Montana Fish, Wildlife & Parks

2017 Report on
Aquatic Invasive Species Monitoring

A native fatmucket mussel found during diver searches in Tiber Reservoir for invasive mussels

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Aquatic Invasive Species Program
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Table of Contents

The Montana Fish, Wildlife & Parks (FWP) Aquatic Invasive Species (AIS) Program works to implement 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. The report for the Early Detection and Monitoring program for 2017 follows.

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), Eurasian watermilfoil (EWM), flowering rush (FR), curlyleaf pondweed (CLPW), 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 program in addition to an increase in volunteer sampling efforts. To aid in AIS monitoring, the AIS program has trained other FWP employees, including fish health staff and regional biologists and technicians, in AIS species identification and often contribute to or assist program staff with sampling efforts. FWP fisheries staff are often sampling high-risk waters for other purposes, and additional AIS sampling increases overall efforts with less travel cost for AIS staff. Overall monitoring and early detection efforts have increased steadily over the years but nearly tripled in 2017.

II. 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 as well as 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 field season. 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 1 with criteria metrics in Appendix I.

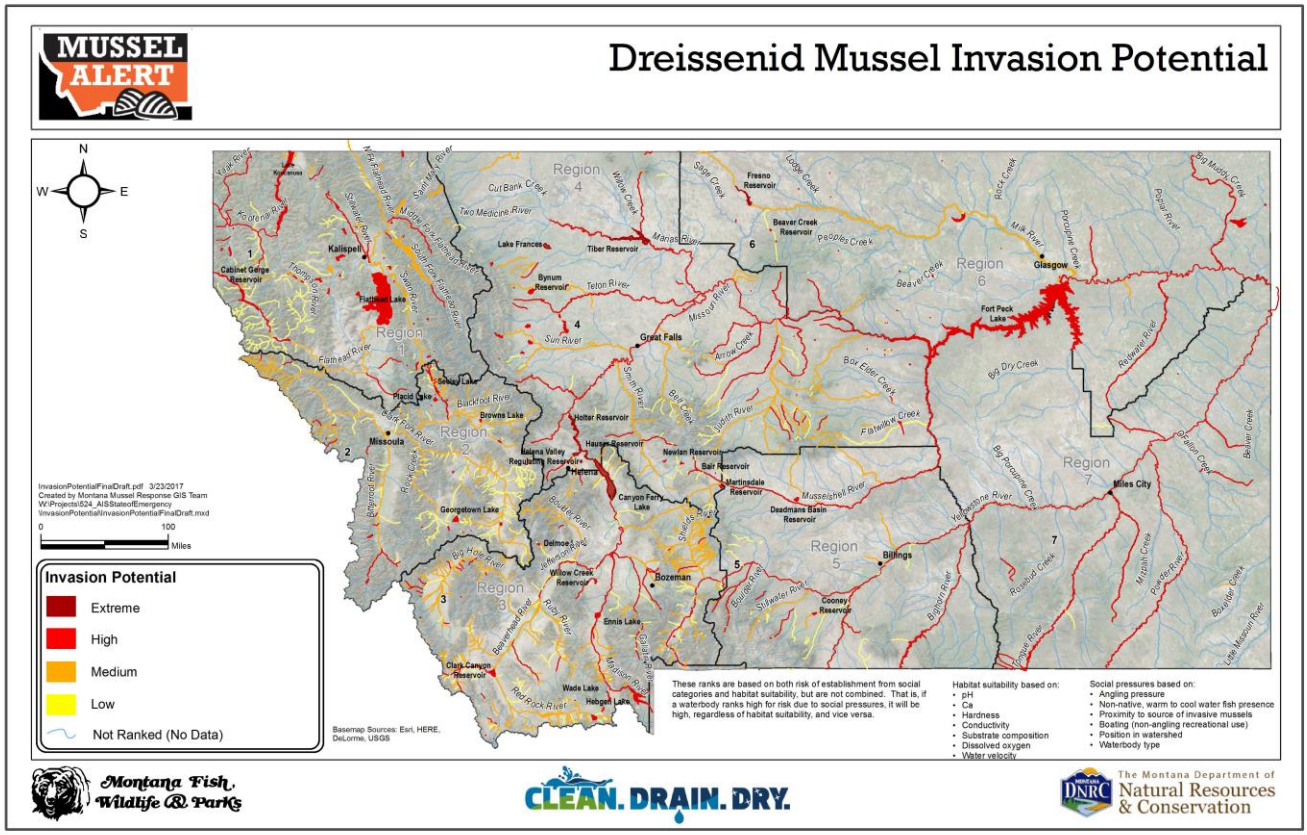


Figure 1: Map of Dreissenid Mussel Invasion Potential used to rank waters in Montana for overall AIS introduction threat and help determine frequency and quantity of sampling events.

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 organism for that area or one they believe to be an invasive species. Samples are often brought in to offices where regional staff will either identify them or send them to the AIS staff in Helena. Photos are often emailed to FWP where they are forwarded to AIS staff. If an organism can't be identified either by looking at photos or getting a good description from the reporting party, FWP staff will travel to the location to investigate the report.

In 2017, 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. One of the Helena permanent staff hired, trained and supervised three seasonal teams of two

employees each based in Missoula, Helena and Billings. Additionally, the other Helena permanent staff hired and trained 2 permanent/seasonal, part-time lab technicians to assist the one permanent lab position in Helena.

Montana utilizes a variety of techniques in monitoring for AIS populations. All of Montana's monitoring protocols have been scientifically reviewed, are updated annually, 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 in-house 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 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 is conducted in-house at the main FWP AIS lab in Helena, MT, or the satellite lab in Kalispell, MT. Polymerase Chain Reaction (PCR) testing, or the amplification of environmental deoxyribonucleic acid (eDNA), is used as a confirmation of microscopy findings for verification, if necessary, by the Montana FWP AIS Laboratory. Any DNA tests are conducted by independent laboratories as the FWP AIS laboratories do not have the equipment or training to conduct this type of analysis in-house.

Montana FWP began utilizing other sampling methods more frequently to search for adult populations of mussels in waters where larvae were either detected or suspected with the 2016 detection of invasive mussel larvae in the state. These methods include scuba diving, snorkeling, placing artificial substrates, and mussel-sniffing dogs. In 2017, MT FWP also conducted a side-by-side comparison of environmental DNA (eDNA) testing at Tiber Reservoir to look at the efficacy of both methods in partnership with the U.S. Geological Survey.

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 aren't just 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 be collected with any one method.

The simplest method is called rock picking. This is when you reach into the water and pick up pieces of the substrate (such as rocks, sticks, leaves, and plants). You can then either look at the larger objects, as most invertebrates will either live on or retreat to the underside of the object or remove organisms from the object and place them in a tub. Some freshwater invertebrates create structures that are adhered to rocks (such as bryozoans, netspinner caddisfly larvae, black fly pupae, and invasive mussels) and aren't easily brushed off like most other species. With plants and other detritus, you can collect a clump of vegetation and loosen organisms from it by shaking it in a water-filled tub (Figure 2).

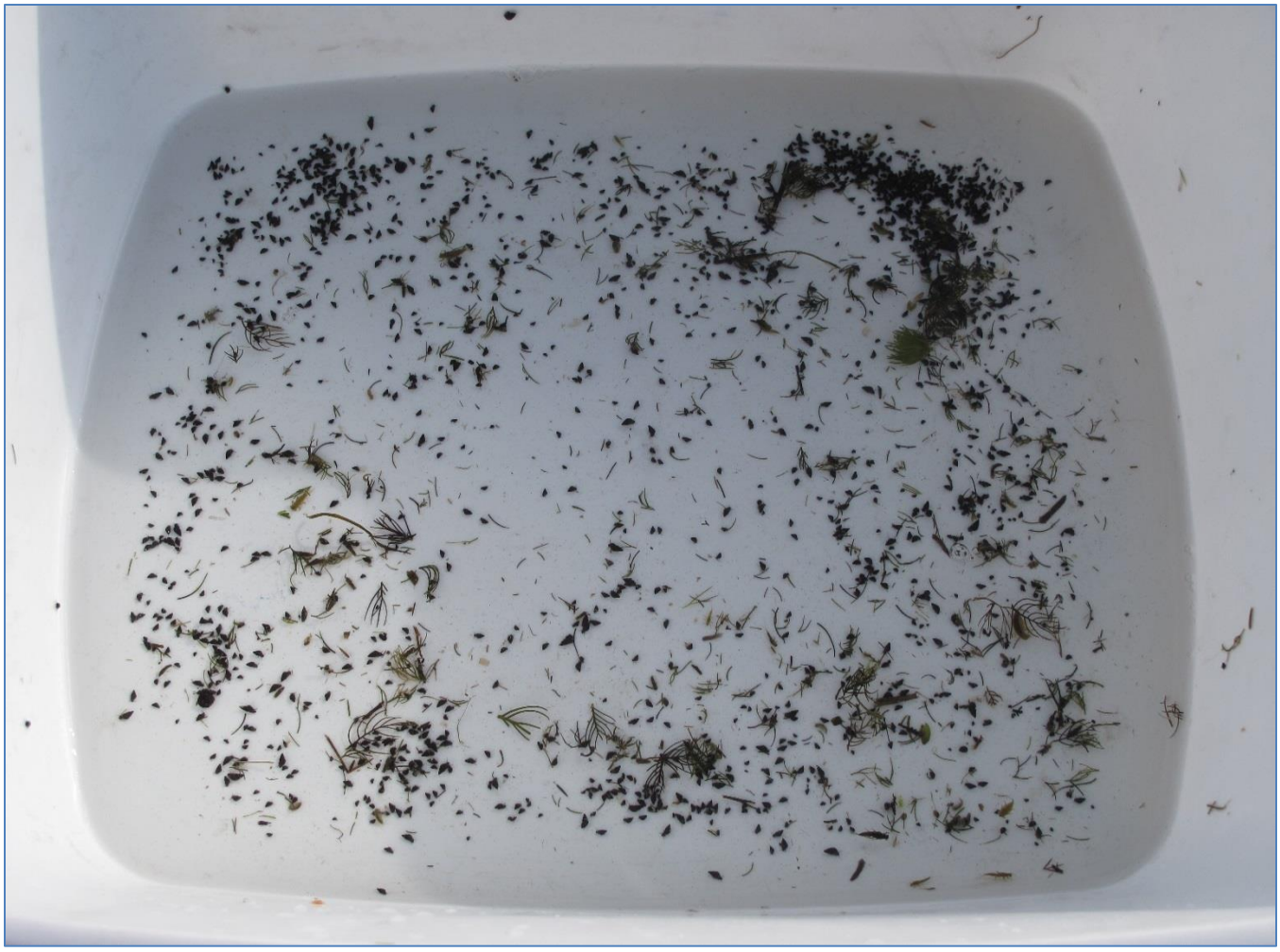


Figure 2: Invertebrates shaken from a plant sample into a white bucket (including many New Zealand mudsnails).

Kick nets (or dip nets) are another good means to collect organisms from vegetation mats or loose substrate. They are used in flowing water to collect invertebrates that are pushed into the net by the current or the sampler. Kick nets are used in slow or still water to remove invertebrates from vegetation or by quick jabs along the bottom to capture invertebrates living in the top 1-2 cm of finer sediment. The macroinvertebrates are placed and examined in a water-filled tub.

The last method to sample invertebrate is to examine all structures nearest to access points, such as docks, boat ramps and buoys. Viewing tools will aid in these efforts (such as a snorkel mask or viewing tube). In areas where there are water level changes, it is important to sample from the high-water line down to a depth where you can reach safely.

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. 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

AIS Sampling Prior to Wild Fish Transfers

The movement of fish could also be a substantial vector for transferring AIS. FWP moves large numbers of fish through both its hatchery and wild fish transfer programs. Hatcheries cannot receive certification to sell or move fish without passing an AIS inspection. To accomplish this, the FWP 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 transfers. These 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 increased the number of hatcheries that they assisted the AIS bureau with inspection due to the time constraints of AIS staff in 2017.

Plant Sampling

FWP has always sampled for macrophytes at high-risk sites as part of the departments all-taxa AIS sampling unless assisting partners with in-depth point-intercept plant mapping. 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.

2017 AIS Sampling Results

In 2017, a total of 260 waterbodies were inspected in Montana. Appendix A provides a listing of all water surveyed during the 2017 field season. It also shows the extent of the effort at each of these locations (type of survey conducted and how many times it was conducted at that waterbody). More sampling details for Tiber Reservoir and Canyon Ferry Reservoir are available in Appendix C and D, respectively. For more specific information on individual waters or areas, send a specific information request to either Craig McLane or Stacy Schmidt (email addresses are on title page).

No new populations of any AIS species were detected in 2017. Locations details of AIS in MT can be found in Appendices E and F. Findings in 2017 also include the following:

- No <u>adult</u> populations of ZM/QM or Asian clams were detected this year or in previous years on Montana waters, including in Tiber Reservoir or Canyon Ferry Reservoir.
- No new Dreissenid mussel larvae were detected in Montana waters in 2017 including samples from Tiber Reservoir and Canyon Ferry Reservoir.
- No Asian clam (*Corbicula spp*.) veligers were detected in the Montana plankton samples processed by the FWP AIS Laboratory in Helena in 2017 or in previous years.
- New Zealand mudsnails persists in sampled locations in Darlington Ditch, Hauser Lake, Bluewater Creek, the Yellowstone River, the Beaverhead River, the Jefferson River, the Ruby River and on the Missouri River below Holter Dam.

- Eurasian watermilfoil persists at sampled locations in Fort Peck Reservoir, Noxon Rapids Reservoir, Cabinet Gorge Reservoir, Beaver Lake, Jefferson Slough, Jefferson River, and the upper Missouri River.
- Curlyleaf pondweed persists in the Bitterroot River, Cabinet Gorge Reservoir, Canyon Ferry Reservoir, Clark Canyon Reservoir, Beaverhead River, Jefferson River, Fourchette Bay of Fort Peck Lake, Hauser Lake, Holter Lake, Ennis Lake, Hebgen Lake, Madison River, Missouri River, Noxon Rapids Reservoir, Clark Fork River, and Post Creek.

Figure 3 illustrates the statewide emphasis placed on AIS monitoring, which includes AIS monitoring sites for 2017 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. The program goal is to comprehensively monitor the state every year, which includes all types of waterbodies (lakes, reservoirs, ponds, creeks, rivers, etc.).

