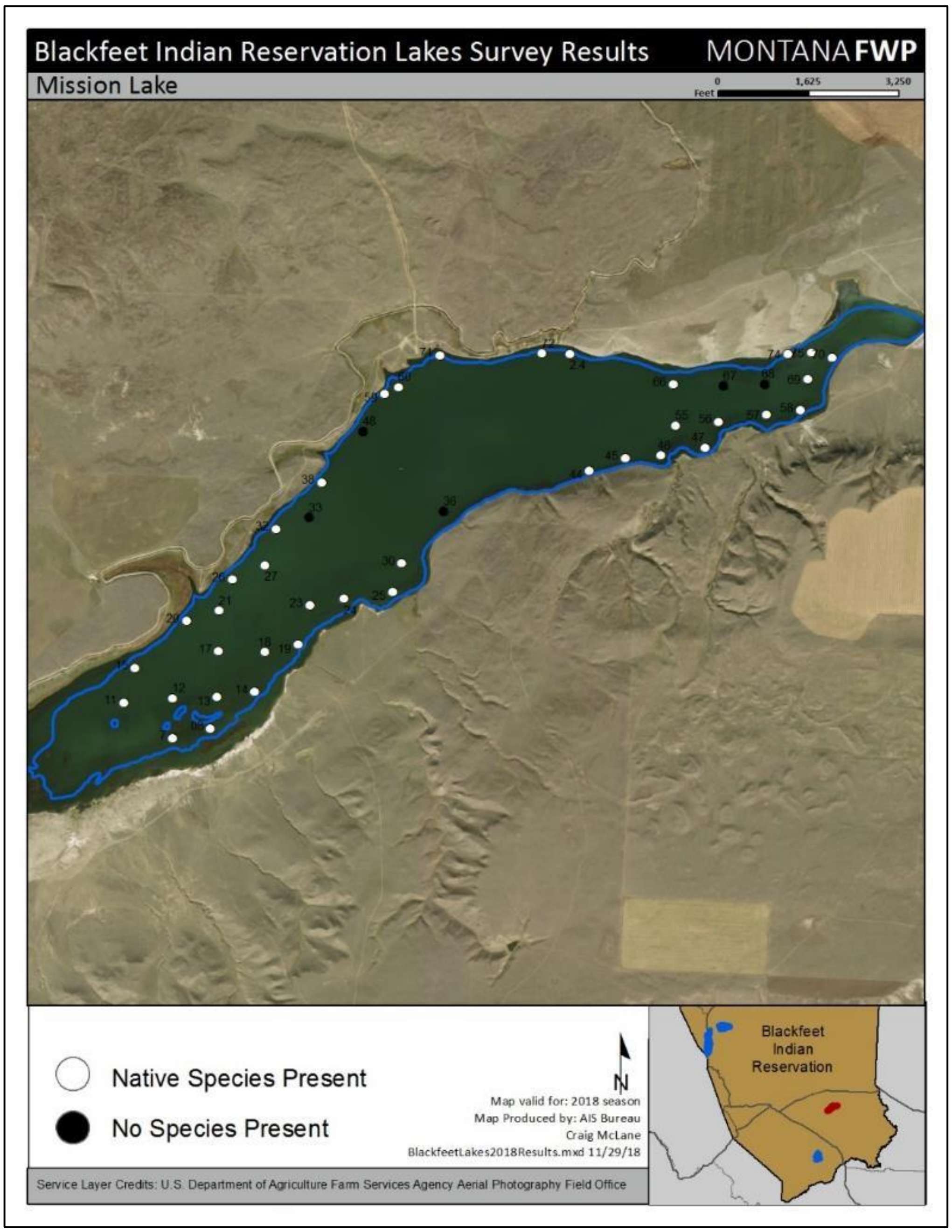
5. Lower Saint Mary Lake

Frequency of Species in Waterbody	Percent Occurrence of Points in Waterbody	Common Name	Scientific Name
49	28.0%	No Species	No species present
44	25.1%	Richardson's Pondweed	*Potamogeton richardsonii*
23	13.1%	Northern Watermilfoil	*Myriophyllum sibiricum*
13	7.4%	Muskgrass	Chara species
12	6.9%	White water buttercup	*Ranunculus aquatilis*
9	5.1%	White-stemmed Pondweed	*Potamogeton praelongus*
8	4.6%	Slender-leaved Pondweed	*Potamogeton filiformis*
5	2.9%	Bigleaf Pondweed	*Potamogeton amplifolious*
4	2.3%	Quillwort	Isoetes species
3	1.7%	Unidentified Pondweed	Potamogeton species
3	1.7%	Sago Pondweed	*Stuckenia pectinata*
1	0.6%	Common Water Moss	*Fontinalis antipyretica*
1	0.6%	Robbin's Pondweed	*Potamogeton robbinsii*



6. Mission Lake

Frequency of Species in Waterbody	Percent Occurrence of Points in Waterbody	Common Name	Scientific Name
5	4.5%	No Species	No species present
28	25.5%	Ditchgrass	*Ruppia cirrhosa*
21	19.1%	Northern Watermilfoil	*Myriophyllum sibiricum*
18	16.4%	Coontail	*Ceratophyllum demersum*
9	8.2%	Sheathed Pondweed	*Potamogeton vaginatus*
7	6.4%	Muskgrass	Chara species
7	6.4%	Sago Pondweed	*Stuckenia pectinata*
4	3.6%	Leafy Pondweed	*Potamogeton foliosus*
4	3.6%	Richardson's Pondweed	*Potamogeton richardsonii*
3	2.7%	Freshwater Sponge	Spongillidae species
2	1.8%	Unidentified Pondweed	Potamogeton species
1	0.9%	Slender-leaved Pondweed	*Potamogeton filiformis*
1	0.9%	White-stemmed Pondweed	*Potamogeton praelongus*

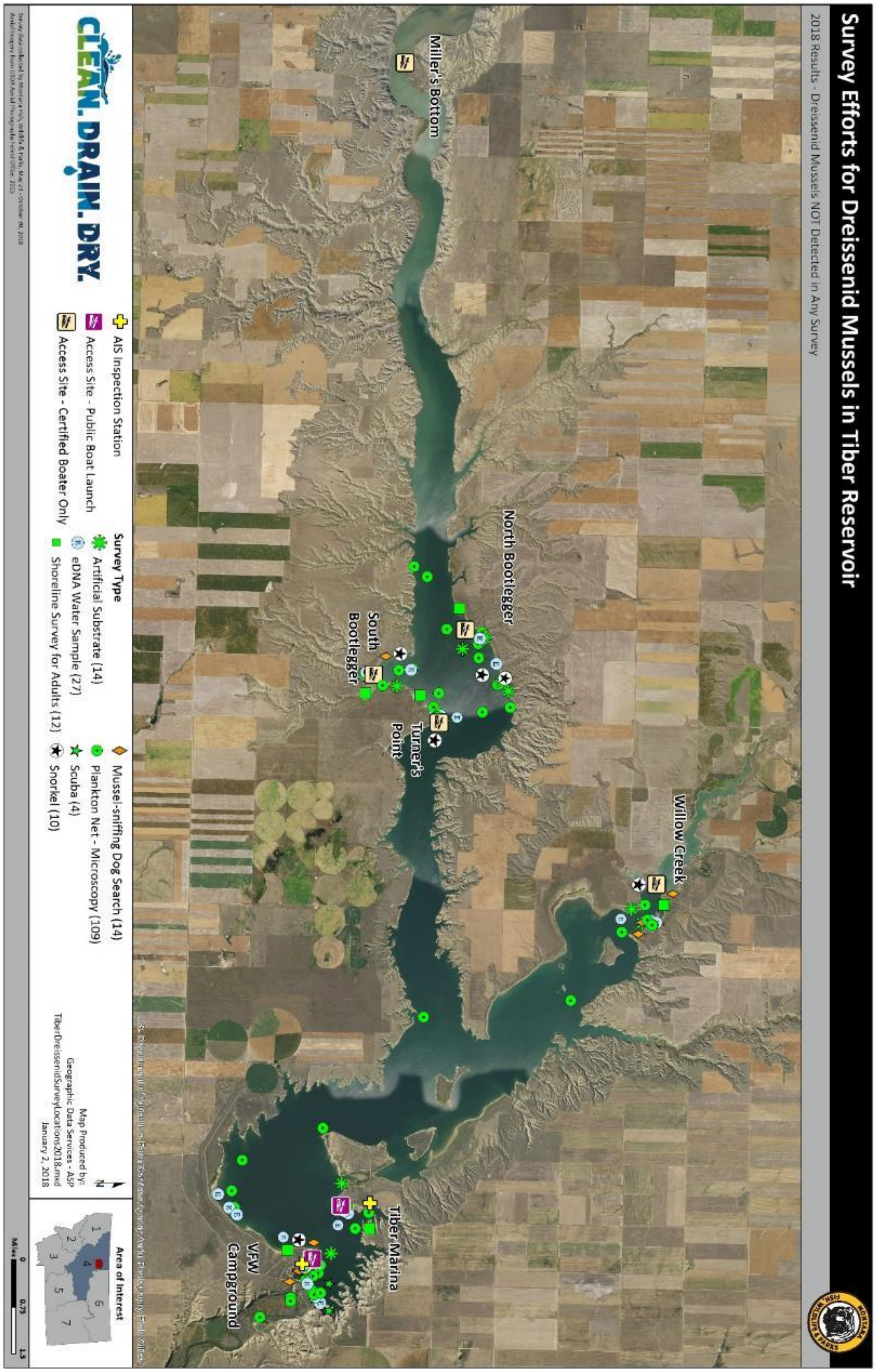


7. Noxon Rapids Reservoir

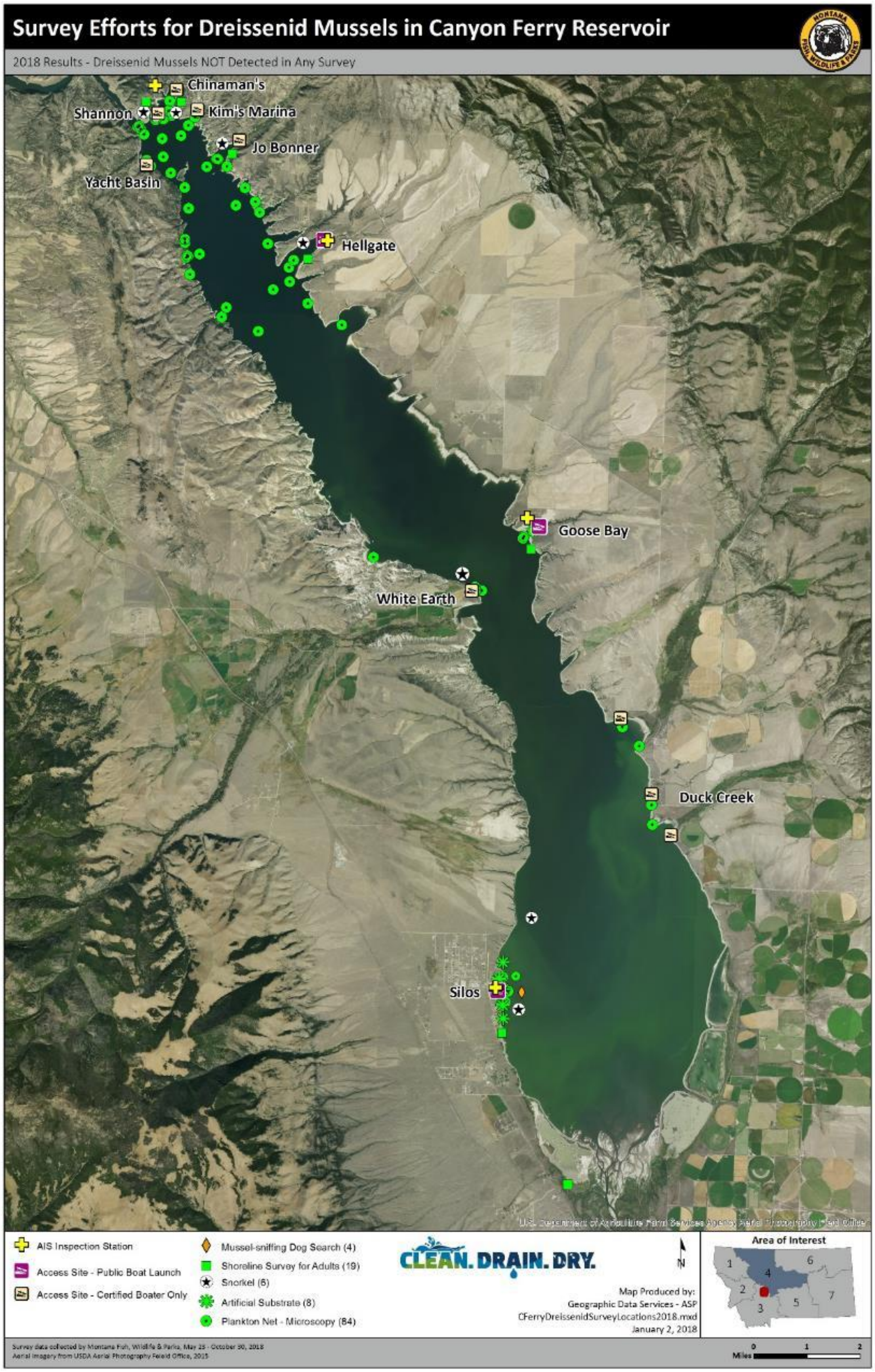
Detailed maps of sampling results for the pre-treatment survey, post-treatment survey, and reservoir-wide surveys can be obtained by emailing Craig McLane cmclane@mt.gov. 649 sample points for reservoir-wide survey.