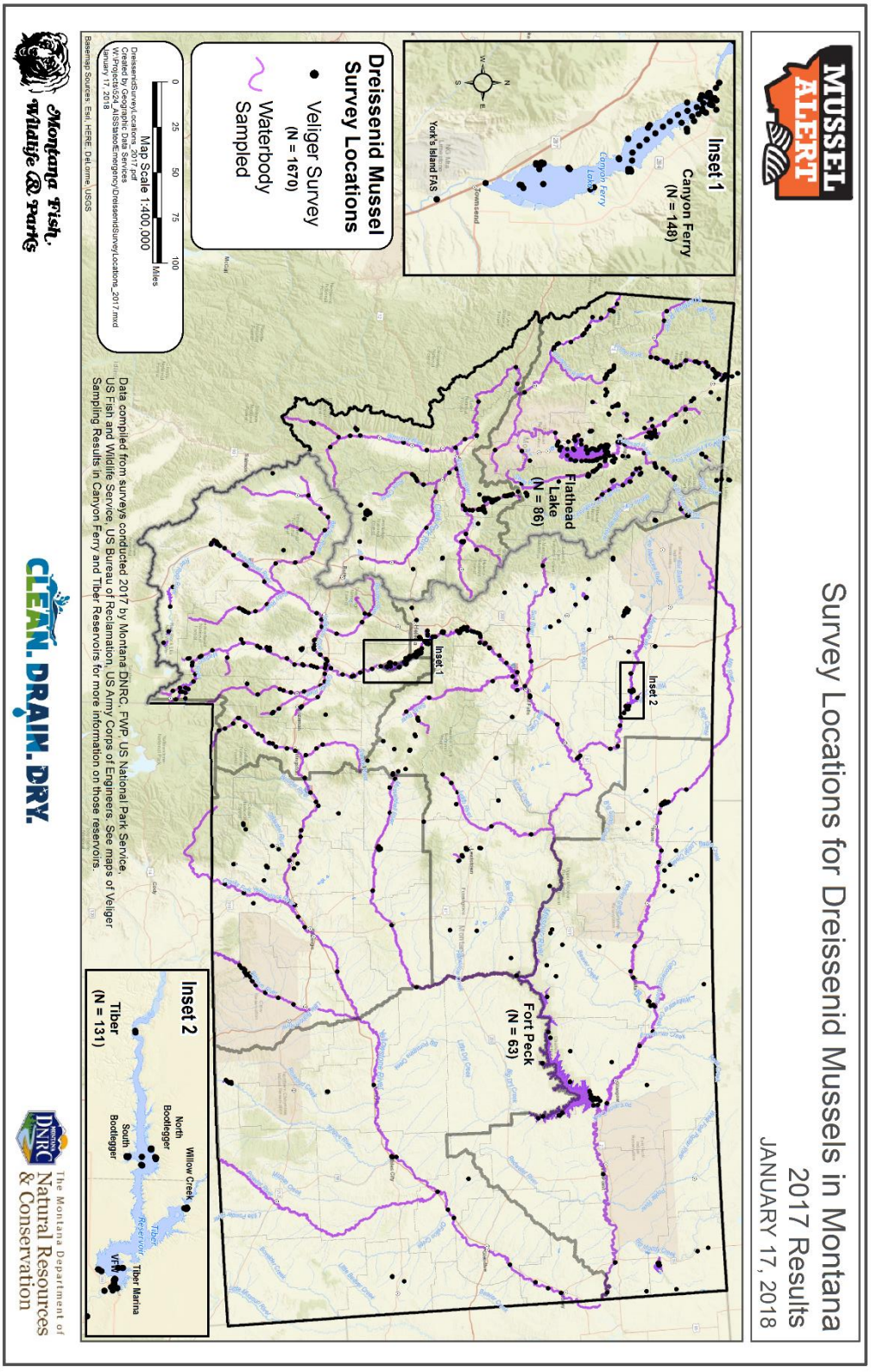


Figure 3: Map of AIS plankton sampling locations, 2017

With the new detection of Dreissenid mussel larvae within the state, the agency has nearly tripled its efforts. Partners are increasing efforts in invasive species detection as well. Figure 4 illustrates how many MT samples the FWP lab received and processed in 2017 from FWP (AIS staff, fisheries staff) as well as outside entities. FWP anticipates working more closely with existing partners and create new partnerships to encourage AIS sampling on a local level.

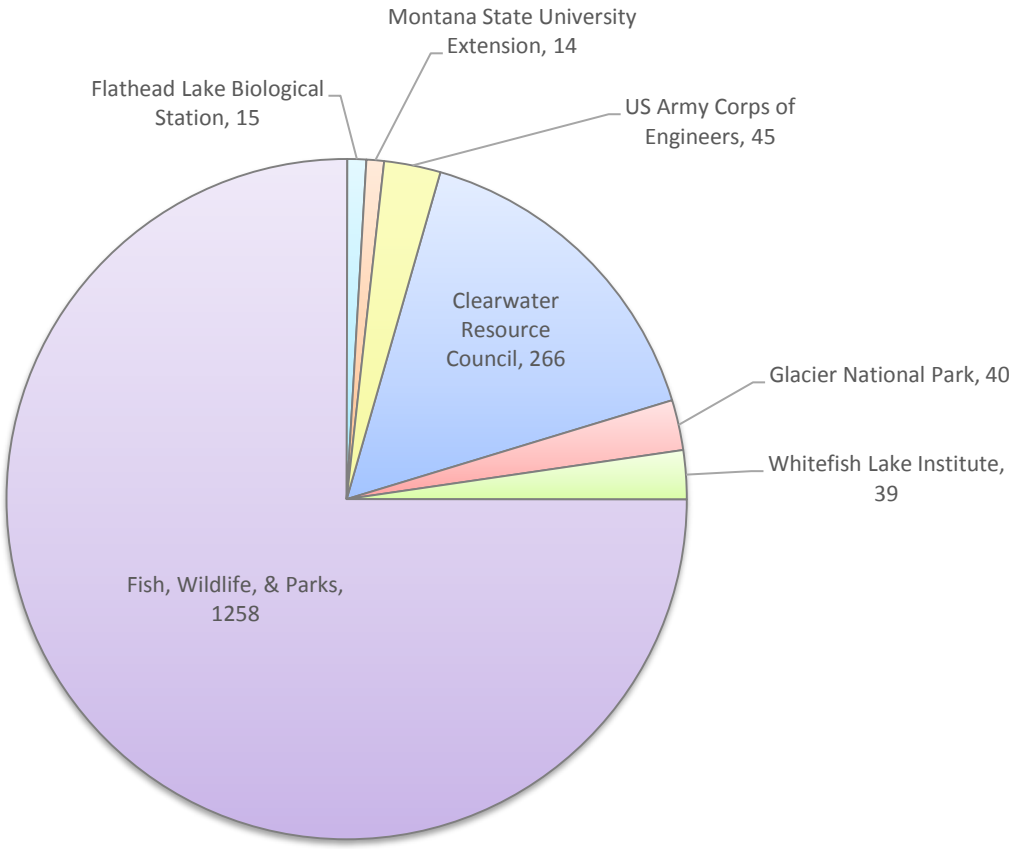


Figure 4: 2017 AIS Plankton Sampling Effort by All Reported Entities Breakdown

Bryozoan Sampling with FWP Region 3 Fisheries Staff

FWP AIS staff assisted the FWP Region 3 fisheries biologists with bryozoan sampling on the Yellowstone River in response to the 2016 mountain whitefish kill. Bryozoan sampling was competed at the Dan Bailey FAS and Pig Farm FAS on September 8, 2017. This was an effort to verify the presence and location of bryozoans in the Yellowstone River system. The effort consisted of turning over several types of substrate both near shore and in the main channel. Bryozoans that were located, collected, and preserved in water for identification at the FWP lab. No bryozoans were located at the Dan Bailey FAS. This may have been due to sampling

occurring too late in the year or that this was not an ideal sampling location. Five separate colonies of bryozoans were found at the Pig Farm FAS. The bryozoans were collected and taken to the FWP lab for identification. The bryozoans were no longer alive and were not able to be identified to species. This was likely the result of bryozoans entering their dormant stage in colder water temperatures. FWP plans to do further work to document distribution and abundance of bryozoans in the Yellowstone River. More information on this sampling and the fish kill on the Yellowstone River can be obtained from the Region 3 fisheries staff.

III. Aquatic Plant Sampling Results

FWP surveyed waterbodies that were suspect to contain AIS, high-risk, or locations needing confirmation of AIS. In addition, several locations were resurveyed to examine the dynamics and abundance of established AIS populations. In all, FWP crews surveyed 16 waterbodies. Table 1 shows the locations of FWP sampling for aquatic invasive plants in 2017. No new invasive plant populations were found this year. More detailed results for each water sampled are available in Appendix B.

Table 1. 2017 Aquatic plant sampling locations

Water Body	County	Sampling Type	Sampling Days	Sampling Points	Findings
Bighorn River	Bighorn	Whole Reach Survey	2	243	No AIS found
Brush Lake	Sheridan	Point-Intercept	1	73	No AIS found
Bull Lake	Lincoln	Point-Intercept	2	172	No AIS found
Cooney Reservoir	Carbon	Point-Intercept	1	73	No AIS found
Deadman's Basin Reservoir	Wheatland	Point-Intercept	2	97	No AIS found
Ennis Lake	Madison	Point-Intercept	2	117	Existing Curlyleaf pondweed
Fresno Reservoir	Hill	Point-Intercept	3	164	No AIS found
Hebgen Lake	Gallatin	Point-Intercept	1	47	No AIS found
Holter Lake	Lewis & Clark	Point-Intercept	1	36	Existing Curlyleaf pondweed
Jefferson River	Jefferson/ Madison/ Gallatin/ Broadwater	Whole Reach Survey	3	1410	Existing curlyleaf pondweed and Eurasian watermilfoil
Lake Como	Ravalli	Point-Intercept	3	41	No AIS found
Marias River	Liberty/Choteau	Whole Reach Survey	2	18	No AIS found
Nelson Reservoir	Phillips	Point-Intercept	2	125	No AIS found
Upper Holter	Lewis & Clark	Point-Intercept	2	128	Existing curlyleaf pondweed
Yellowstone River	Richland/Dawson	Point-Intercept	1	26	No AIS found
Yellowtail Afterbay Reservoir	Bighorn	Point-Intercept	2	50	No AIS found

IV. Aquatic Invasive Species Laboratory

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 (veliger) (Figure 5) for New Mexico and the Missouri River Basin (MRB), including Colorado, Kansas, Nebraska, North Dakota, Wyoming, and Montana. It is in Montana's best interest to know what AIS may exist downstream and near its borders, and as such, samples are processed 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. Figures 6 and 7 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 (Frischer et al, 2011).

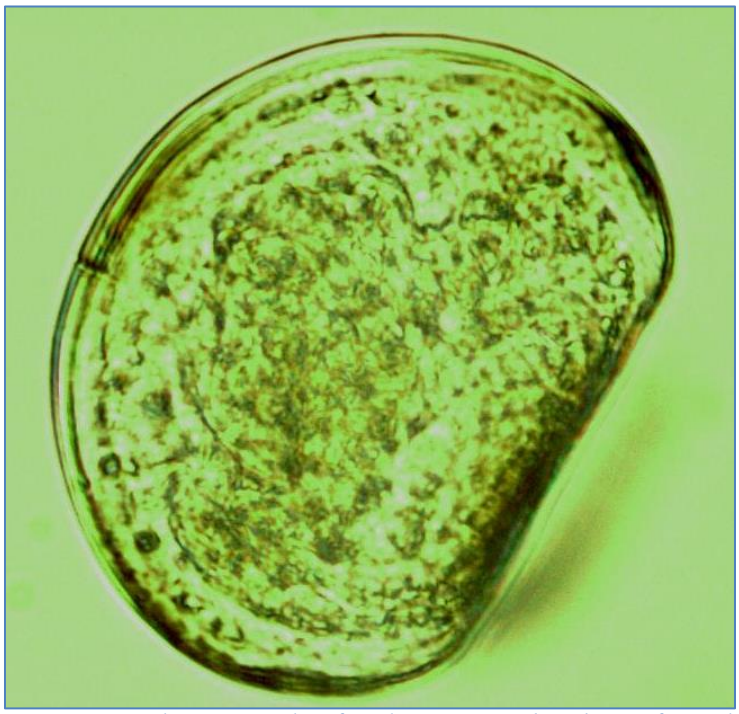


Figure 5: Photograph of Zebra mussel veliger found in an out-of-state sample processed in 2017 by FWP AIS Laboratory in Helena. Length of veliger = 111 µm.

In 2017, no Dreissenid veligers were found in any samples collected in MT, including from Tiber Reservoir and Canyon Ferry Reservoir by either FWP or BOR.

FWP staff are also participating in workgroups organized by the Western Regional Panel to standardize both laboratory and sampling techniques across western states. This is an ongoing project.

All Montana samples were completed by November 17th, 2017. High priority Montana samples were processed within a turnaround time of two weeks. Lower priority Montana

samples had a longer turnaround time and out-of-state samples took the longest to process. Out-of-state samples were completed on December 28th, 2017. Overall, samples were processed in a shorter timeframe than prior recent years. The FWP AIS laboratories are continuing to work on methods to improve sample processing time.

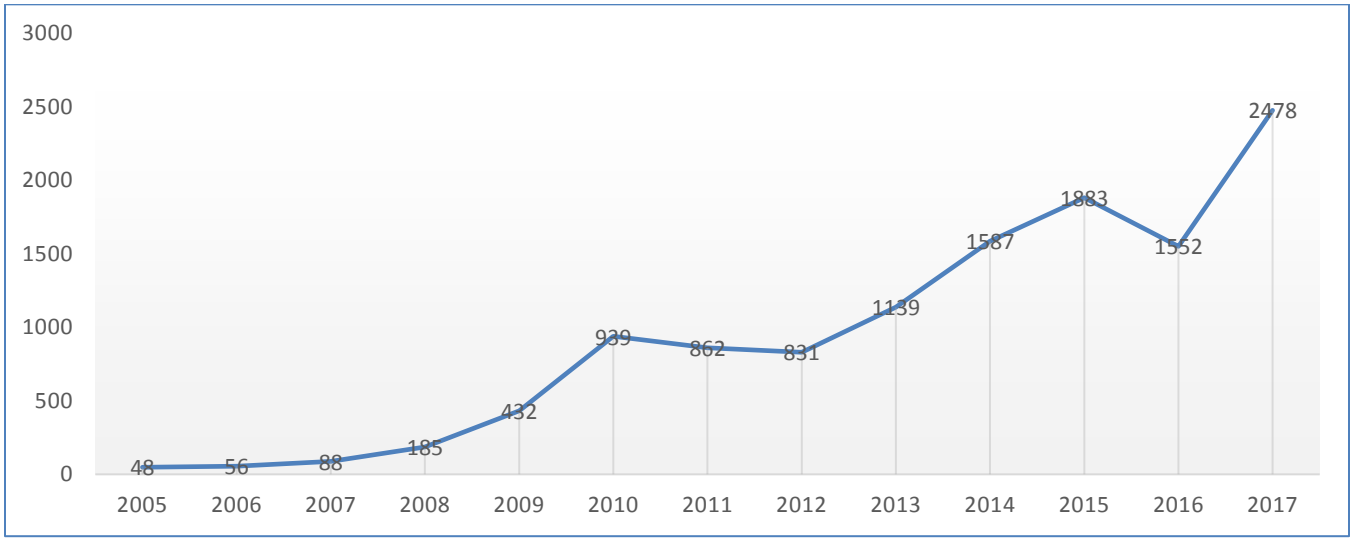


Figure 6: Number of samples processed by FWP AIS lab each year.

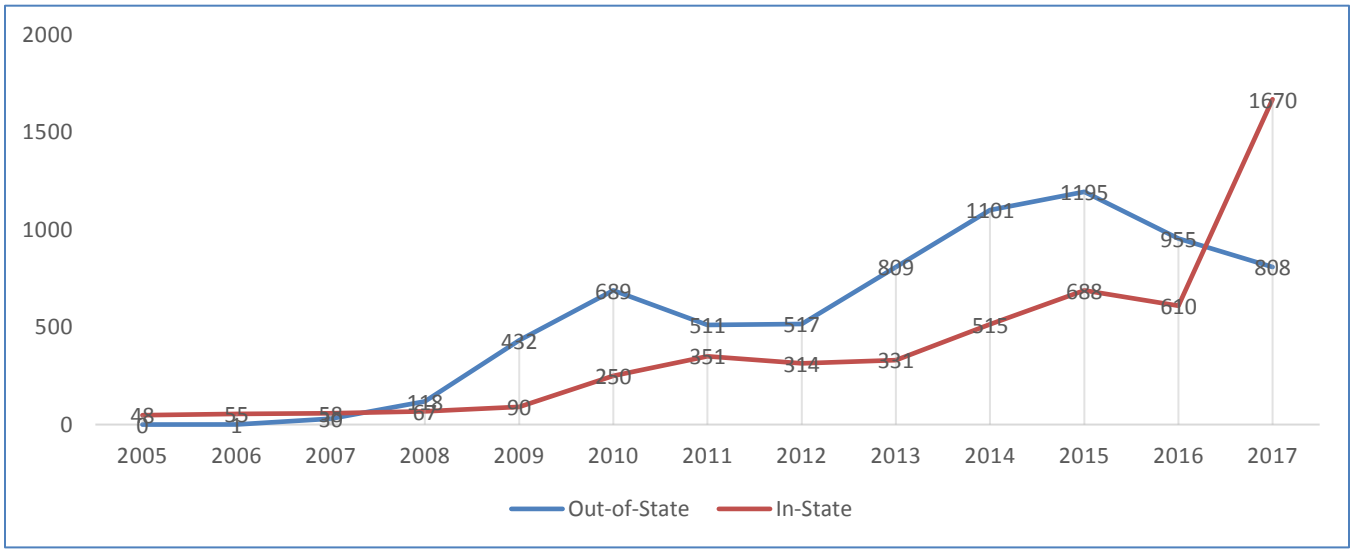


Figure 7: Number of plankton samples processed by year: in-state vs. out-of-state.

In 2015-2016, the AIS laboratory was over its capacity to process samples in a timely manner. Starting in the winter of 2015, FWP trained an existing permanent staff member in laboratory sample processing techniques. The newly established, secondary AIS lab is in Kalispell, MT,

and has become very proficient in veliger identification. It can take two to three years for a lab technician to become proficient. In 2017, this became that staff member's primary role in the program and that will continue into the 2018 field season. 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. Both employees excelled in learning lab protocol and they are becoming proficient in sample analysis and may be able to process samples unsupervised in the 2018 sampling season. Additional equipment was purchased in 2017 to be used in a newly renovated, larger lab space in the FWP office space building in Helena. The lab space will be completed in spring of 2018. This will allow for two employees to process samples at the same time versus one. Lastly, a contract was written to serve as a backup for sample processing if both MT labs became inundated with samples and may be used in the 2018 sampling season if needed. That contract was awarded to Aquaticus, LLC in Florida. There are still some issues with sample processing time and reporting, but FWP is continually making improvements.

V. Mussel Response – the battle wages on

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. Additional help was brought in to assist with the planning and implementation of the 2017 field season both from within the agency and from outside.

Figure 8: 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 find any further presence of mussels. These efforts included plankton tow sampling, artificial substrate sampling (Figure 8), underwater inspections using scuba divers (Figure 9) and snorkelers, mussel detecting dogs (Figure 10) and the use of environmental DNA (eDNA) sampling. This year, 131 plankton tow

samples taken at Tiber and 148 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.

FWP, in cooperation with the U.S. Geological Survey, collected eDNA samples from Tiber Reservoir this season to compare invasive mussel early detection sampling methods. Several of the eDNA samples collected from Tiber indicated the potential presence of invasive mussel DNA.

Figure 9: FWS divers (top to bottom): Nicole Prescott, Jackie Wichman, Deborah Goeb. Taking a break between dives at Tiber Dam. August 2017.

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 G and H). Due to questions surrounding this method, FWP and the Montana Invasive Species Council are forming a scientific advisory panel to provide guidance on the use of DNA methods for early detection of invasive mussel. The first meeting of the workgroup, who are working to identify panel members and questions to ask the panel, occurred in January of 2018. The presence of invasive mussel DNA in the eDNA samples from Tiber are not conclusive about the presence of invasive mussels in the reservoir. The most conclusive and accepted test is the plankton tow sampling and cross polarized light microscopy for veligers as well as adult surveys, all of which came back negative for the presence of invasive mussels in 2017.

Continued Efforts

FWP is continually trying to improve efforts in all taxa AIS early detection and monitoring. Due to the newly hired staff in 2017 (including the watercraft inspection station area supervisors), already existing permanent staff can spend more time on this aspect of the AIS Bureau. Plans for the remaining winter of 2017-2018 are to provide additional training to lab technicians. Instead of focusing on presence/absence of veligers, they will spend some time

on native species in plankton samples. They will catalogue organisms present in plankton tow samples, practice photographing these organisms to begin to create a comprehensive reference library, and spend time using taxonomic keys to identify them.

Additionally, as part of expanding laboratory tools, FWP has enlisted the help of the University of Montana's EMTRIX Electron Microscopy facility to use a Scanning Electron Microscope (SEM) to photograph known veligers of Zebra mussels, Quagga mussels and Asian clams collected from outside Montana with different age classes and morphologies to see if SEM photographs could be used as another confirmatory tool for FWP suspect samples.

Figure 10: Alberta team Cindy Sawchuk and Hilo searching the shoreline at South Bootlegger Boat Ramp, Tiber Reservoir, October 2017.

Efforts during the field season of 2018 will be like 2017 with some changes and improvements. There will still be one permanent staff who will be the primary person responsible for sampling Tiber and Canyon Ferry Reservoirs. This will allow for consistency throughout different sampling seasons while others will still be brought in for consultation and additional manpower. FWP's AIS Bureau will continue to work closely with local FWP fisheries staff on both these waters. During the field season of 2017, the local fisheries biologists and technicians were invaluable to sampling efforts because of their knowledge of the reservoirs. FWP will continue to work with existing partners on sampling 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. These crews will be conducting less entire lake/river type surveys with boats and focusing more intently on access point surveying from shore (such as at fishing access sites, boat ramps, and recreation areas). All crews will continue FWP's all-taxa survey approach with an emphasis on invasive mussels.

FWP will rely on the advice of the scientific advisory panel to make decisions regarding the use of eDNA during the 2018 sampling season but will continue with the other sampling techniques used in prior years and widely accepted in western states as the standard for early detection methods.

Looking Forward

A nearly threefold increase in plankton sampling statewide cannot occur without experiencing some growing pains. Fish, Wildlife, & Parks is evaluating the AIS Early Detection and Monitoring Program and working to improve its protocols and training processes to continually improve its AIS monitoring program. These improvements will lead to more reliable sampling efforts, data collection, sampling handling, and loss of samples during transfer from collection to the lab. Plans to improve FWP's monitoring program are (in no order):

- Review annual monitoring plan to ensure adequate frequency and intensity at highest priority waterbodies sampling occurs.
- Implement and refine a mobile data that will be available to the agency and partners will help reduce error in the data as well, while making the data available in a timelier manner.
- Hire seasonal crew to begin earlier in the season (May) to allow more sampling statewide.
- Include more in-depth training for seasonal crews (Figure 11), which likely will require more days to prepare the crews for the sampling season.

- Permanent staff will spend more time with the crews while in the field to help with QA/QC.
- Seasonal crews will start in early May to provide more time for monitoring and surveys.
- Reduce loss of plant, macroinvertebrate, and plankton samples between collection and delivery at the lab. This process is yet to be finalized but would likely include a chain-of-custody, having crews mail samples directly in place of relying on FWP traveling between regions, and better communication with crews on progress of sampling efforts.
- Crews and members of the public will be required to send in a minimum of photographs of organisms so FWP can verify identification. Some collectors may also be required to collect and send in voucher samples of organisms. Additional tools will be created to provide to crews to reference while in the field.
- Help partners improve their all-taxa AIS monitoring efforts.

Figure 11: FWP AIS sampling crew training on Missouri River, June 2017.

Statewide monitoring efforts by FWP, private sector, organizations, and government continue 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 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

Frischer, M.E., Nierzwicki-Bauer, S.A., Kelly, K.L. 2011. Reliability of Early Detection of *Dreissena* spp. Larvae by Cross Polarized Light Microscopy, Image Flow Cytometry, and Polymerase Chain Reaction Assays: Results of a Community Double-Blind Round Robin Study (Round Robin Study Phase II).

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

Water Body Name	Hand Grab	eDNA Water Grab	Kick Net	Other	Plankton Net - eDNA/ qPCR	Plankton Net - Microscopy	Rake Toss	Rock Pick	Snorkel	Substrate	Water Total
Abbot Lake						1					1
Ackley Lake			1			1	1	1			4
AMC Settling Pond #3	2					2	2	2			8
Anderson Reservoir			1			1	1	1			4
April Reservoir			1			1	1				3
Ashley Lake	1					3		1			5
Bailey Reservoir			2			2	2	2			8
Bair Reservoir			5			5	5	5			20
Bean Lake	1		1			1	1				4
Bearpaw Lake			1			1	1	1			4
Beaver Creek Reservoir	1		1			2		2			6
Beaver Lake	1					3		2			6
Beaverhead River			6			6	6	6			24
Big Casino Creek Reservoir			1			1	1	1			4
Big Hole River	1		6			6	3	6			22
Big Spring Creek	1		4			5	3	1			14
Big Therriault Lake						1					1
Bighorn Lake			1			18	1				20
Bighorn River*	243		4			8	8	8			271
Bison Bone Reservoir			1			1	1	1			4
Bitterroot River	8		1			9	1	8			27
Black Sand Spring	1							1			2
Blackfoot River	2					3		2			7
Blacktail Meadows Kids Pond			1			1	1	1			4
Blaine Spring Creek			1			2	1				4
Blanchard Lake	1					2		1			4
Bluewater Creek	1		1			1	1	1		1	6
Bonanza Reservoir			1			1	1	1			4
Bootjack Lake						1					1
Boulder River	4		2	1		4	2	4			17
Bowman Lake					4	4					8
Boxelder Lake			2			2	2	1			7
Brownes Lake	1					1		1			3
Browns Lake	7		2			27	1	5			42
Brush Lake*	2		1			2	73	1			79
Bull Lake*	3					1	171				175
Bynum Reservoir	1		3			3	3	3			13
Cabinet Gorge Reservoir	4		1			6	5	5			21

Water Body Name	Hand Grab	eDNA Water Grab	Kick Net	Other	Plankton Net - eDNA/ qPCR	Plankton Net - Microscopy	Rake Toss	Rock Pick	Snorkel	Substrate	Water Total
Canyon Ferry Lake	1		2	2		148	2	2	1	20	178
Carters Pond			1			1	1	1			4
Castle Rock Reservoir			4			4	4	4			16
Chouteau Reservoir			1			1	1	1			4
Cibid Lake	1					1		1			3
Clark Canyon Reservoir	1		11			12	11	11			46
Clark Fork River	7		2			10	3	7			29
Clarks Fork Yellowstone River			2			2	2	2			8
Clearwater Lake	1					1		1			3
Clearwater River	4		2	1		4		3			14
Cliff Lake			1			1	1	1			4
Compton Reservoir			1			1	1				3
Cooney Reservoir*	2		3			3	74	3			85
Coopers Lake	2					18	1	2			23
Crystal Lake	1					2	1	1			5
Dailey Lake			4			4	4	4			16
Darlington Ditch 1	1		1			1		1			4
Dawson Pond #1			1			1	1	1			4
Dawson Pond #2			1			1	1	1			4
Deadmans Basin Reservoir*			9			8	102				119
Dickey Lake	1					2					3
Dollar Lake						1					1
Don Reservoir			1			1	1				3
Drag Creek Reservoir			1			1	1	1			4
Dry Creek			1			1	1	1			4
Duck Creek	1					1		1			3
East Fork Bitterroot River	1					1		1			3
East Fork Reservoir	4		2			6	3	5			20
East Gallatin River			2			2	2	2			8
Echo Lake	2					3	1	1			7
Emerald Lake	1					1		1			3
Ennis Lake*			6			7	120	6			139
Ester Lake			1			1	1	1			4
Eureka Reservoir			2			2	2	2			8
Eyraud Lake, lower	1		1			1					3
Fairy Lake	1		1			1	1	1			5
Fish Lake	1					14					15
Flathead Lake	7					87	2	5			101
Flathead River	5		3			7	2	4			21
Flynn Pond			1			1	1	1			4
Forest Lake			1			1	1	1			4
Fort Peck Dredge Cuts	2		5			9	4	4			24

Water Body Name	Hand Grab	eDNA Water Grab	Kick Net	Other	Plankton Net - eDNA/ qPCR	Plankton Net - Microscopy	Rake Toss	Rock Pick	Snorkel	Substrate	Water Total
Fort Peck Lake			22			48	24	25			119
Fort Peck Trout Pond			2			5	2	2			11
Foy Lake	2					3	2	2			9
Freezeout Lake			1			1	1	1			4
Frenchtown Pond	1		1			2	1	1			6
Fresno Reservoir*			5			16	303	4			328
Gallatin River			7			9	7	7			30
Georgetown Lake	13		1			15	10	11			50
Gibson Reservoir	1		2			2	1	2			8
Glasgow Base Pond			1			1	1				3
Glen Lake	1					2					3
Gullwing Reservoir			1			1	1				3
Halfmoon Lake						1					1
Handkerchief Lake	1					1		1			3
Hanson-doyle Lake						1					1
Harpers Lake	2		1			3	1	1			8
Hauser Reservoir	7		22			23	22	23			97
Hebgen Lake*	1		11			10	58	10			90
Helena Valley Regulating Reservoir	1		6			9	6	6			28
Holgate Reservoir			1			1	1	1			4
Holland Lake	3					19		2			24
Holter Reservoir*	1		7			18	53	7			86
Homestead Reservoir			1			1	1				3
Horseshoe Lake	2					2		2			6
Hubbart Reservoir	1					1					2
Hundred Dollar Bill Pond			1			1	1	1			4
Hungry Horse Reservoir	16		3			20		12			51
Hyalite Reservoir			3			3	3	3			12
Indian Creek						1		1			2
Jefferson River*	1411		3	2		8	3	3			1430
Jessup Mill Pond	1					1		1			3
Jette Lake						1					1
Jocko River	1		1			1		1			4
Judith River			4	1		4	4	4			17
Karsten Coulee Reservoir			1			1	1	1			4
Kolar Reservoir 1			1			1	1	1			4
Kolar Reservoir 2			1			1	1	1			4
Kootenai River	4					4		4			12
Lake Alva	2					22	2	2			28
Lake Blaine						1					1
Lake Como*	3					3	41	3			50
Lake Five	1					2		1			4