Frequency of Species in Waterbody	Percent Occurrence of Points in Waterbody	Common Name	Scientific Name
56	8.6%	no species found at point	No Species
418	64.4%	coontail	*Ceratophyllum demersum*
388	59.8%	Eurasian watermilfoil	*Myriophyllum spicatum*
314	48.4%	elodea	*Elodea canadensis*
132	20.3%	curlyleaf pondweed	*Potamogeton crispus*
126	19.4%	leafy pondweed	*Potamogeton foliosus*
101	15.6%	muskgrass	*Chara spp.*
87	13.4%	Richardson's pondweed	*Potamogeton richardsonii*
77	11.9%	northern watermilfoil	*Myriophyllum sibiricum*
61	9.4%	flowering rush	*Butomus umbellatus*
43	6.6%	unspecified pondweed	*Potamogeton spp.*
32	4.9%	sago pondweed	*Stuckenia pectinata*
27	4.2%	white water buttercup	*Ranunculus aquatilis*
14	2.2%	whitestem pondweed	*Potamogeton praelongus*
5	0.8%	ribbon-leaved pondweed	*Potamogeton epihydrus*
4	0.6%	Unidentified species	*Unidentified spp.*
2	0.3%	common waternymph	*Najas guadalupensis*
2	0.3%	variableleaf pondweed	*Potamogeton gramineus*
2	0.3%	sheathed pondweed	*Potamogeton vaginatus*
2	0.3%	arumleaf arrowhead	*Sagitaria cunneata*
1	0.2%	water stargrass	*Heteranthera dubia*
1	0.2%	largeleaf pondweed	*Potamogeton amplifolius*
1	0.2%	horned pondweed	*Zannichellia palustris*