Water Body Name	Hand Grab	eDNA Water Grab	Kick Net	Other	Plankton Net - eDNA/ qPCR	Plankton Net - Microscopy	Rake Toss	Rock Pick	Snorkel	Substrate	Water Total
Lake Frances			2			10	2	2			16
Lake Helena			10			10	10	10			40
Lake Inez	1					26	1	1			29
Lake Josephine			1			1	1	1			4
Lake Koocanusa	19		4			71	7	11			112
Lake Mary Ronan	3					4	2	2			11
Lake Shel-oole			1			1	1	1			4
Lake Sutherlin			4	2		4	4	4			18
Laurel Pond			1			1	1	1			4
Lima Reservoir	1					1					2
Lindbergh Lake	3					17	1	2			23
Little Bitterroot Lake	3					4	1	1			9
Little Blackfoot River	1					1		1			3
Little Boulder River	1		1			1	1	1			5
Little Mcgregor Lake	1					1		1			3
Loon Lake	1					2					3
Lower Glaston Lake						1					1
Lower Stillwater Lake	1					2	1				4
Luloff Pond						1					1
Madison River	4		17			21	17	17			76
Maier Reservoir			1			1	1				3
Marias River*	14		13			15	14	15			71
Martinsdale Reservoir			4			4	3	4			15
Mcdonald Lake					4	5					9
Mcgilvray Lake						1					1
Mcgregor Lake	3					4		1			8
Medicine Lake			4			4	4	3			15
Middle Creek			1			1	1	1			4
Middle Fork Judith River			1			1	1	1			4
Miles City Hatchery Pond						2					2
Milk River			10			10	9	10			39
Missouri River	8		43	2		44	42	42			181
Mollman Creek	1		1			1		1			4
Murphy Lake	1					2		1			4
Murray Lake	1					2					3
Musselshell River	3		5			5	5	5			23
Nelson Dredge			1			2	1	1			5
Nelson Reservoir*			5			7	126	1			139
Nevada Reservoir	1		1			4	2	3			11
Newlan Reservoir			6			6	6	6			24
Nilan Reservoir	1		1			1		1			4
North Fork Big Hole River			1			1	1	1			4

Water Body Name	Hand Grab	eDNA Water Grab	Kick Net	Other	Plankton Net - eDNA/ qPCR	Plankton Net - Microscopy	Rake Toss	Rock Pick	Snorkel	Substrate	Water Total
North Fork Blackfoot River	1					1					2
North Fork Musselshell River	1		1			1		1			4
North Polly Reservoir						1		1			2
Noxon Rapids Reservoir	12		3			18	14	12			59
Ostle Reservoir	1		1			1	1	1			5
Painted Rocks Reservoir	4		1			4	2	2			13
Paulo Reservoir			1			1	1				3
Payola Reservoir			1			1		1			3
Pelican Point #1	1		1			2	1	1		1	7
Pelican Point #2			1			1	1				3
Peterson Lake						1					1
Peterson Reservoir			1			1	1	1			4
Pishkun Reservoir	1		1			2		2			6
Placid Lake	6					30	4	3			43
Post Creek	1					1		1			3
Powder River			1			1	1	1			4
Priest Butte Lake			1			1	1	1			4
Quake Lake			2	1		2	2	2		1	10
Rainy Lake	2					5	1	2			10
Raymond Dam			1			1	1	1			4
Red Rock Lake, Lower	1					1					2
Red Rock Lake, Upper	1					1					2
Red Rock River	1					1					2
Reser Reservoir			1			1	1	1			4
Rock Creek	5					9	1	2			17
Roe River	1		1			1	1	1		1	6
Rogers Lake	1					2		1			4
Rose Creek	1					1					2
Rosebud Creek						1		1			2
Rostad Reservoir			1			1	1	1			4
Ruby River	1		6			6	5	6			24
Ruby River Reservoir			6			6	6	5			23
Sagebrush Reservoir						1	1	1			3
Salmon Lake	6					33	1	5			45
Seeley Lake	6					47	2	4			59
Shelby Kids Pond			1				1	1			3
Shields River	1		4			4	3	4			16
Silver Lake	1					1	1	1			4
Skyles Lake						1					1
Smith River	2		4			4	3	4			17
Sophie Lake						1					1
South Fork Dry Fork Marias River			1			1	1	1			4

Water Body Name	Hand Grab	eDNA Water Grab	Kick Net	Other	Plankton Net - eDNA/ qPCR	Plankton Net - Microscopy	Rake Toss	Rock Pick	Snorkel	Substrate	Water Total
South Fork Madison River	1		1			2	1	2			7
South Fork Musselshell River			1			1		1			3
South Sandstone Reservoir			2			2	2	2			8
Spencer Lake						1					1
Spook Lake	1					1					2
Spotted Eagle Lake			2			2	2	2			8
Spring Meadow Lake			5			5	5	5			20
St. Mary Lake					5	4					9
Stillwater River			2			2	2	2			8
Summit Lake						5					5
Sun River	1		4			4	4	4			17
Swan Lake	2					27	1	3			33
Swift Reservoir			1			2	1	1			5
Taint Reservoir			1			1	1	1			4
Tally Lake	1					2	1				4
Tenmile Creek	1					1		1			3
Tetrault Lake	1					2		1			4
Thompson Falls Reservoir	6					8	7	5			26
Thompson Lake, Lower	4		1			6	1	4			16
Thompson Lake, Middle	4					4		1			9
Thompson Lake, Upper	4					4		3			11
Three Forks Pond			1			1	1	1			4
Three Forks Pond East			1			1	1	1			4
Tiber Reservoir		28	16	7		131	16	5	35	4	242
Tongue River Reservoir			10			12	10	10			42
Topless Lake	1					1	1				3
Tunnel Lake	1		1			1		1			4
Tuppers Lake	1					1					2
Twin Lakes	1					1		1			3
Two Medicine Lake					3	3					6
Upper Carters Pond			1			1	1	1			4
Upper Holter Lake*	1		4			14	140	5			164
Upper Stillwater Lake	1					2	1	1			5
Upper Whitefish Lake	1					2		1			4
Upsata Lake	2		1			18		2			23
Valley Reservoir			1			1	1				3
Van Lake	1					13		1			15
Wade Lake			1			1	1	1			4
Wapiti Reservoir			1			1	1				3

Water Body Name	Hand Grab	eDNA Water Grab	Kick Net	Other	Plankton Net - eDNA/ qPCR	Plankton Net - Microscopy	Rake Toss	Rock Pick	Snorkel	Substrate	Water Total
Warm Springs Creek			1			1	1	1			4
Waterton Lake					6	6					12
Wayne Edsall Pond			2			2	2	2			8
West Fork Bitterroot River	1					1		1			3
West Fork Gallatin River			2			2	2	2			8
West Fork Madison River	1					1		1			3
West Rosebud Creek						1		1			2
West Rosebud Lake						1		1			2
Whitefish Lake	3					7	2	2			14
Willow Creek Reservoir	1		4			6	3	3			17
Winter Harbor Pond			2			2	2	2			8
Wise River			1			1	1	1			4
Yellow Water Reservoir			1			1	1	1			4
Yellowstone River*	4		37			39	59	35			174
Yellowtail Afterbay Reservoir*			6			6	56	5			73
Grand Total	2014		531	19	22	1655	1860	659	36	28	6824

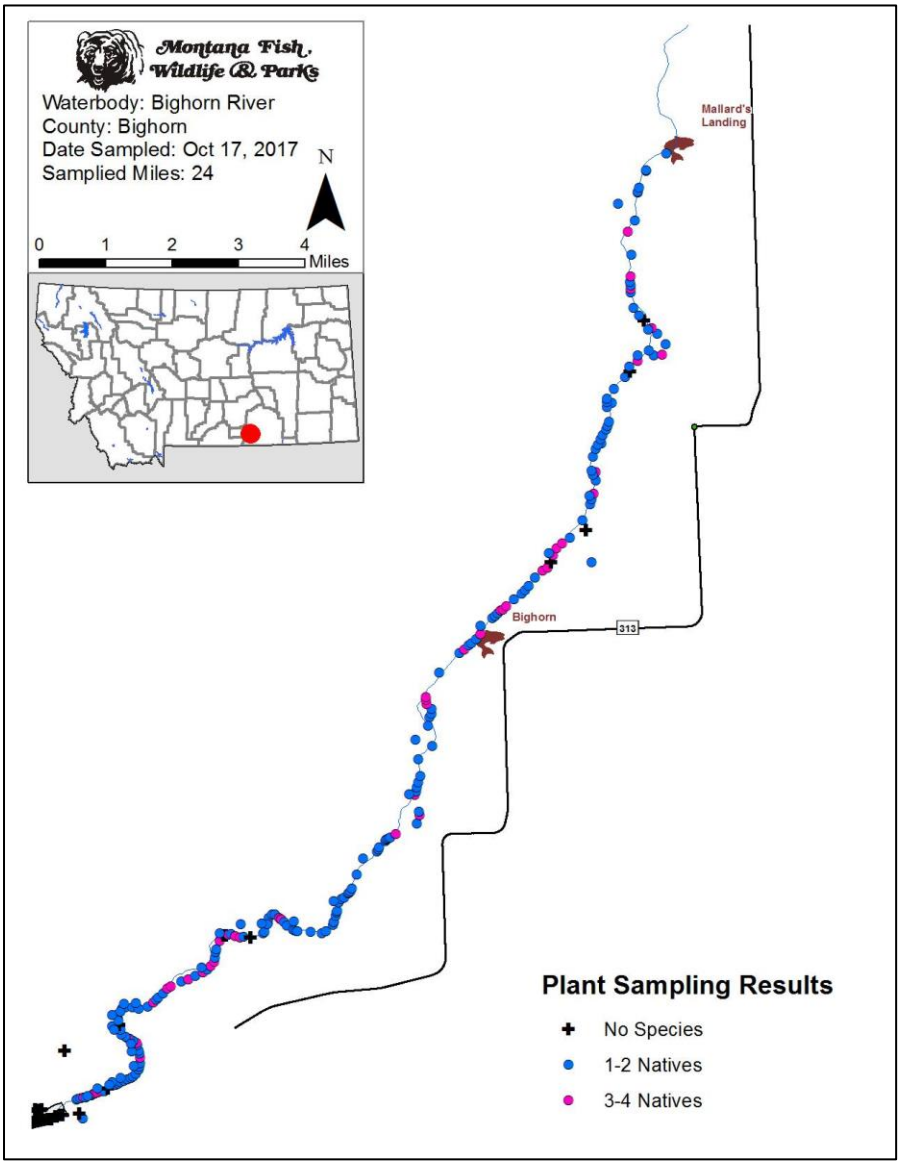
* Indicates waters where comprehensive macrophyte surveys were conducted. See Appendix B.

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.

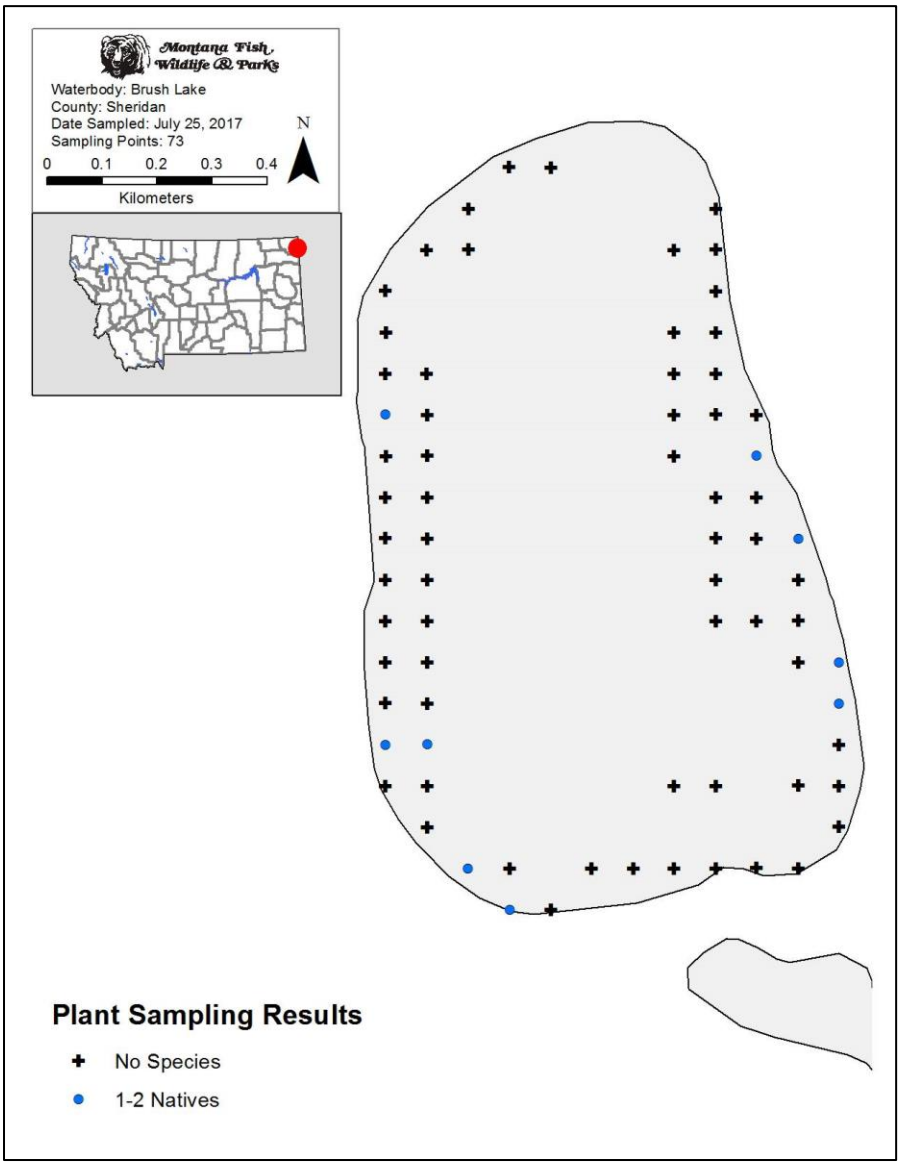
1. Bighorn River

Bighorn	**n=243**		
Common Name	**Scientific Name**	**Count**	**Frequency**
No species	-	12	5%
Leafy pondweed	*Potamogeton foliosus*	208	86%
Duckweed	*Lemna spp.*	53	22%
Unidentified Plant	-	39	16%
Sago pondweed	*Stuckenia pectinatus*	28	12%
Bulrush species	*Scirpus spp.*	5	2%
White-stemmed pondweed	*Potamogeton praelongus*	2	1%



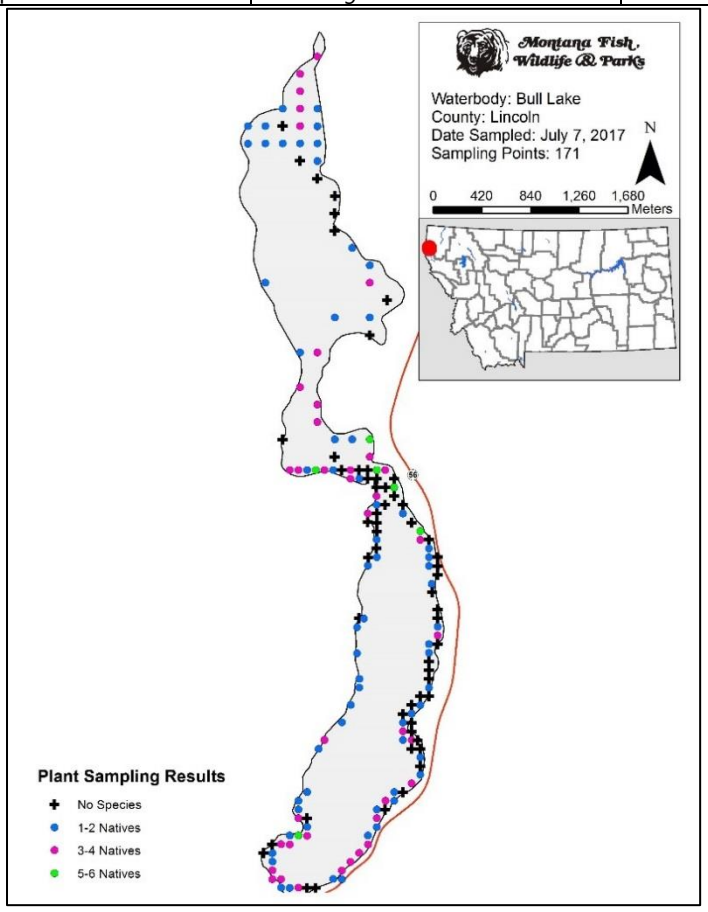
2. Brush Lake

Brush Lake	**n=73**		
Common Name	**Scientific Name**	**Count**	**Frequency**
No species	-	64	88%
Sago Pondweed	*Stuckenia pectinatus*	8	11%
Leafy pondweed	*Potamogeton foliosus*	1	1%



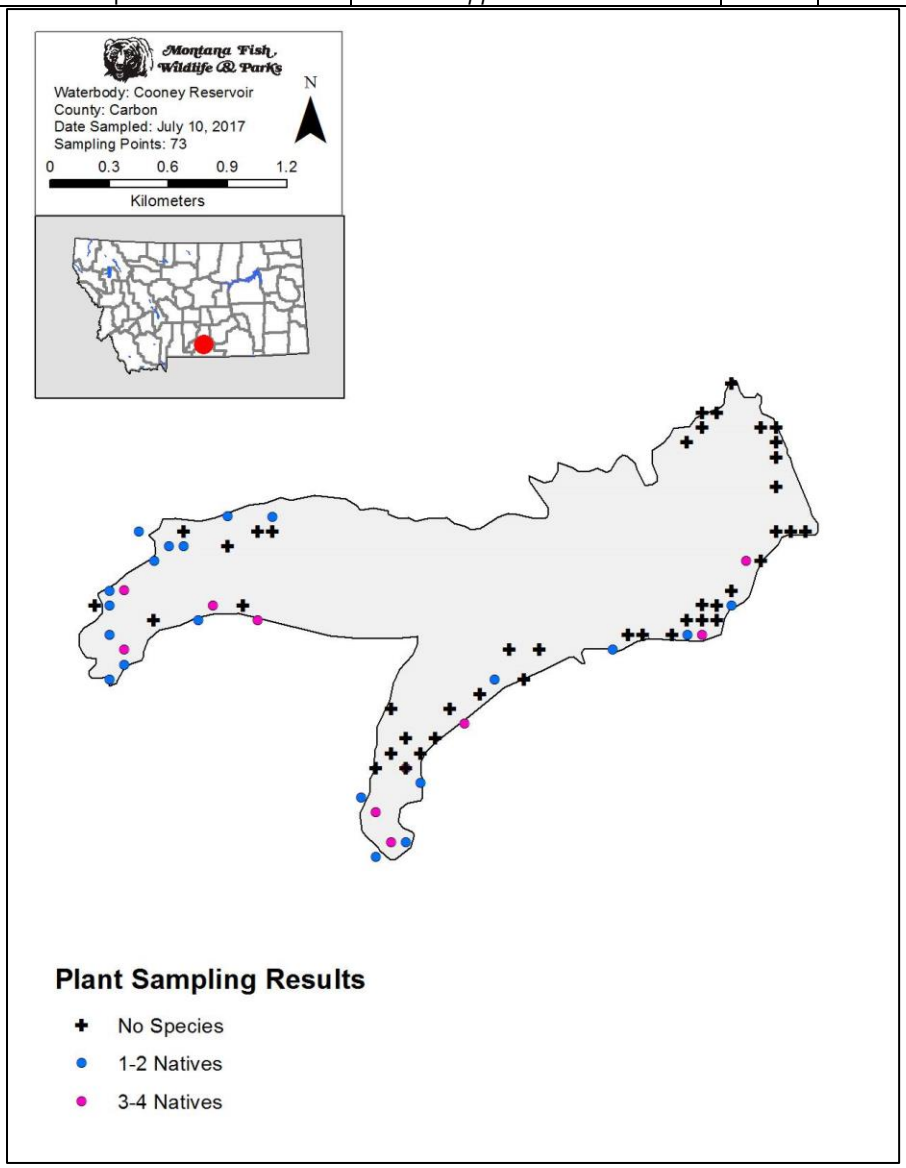
3. Bull Lake

Bull Lake	**n=172**		
Common Name	**Scientific Name**	**Count**	**Frequency**
No species	-	88	51%
Elodea species	*Elodea spp.*	47	27%
Muskgrass	*Chara spp.*	42	24%
Fern-leaved pondweed	*Potamogeton robbinsii*	30	17%
White-stemmed pondweed	*Potamogeton praelongus*	22	13%
Horned pondweed	*Zannichellia palustris*	21	12%
Northern watermilfoil	*Myriophyllum sibiricum*	9	5%
Leafy pondweed	*Potamogeton foliosus*	8	5%
Alpine Pondweed	*Potamogeton alpinus*	7	4%
Bulrush spp	*Scirpus spp.*	6	3%
Stonewort	*Nitella spp.*	6	3%
Large-leaf pondweed	*Potamogeton amplifolius*	4	2%
Beck's Water-marigold	*Bidens beckii*	3	2%
White waterbuttercup	*Ranunculus aquatilis*	3	2%
Floating-leaved pondweed	*Potamogeton natans*	2	1%
Unidentified Plant	-	2	1%
Coontail	*Ceratophyllum demersum*	1	1%
Grass-leaved pondweed	*Potamogeton gramineus*	1	1%
Unidentified Pondweed species	*Potamogeton spp.*	1	1%
Richardson's pondweed	*Potamogeton richardsonii*	1	1%



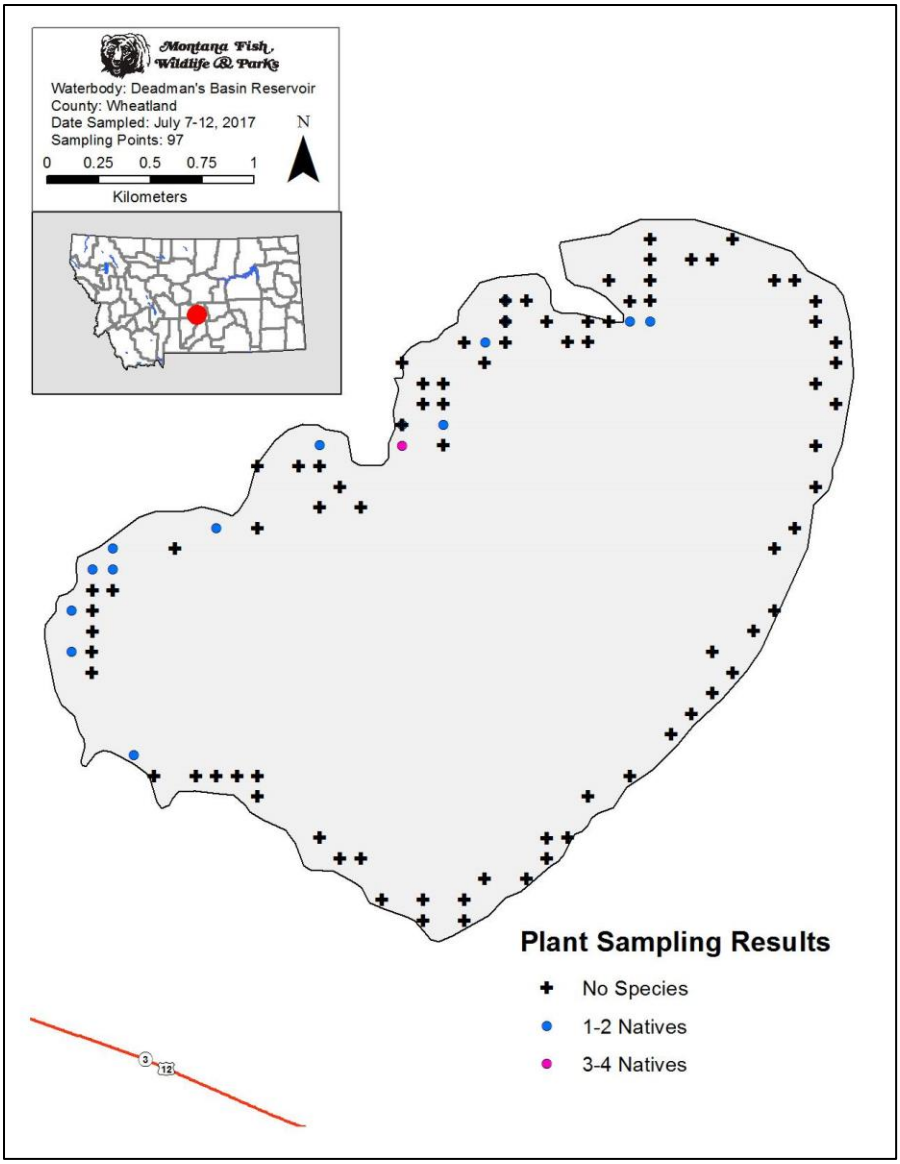
4. Cooney Reservoir

Cooney Reservoir	**n=73**		
Common Name	**Scientific Name**	**Count**	**Frequency**
No species	-	43	58%
Sago pondweed	*Stuckenia pectinatus*	15	20%
Elodea species	*Elodea spp.*	11	15%
Stonewort	*Nitella spp.*	8	11%
Leafy pondweed	*Potamogeton foliosus*	6	8%
White waterbuttercup	*Ranunculus aquatilis*	6	8%
Muskgrass	*Chara spp.*	4	5%
Unidentified Plant	-	3	4%
Richardson's pondweed	*Potamogeton richardsonii*	1	1%
Water Clover species	*Marsilea spp.*	1	1%



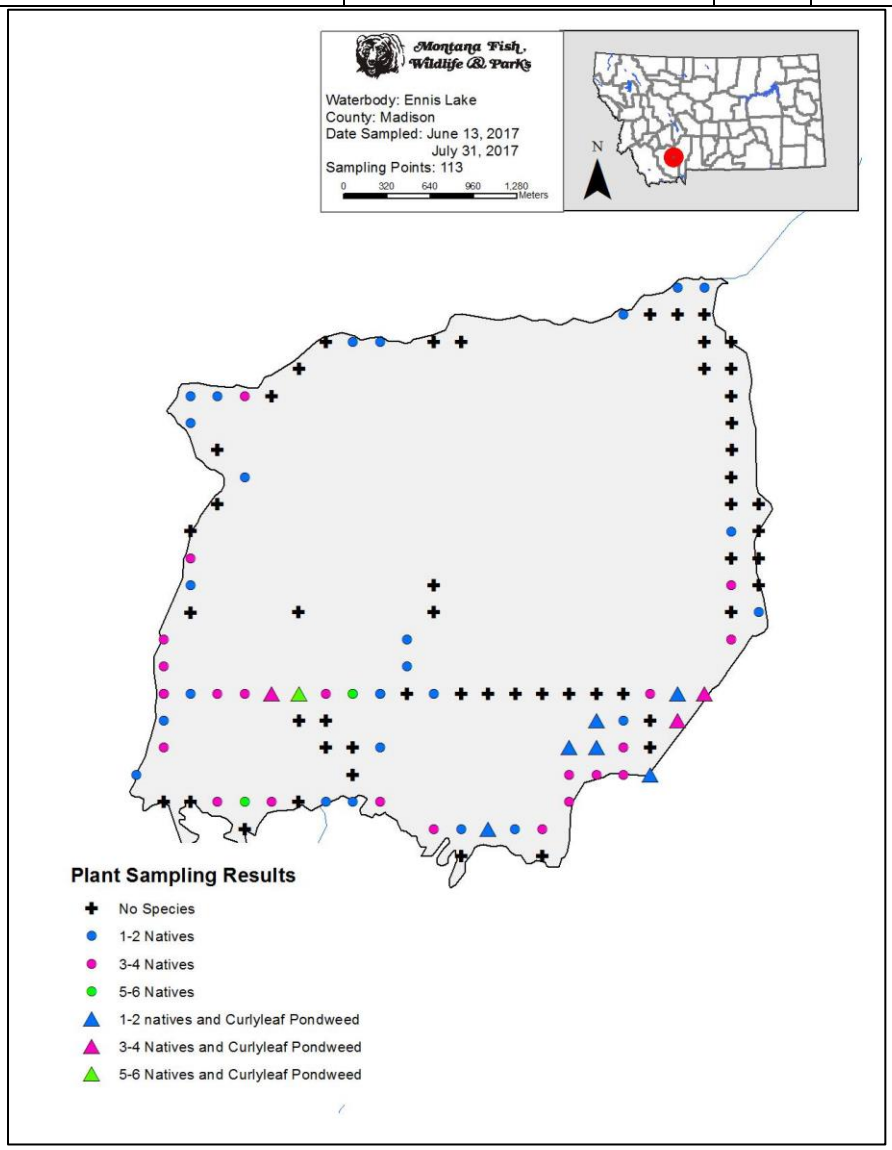
5. Deadman's Basin Reservoir

Deadman's Basin Reservoir	**n=97**		
Common Name	**Scientific Name**	**Count**	**Frequency**
No species	-	81	84%
Leafy pondweed	*Potamogeton foliosus*	9	9%
Unidentified Plant	-	6	6%
Illinois pondweed	*Potamogeton illinoensis*	4	4%
Sago pondweed	*Stuckenia pectinatus*	1	1%