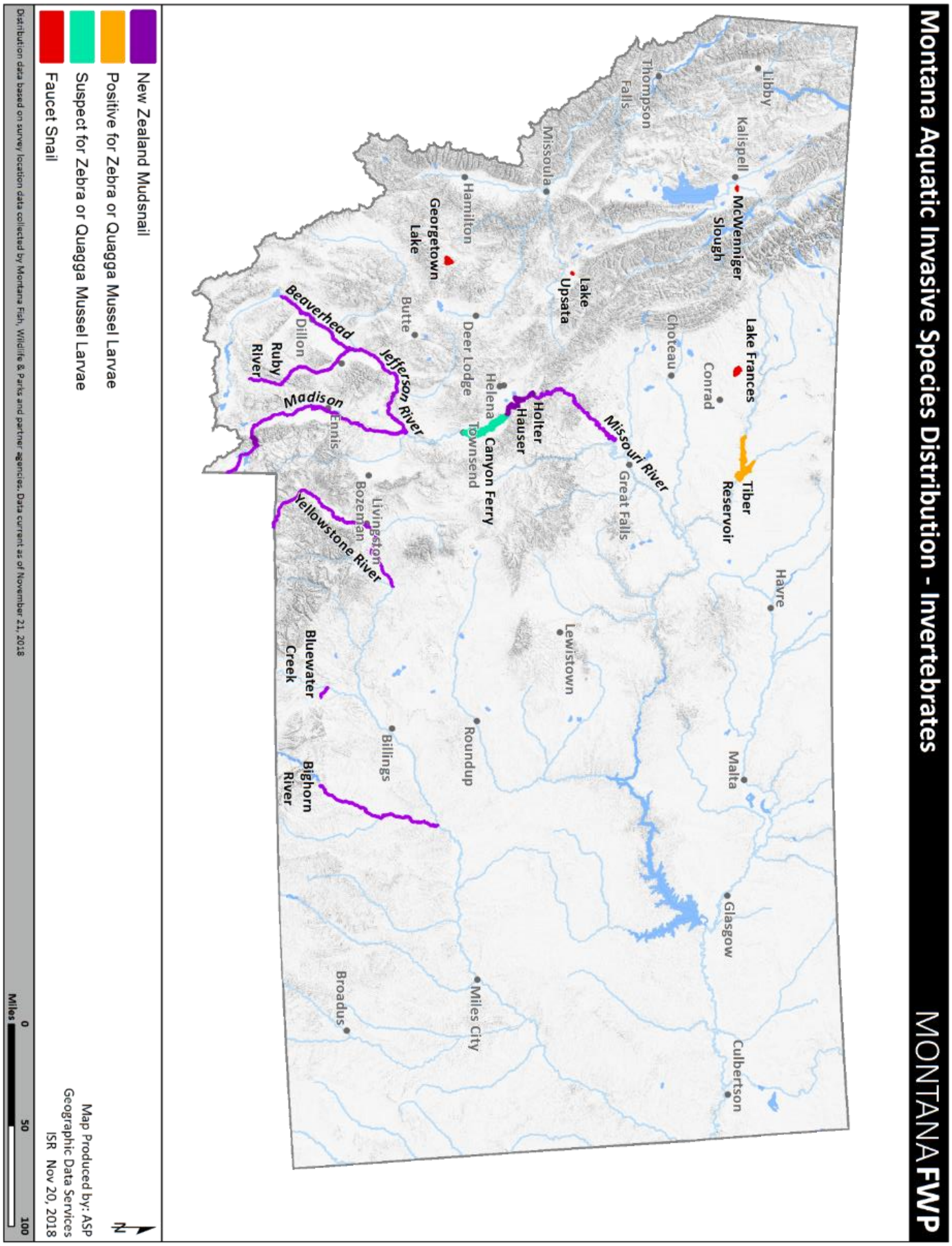
Appendix C. Mussel response sampling events on Tiber Reservoir