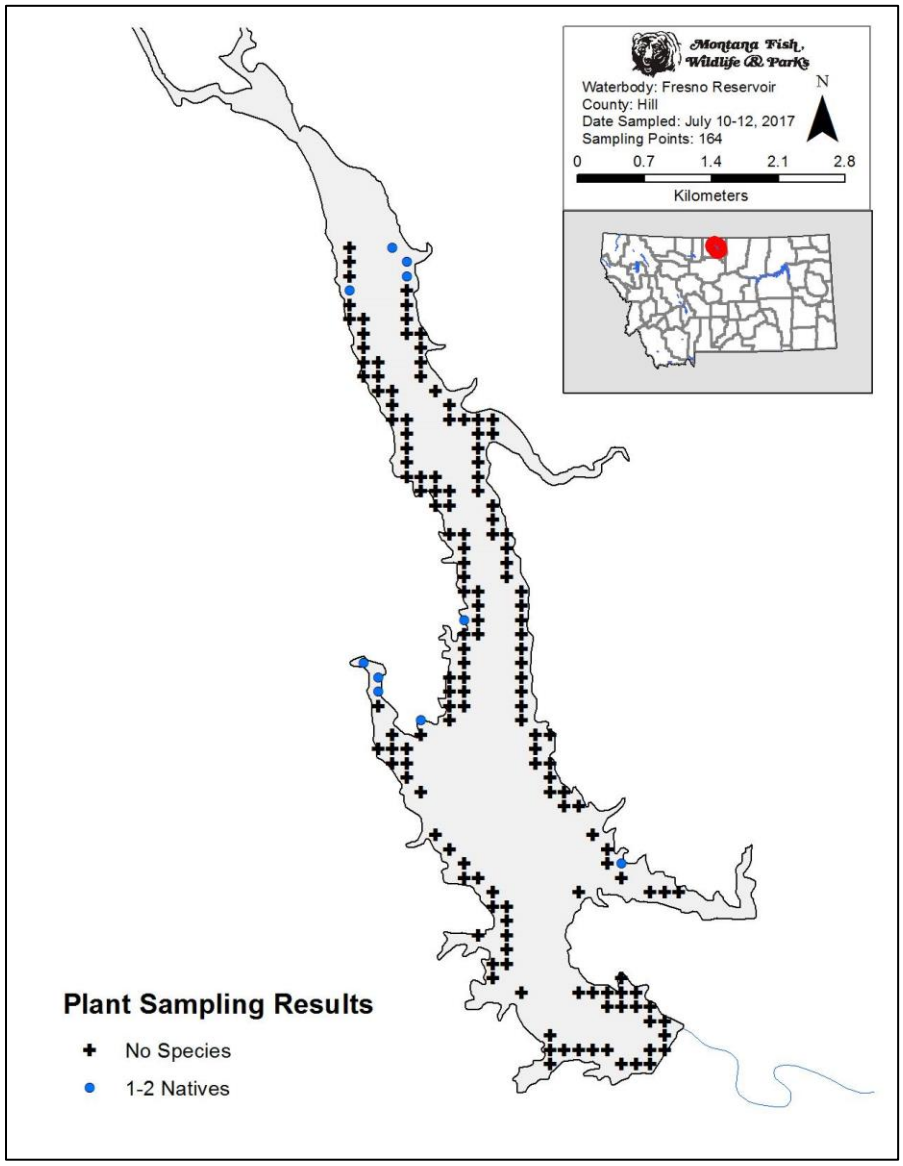
6. Ennis Lake

Ennis Lake	**n=117**		
Common Name	**Scientific Name**	**Count**	**Frequency**
No species	-	51	44%
Leafy pondweed	*Potamogeton foliosus*	42	36%
Elodea species	*Elodea spp.*	27	23%
White waterbuttercup	*Ranunculus aquatilis*	22	19%
Chara species	*Chara spp.*	16	14%
Curlyleaf pondweed	*Potamogeton crispus*	10	9%
Northern watermilfoil	*Myriophyllum sibiricum*	10	9%
Richardson's pondweed	*Potamogeton richardsonii*	10	9%
Northern arrowhead	*Sagittaria cuneata*	3	3%
Coontail	*Ceratophyllum demersum*	2	2%
Sago pondweed	*Stuckenia pectinatus*	2	2%
Unidentified Plant	-	2	2%



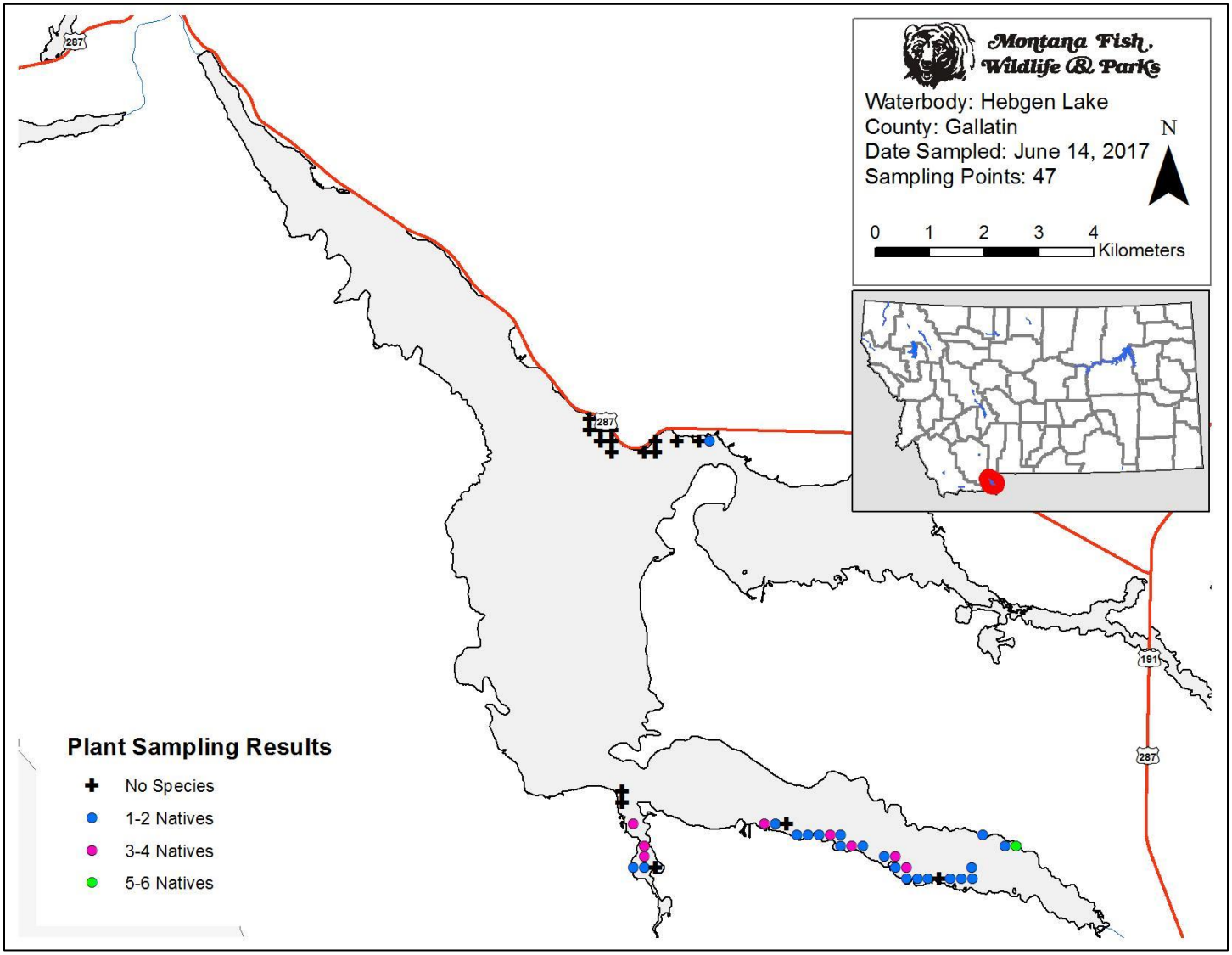
7. Fresno Reservoir

Fresno Reservoir	**n=164**		
Common Name	**Scientific Name**	**Count**	**Frequency**
No species	-	153	93%
Leafy Pondweed	*Potamogeton foliosus*	7	4%
Elodea species	Elodea spp.	1	1%
Richardson's pondweed	*Potamogeton richardsonii*	1	1%
Unidentified plant	-	1	1%



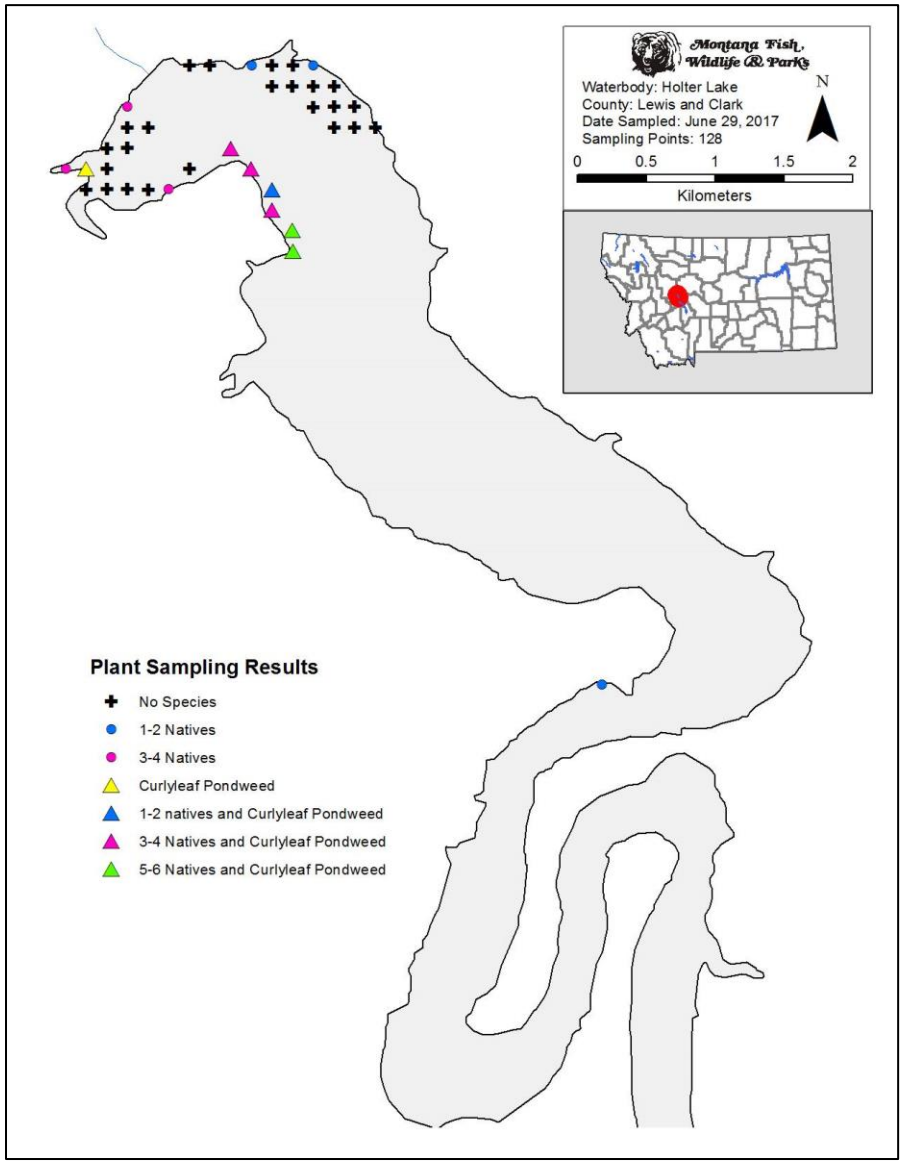
8. Hebgen Lake

Hebgen Lake	**n=47**		
Common Name	**Scientific Name**	**Count**	**Frequency**
No species	-	17	36%
Elodea species	*Elodea spp.*	14	30%
Northern watermilfoil	*Myriophyllum sibiricum*	13	28%
Unidentified Plant	-	12	26%
Leafy pondweed	*Potamogeton foliosus*	9	19%
Coontail	*Ceratophyllum demersum*	4	9%
Richardson's pondweed	*Potamogeton richardsonii*	3	6%
Chara species	*Chara spp.*	2	4%