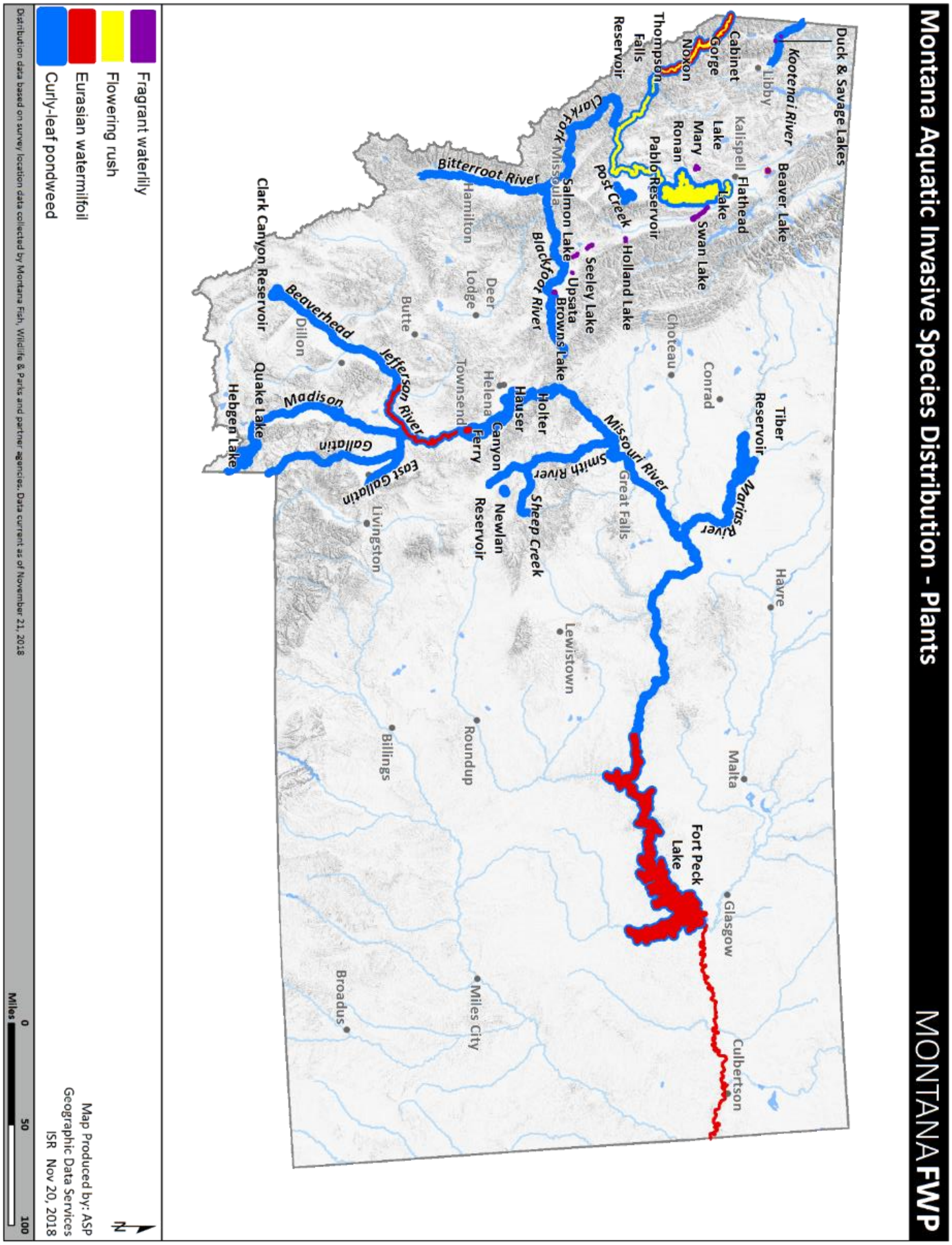
Appendix D. Mussel response sampling events on Canyon Ferry Reservoir