9. Holter Lake

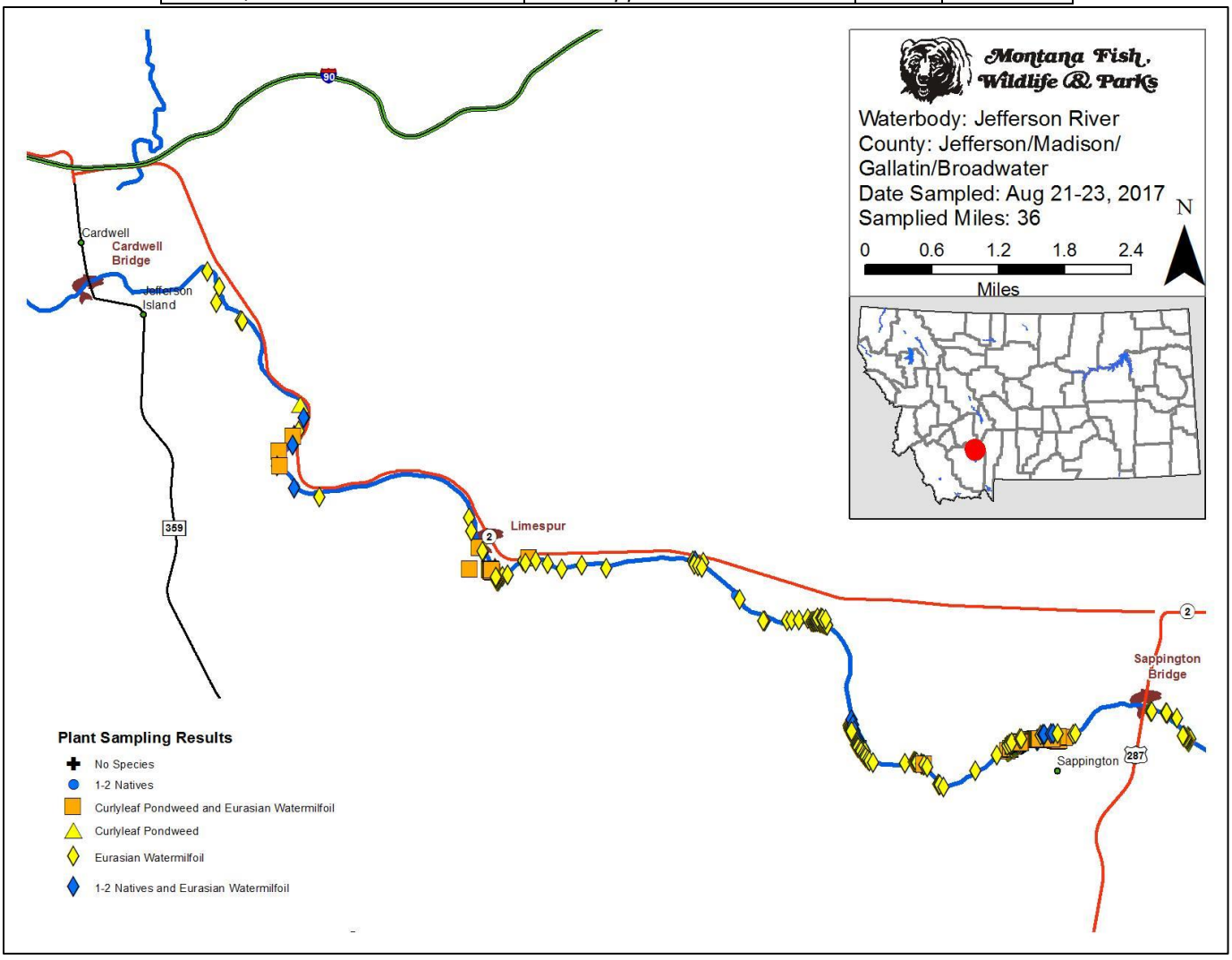
Holter Lake	**n=36**		
Common Name	**Scientific Name**	**Count**	**Frequency**
No species	-	24	67%
Elodea species	*Elodea spp.*	9	25%
Curlyleaf pondweed	*Potamogeton crispus*	7	19%
Leafy pondweed	*Potamogeton foliosus*	7	19%
Muskgrass	*Chara spp.*	6	17%
Sago pondweed	*Stuckenia pectinatus*	5	14%
Stonewort	*Nitella spp.*	3	8%
Unidentified Plant	-	3	8%
White waterbuttercup	*Ranunculus aquatilis*	1	3%

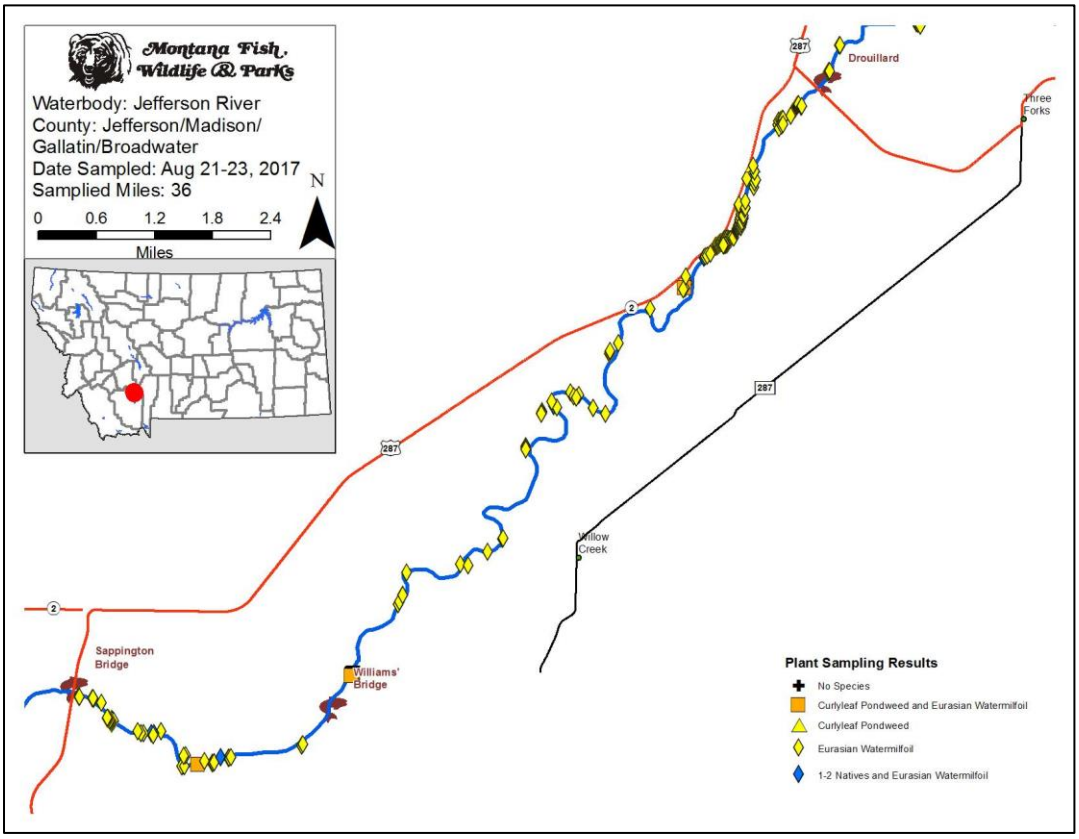


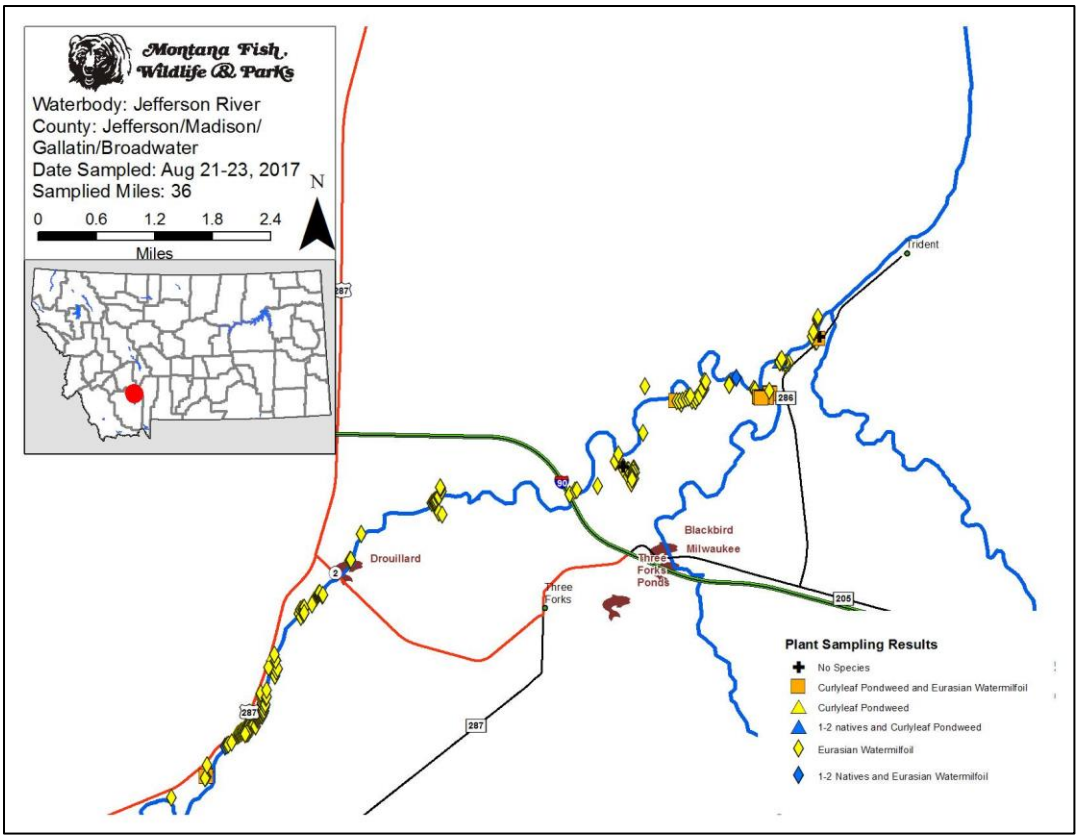
10. Jefferson River (Downstream of Cardwell)

This sampling effort was to delineate Eurasian watermilfoil populations. As a result, mapping occurred at locations with Eurasian watermilfoil. Native species were not recorded unless they occurred with Eurasian watermilfoil populations. As such, native species are underrepresented in these data.

Jefferson River	**n=1410**		
Common Name	**Scientific Name**	**Count**	**Frequency**
No species	-	5	0.4%
Eurasian watermilfoil	Myriophyllum spicatum	1400	99.3%
Curlyleaf pondweed	Potamogeton crispus	312	22.1%
Northern watermilfoil	*Myriophyllum sibiricum*	217	15.4%
Leafy pondweed	*Potamogeton foliosus*	10	0.7%
Elodea species	*Elodea spp.*	5	0.4%
Richardson's pondweed	*Potamogeton richardsonii*	3	0.2%
Chara species	*Chara spp.*	1	0.1%

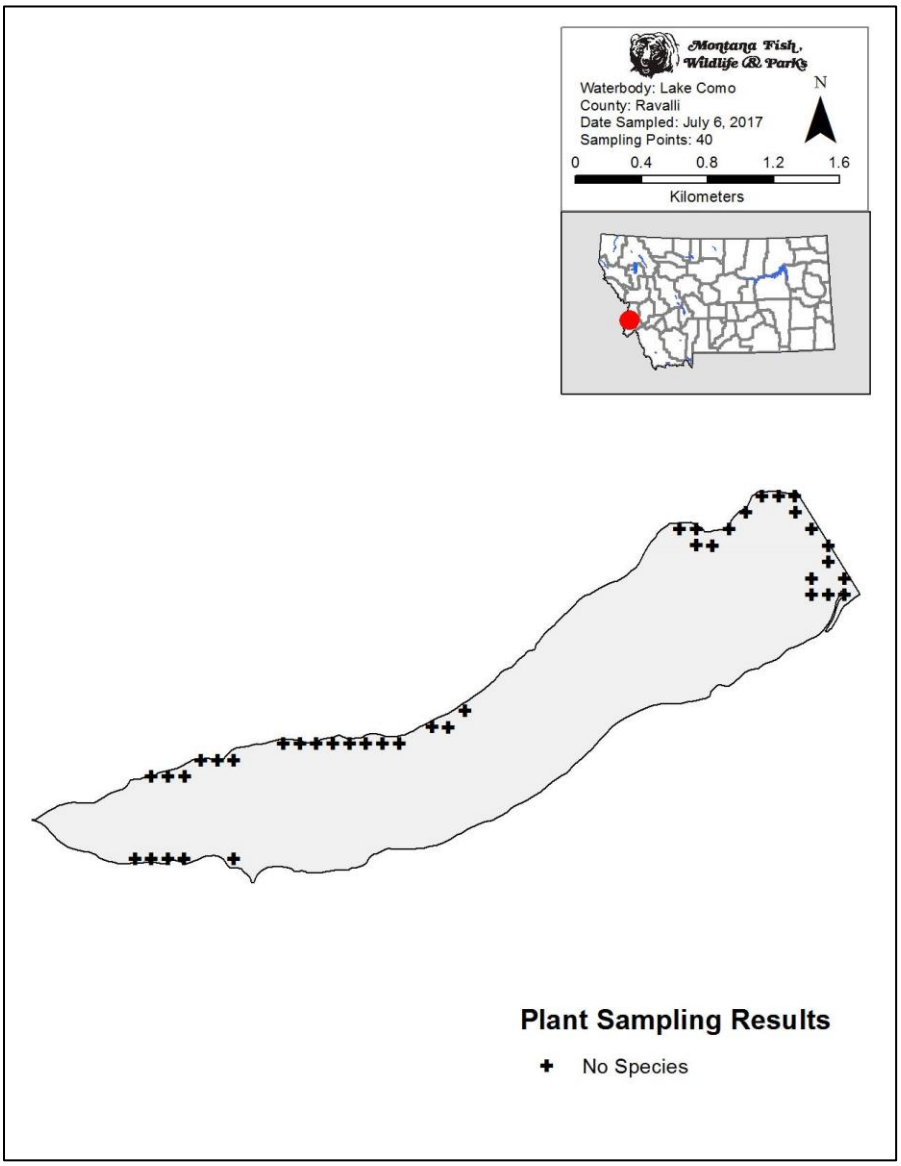



Montana Fish, Wildlife & Parks
Waterbody: Jefferson River
County: Jefferson/Madison/
Gallatin/Broadwater
Date Sampled: Aug 21-23, 2017
Samplied Miles: 36
N
0 0.6 1.2 1.8 2.4
Miles
Drouillard
Three Forks
Willow Creek
Sappington Bridge
Williams' Bridge
Plant Sampling Results
No Species
Curlyleaf Pondweed and Eurasian Watermilfoil
Curlyleaf Pondweed
Eurasian Watermilfoil
1-2 Natives and Eurasian Watermilfoil


Montana Fish, Wildlife & Parks
Waterbody: Jefferson River
County: Jefferson/Madison/
Gallatin/Broadwater
Date Sampled: Aug 21-23, 2017
Samplied Miles: 36
N
0 0.6 1.2 1.8 2.4
Miles
Trident
Blackbird
Milwaukee
Three Forks Ponds
Drouillard
Three Forks
Plant Sampling Results
No Species
Curlyleaf Pondweed and Eurasian Watermilfoil
Curlyleaf Pondweed
1-2 natives and Curlyleaf Pondweed
Eurasian Watermilfoil
1-2 Natives and Eurasian Watermilfoil