Appendix E. Map of invasive mollusks in Montana



Appendix F. Map of invasive plants in Montana



Appendix G. Dreissenid Mussel Invasion Potential Criteria

Yellow: Habitat Variable

Green: Social Variable

Red: Final Rank either Habitat or Social

ID	MinValue	Max Value	Parameter	Score
1	0	40	Water Temp	1
2	40.1	46	Water Temp	2
3	46.01	56	Water Temp	3
4	56.01	71	Water Temp	4
5	71.01	75	Water Temp	3
6	75.01	83	Water Temp	2
7	83.01	120	Water Temp	1
8	0	3.9	pH	1
9	4	5.4	pH	2
10	5.5	6.9	pH	3
11	7	9.9	pH	4
12	10	11	pH	3
13	11.1	12.9	pH	2
14	13	14	pH	1
15	0	50	Hardness	1
16	50.1	99	Hardness	2
17	99.1	125	Hardness	3
18	125	1000	Hardness	4
19	0	4	Calcium	1
20	4.1	13	Calcium	2
21	13.1	24	Calcium	3
22	24.1	100	Calcium	4
23	0	490	Conductivity	1
24	491	989	Conductivity	2
25	990	1499	Conductivity	3
26	1500	3000	Conductivity	4
27	0	3	Dissolved Oxygen	1
28	3.1	7	Dissolved Oxygen	2
29	7.01	12	Dissolved Oxygen	3
30	12.01	50	Dissolved Oxygen	4
31	1	100	Percent Boulder	4
32	1	100	Percent Cobble	4
33	1	100	Percent Bedrock	4
34	Low use <25% quartile		Angler Days	1
35	Medium Low 26- 50% quartile		Angler Days	2

ID	MinValue	Max Value	Parameter	Score
36	Medium High 51-75% quartile		Angler Days	3
37	High Use > 75% quartile		Angler Days	4
38	very far away		Mussel Proximity	1
39	not close but still accessible		Mussel Proximity	2
40	nearby, but may not be as easily accessible		Mussel Proximity	3
41	downstream, connected, or within easy drive		Mussel Proximity	4
42	Very Low Use - subjective		Recreational Boat Use	1
43	Medium Low Use - subjective		Recreational Boat Use	2
44	Medium High Use - subjective		Recreational Boat Use	3
45	High Use - subjective		Recreational Boat Use	4
46	coldwater stream or small lake		Waterbody Type	1
47	large river		Waterbody Type	2
48	hatchery		Waterbody Type	3
49	warmwater reservoir or large lake or walleye waterbody		Waterbody Type	4
50	headwaters of watershed		Position Rank	1
51	upper end of watershed		Position Rank	2
52	lower end of watershed		Position Rank	3
53	bottom of watershed		Position Rank	4

- Habitat suitability equals the sum of all variable scores for each parameter (max score when multiple samples present), divided by the number of parameters. This number is a percentage from 0 – 100.
- Final habitat suitability rank equals a 1-4 score, broken by quartiles.
- Social Sum = sum of variables

Social Sum	Social Rank
20 - 17	4
16-11	3
10-6	2
0-5	1

Final Rank	Social OR Habitat Rank
Extreme	4
High	3
Medium	2
Low	1

Appendix H. Relative efficacy of eDNA and cross-polarized light microscopy to detect dreissenid mussel presence in a newly positive water, Tiber Reservoir.

Environmental DNA (eDNA) is an emerging technique to determine presence of various aquatic organisms generally by sampling water to obtain a target organism's DNA. In general, the use of eDNA has been desirable because it is easier and quicker to collect water samples, rather than capture animals, which sometime are rare, threatened or endangered, or otherwise difficult to capture in a zooplankton net. It can be less expensive than traditional technique and in many cases it is more effective at detecting the presence of targeted animals. Dreissenid mussels only reproduce in warm water so their larvae cannot be collected in winter months and sampling for adults under ice is also not an effective method of sampling. eDNA could potentially detect mussels when they are not reproducing.

In 2016, larval dreissenid mussels were detected in Tiber Reservoir and a suspect sample was detected in Canyon Ferry Reservoir, these being the first detections in the State of Montana. These discoveries were made using conventional technique (plankton tows followed by cross-polarized light microscopy to identify the target organisms). The sampling concluded that densities were extremely low in both reservoirs, if established at all. To determine the best technique for early detection of dreissenids in Montana's waters to inform management (eradication, control or monitoring) and to protect neighboring waterbodies and states, we propose a comparative study between the two techniques.

Whereas eDNA has been used to detect the presence of mussels in waters that have been colonized, the efficacy of detecting their presence for early detection, that is when densities are extremely low, is unclear. Owing to this, the use of eDNA as an early detection technique is not favored as a primary tool in Montana. However, because no studies exist that provide clear direction, this situation provides for a unique opportunity to evaluate the relative efficacy of the two techniques, which will help to shape future early detection sampling. To our knowledge, the sensitivity of cross-polarized light microscopy has not been compared to eDNA for early detection (i.e., at low densities). This information is critical prior to incorporating eDNA into Montana's standard operating procedures for detecting AIS.

We propose to collect samples for cross-polarized light microscopy and eDNA simultaneously on each reservoir during three time periods in 2017: prior to, during and after water temperatures associated with peak dreissenid veliger presence in the water column (16 – 19 °C). Temporal comparisons may provide insight about when to optimally use each technique. The primary funding need is to pay for genetic analyses of eDNA samples. We propose a budget of $10,000 to pay for ~100 eDNA samples. The microscopy samples will be processed by the Montana FWP AIS laboratory using existing operation funds from FWP's survey and monitoring program.

Appendix I. Use of Environmental DNA in early detection and monitoring of AIS in Montana

Mussel Command Team's Decision

The Montana Mussel Incident Command Team has made the decision to suspend additional sampling and testing using eDNA for the time being (winter 2016-2017). After consultation with the Science Advisory Council, it seems that eDNA testing is unlikely to help us gather any additional information that will inform decisions during the emergency response timeframe. The cost of testing, as well as the potential for false positive results, means that this method of testing must be used in direct support of plankton tow samples whenever possible.

While this method remains a viable option for the future, it does not appear to be a good use of the emergency funding or team effort at this time.

eDNA sampling priorities

When a determination to use eDNA sampling has been made by the incident command team, the following priorities should be referenced when allocating funding and resources.

> Priority 01 -Additional verification of waters where previous plankton samples have been verified by microscopy for the presence of mussels. This testing should be used to provide additional verification as well as to inform responders as to potential locations of adult mussel populations

> Priority 02 - Additional verification of waters where suspect samples were identified by plankton sampling and microscopy and where secondary verification was inconclusive. (i.e. Canyon Ferry, Milk River, Missouri).

eDNA is not considered at this time to be a useful tool for testing waters as a primary detection tool. At this time, the potential for false positives remains too great to allow for it to be considered as a useful tool for this step in the process. The IMT does not intend to use state dollars at this time, for eDNA for testing of waters that have not had suspect samples verified though plankton samples and microscopy.

> The IMT is recommending that all state departments and agencies providing funding for eDNA sampling and testing consider these priorities during the emergency response time frame.

Rationale

Environmental DNA (eDNA) is an emerging technique employed to determine presence of various aquatic organisms generally by filtering water and using genetic techniques to detect DNA from target organisms. Much research and development has occurred recently to identify presence of fish species with eDNA (e.g., Asian carp in the Great Lakes, or brook trout in cutthroat trout restoration areas). In many cases, research has been conducted where water samples are taken prior to electrofishing surveys to evaluate the relative ability of each technique to detect fish, and the results show promise.

Hurdles associated with eDNA are the development of genetic markers that accurately differentiate among con-generic species as well as other non-target taxa. In addition, the markers must be evaluated within the geographic extent of the target species such that markers represent all genetic variants in situations where genetic structuring has occurred. This work is critical in understanding false results of the eDNA testing.

Environmental DNA markers have been developed for invasive mussels (zebra and quagga mussels), and some research has been conducted to compare general polymerase chain reaction (PCR) techniques to eDNA protocols. Results have shown good concurrence among the two techniques. The standard technique used my most governmental entities is cross-polarized light microscopy. To our knowledge, comparisons between cross-polarized light microscopy have not been compared to eDNA for early detection (i.e., at low densities). This information is critical prior to incorporating eDNA into Montana's standard operating procedures for detecting AIS.

Many questions remain to be answered to best understand the utility of eDNA in early detection of AIS. For example, what is the temporal nature of DNA persistence in a natural water body? What is the probability of detecting DNA in low-density early invasion situations? Has there been standardization among field sampling protocols (e.g., how much water to sample) and laboratory protocols? What is the prevalence of false positives, and what factors lead to false positives?

At the current time, Montana does not have the capacity or resources to conduct research to evaluate the efficacy of eDNA relative to cross-polarized light microscopy in early detection of AIS. However, the State of Montana would certainly work collaboratively with researchers that are investigating these questions.

Appendix J. 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