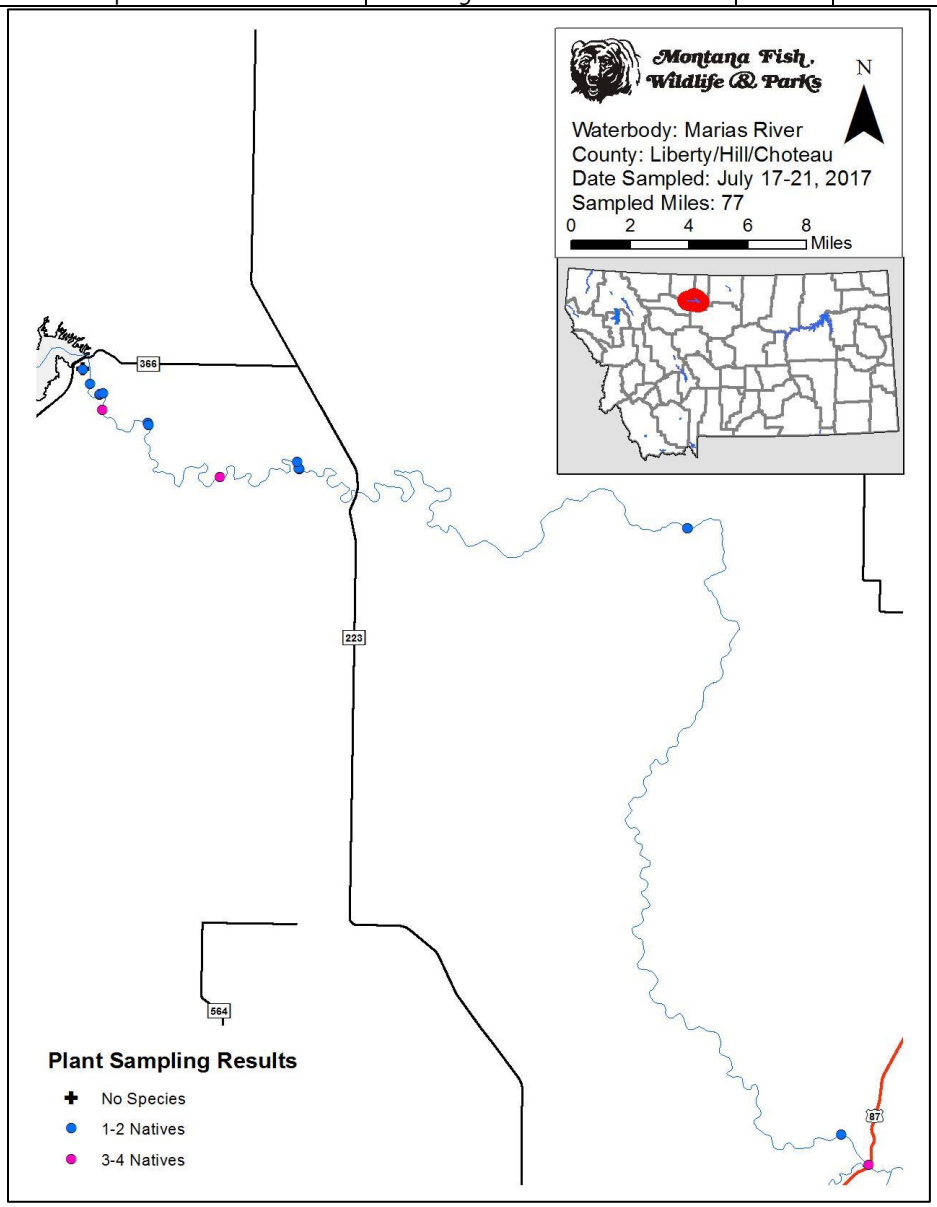
11. Lake Como

Lake Como	n=41		
Common Name	**Scientific Name**	**Count**	**Frequency**
No species	-	41	100%



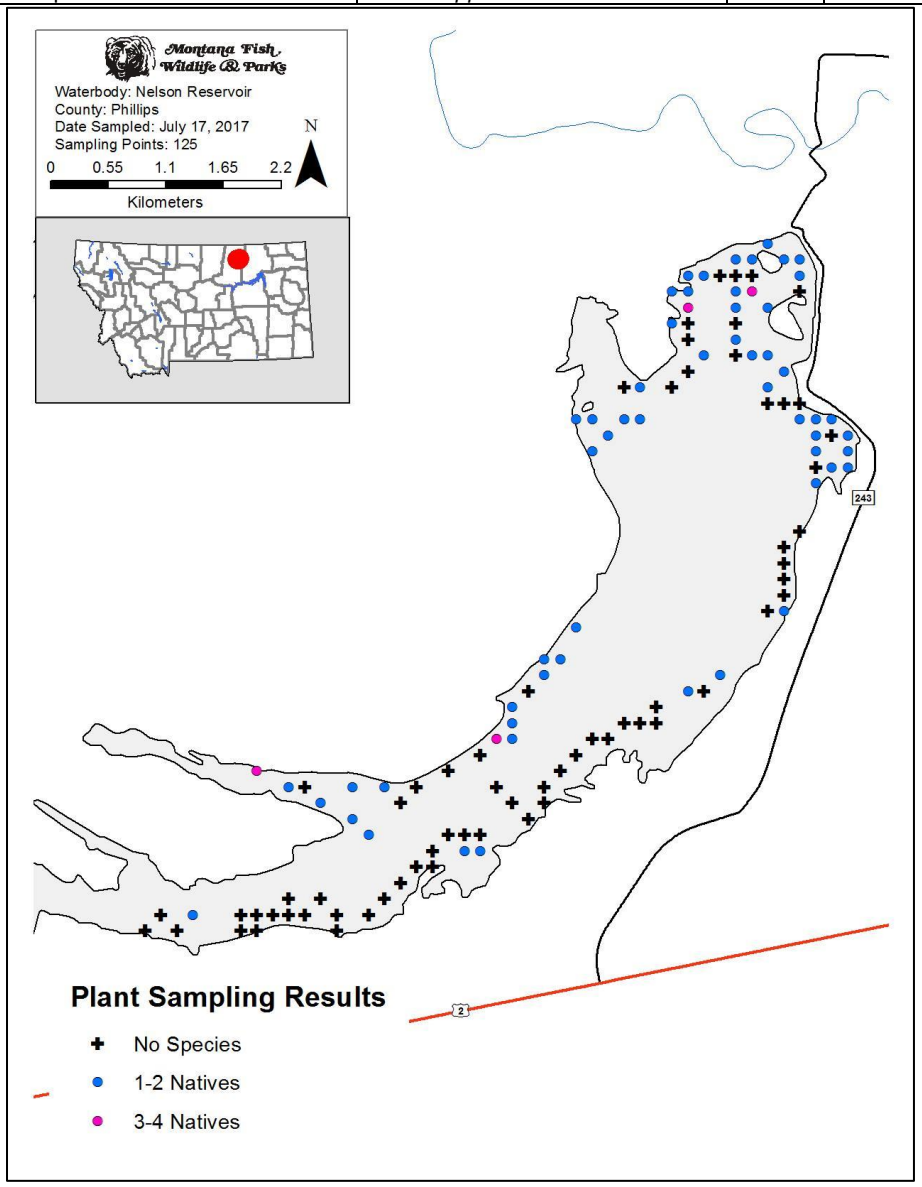
12. Marias River

Marias River	**n=18**		
Common Name	**Scientific Name**	**Count**	**Frequency**
No species	-	1	6%
Muskgrass	*Chara spp*	8	44%
Unidentified Plant	-	7	39%
Elodea species	*Elodea spp.*	5	28%
White waterbuttercup	*Ranunculus aquatilis*	2	11%
Horned pondweed	*Zannichellia palustris*	1	6%
Narrowleaf water-plantain	*Alisma gramineum*	1	6%
Richardson's pondweed	*Potamogeton richardsonii*	1	6%



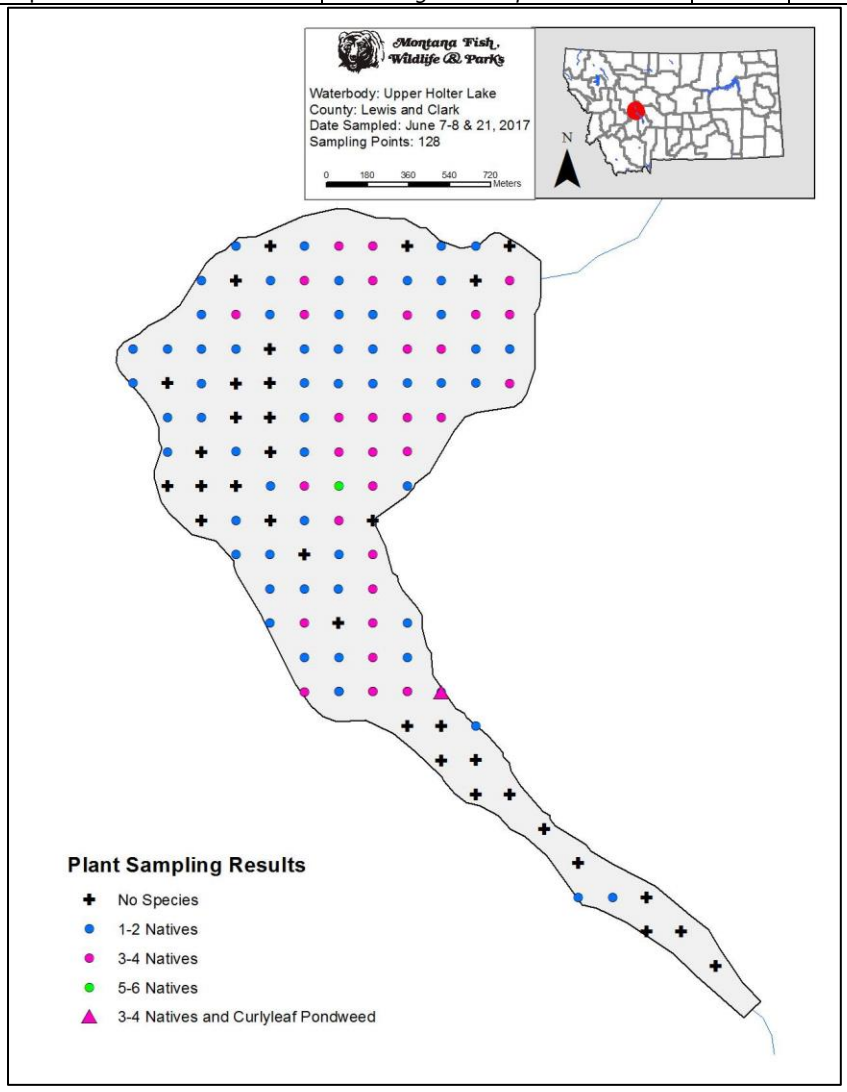
13. Nelson Reservoir

Nelson Reservoir	**n=125**		
Common Name	**Scientific Name**	**Count**	**Frequency**
No species	-	65	52%
Leafy pondweed	*Potamogeton foliosus*	34	27%
Stonewort	*Nitella spp.*	32	26%
Sago pondweed	*Stuckenia pectinatus*	10	8%
White waterbuttercup	*Ranunculus aquatilis*	5	4%
Muskgrass	*Chara spp.*	4	3%
Elodea species	*Elodea spp.*	4	3%



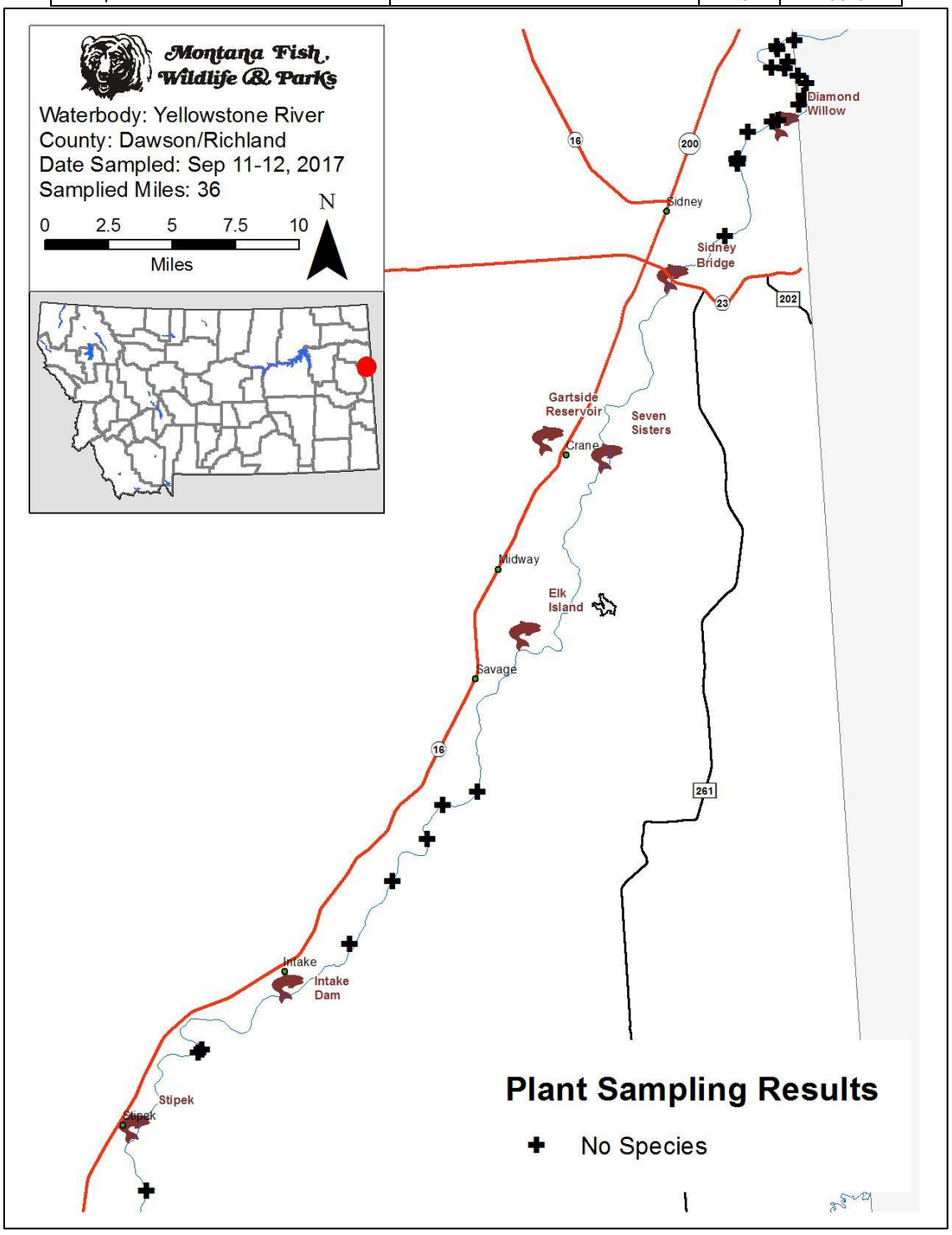
14. Upper Holter Lake

Upper Holter	**n=128**		
Common Name	**Scientific Name**	**Count**	**Frequency**
No species	-	33	26%
Leafy pondweed	*Potamogeton foliosus*	76	59%
Elodea species	*Elodea spp.*	42	33%
Sago pondweed	*Stuckenia pectinatus*	22	17%
White waterbuttercup	*Ranunculus aquatilis*	20	16%
Northern watermilfoil	*Myriophyllum sibiricum*	14	11%
Stonewort	*Nitella spp.*	6	5%
Coontail	*Ceratophyllum demersum*	3	2%
Horned pondweed	*Zannichellia palustris*	3	2%
Richardson's pondweed	*Potamogeton richardsonii*	2	2%
Sheathing pondweed	*Potamogeton vaginatus*	2	2%
Curlyleaf pondweed	*Potamogeton crispus*	1	1%



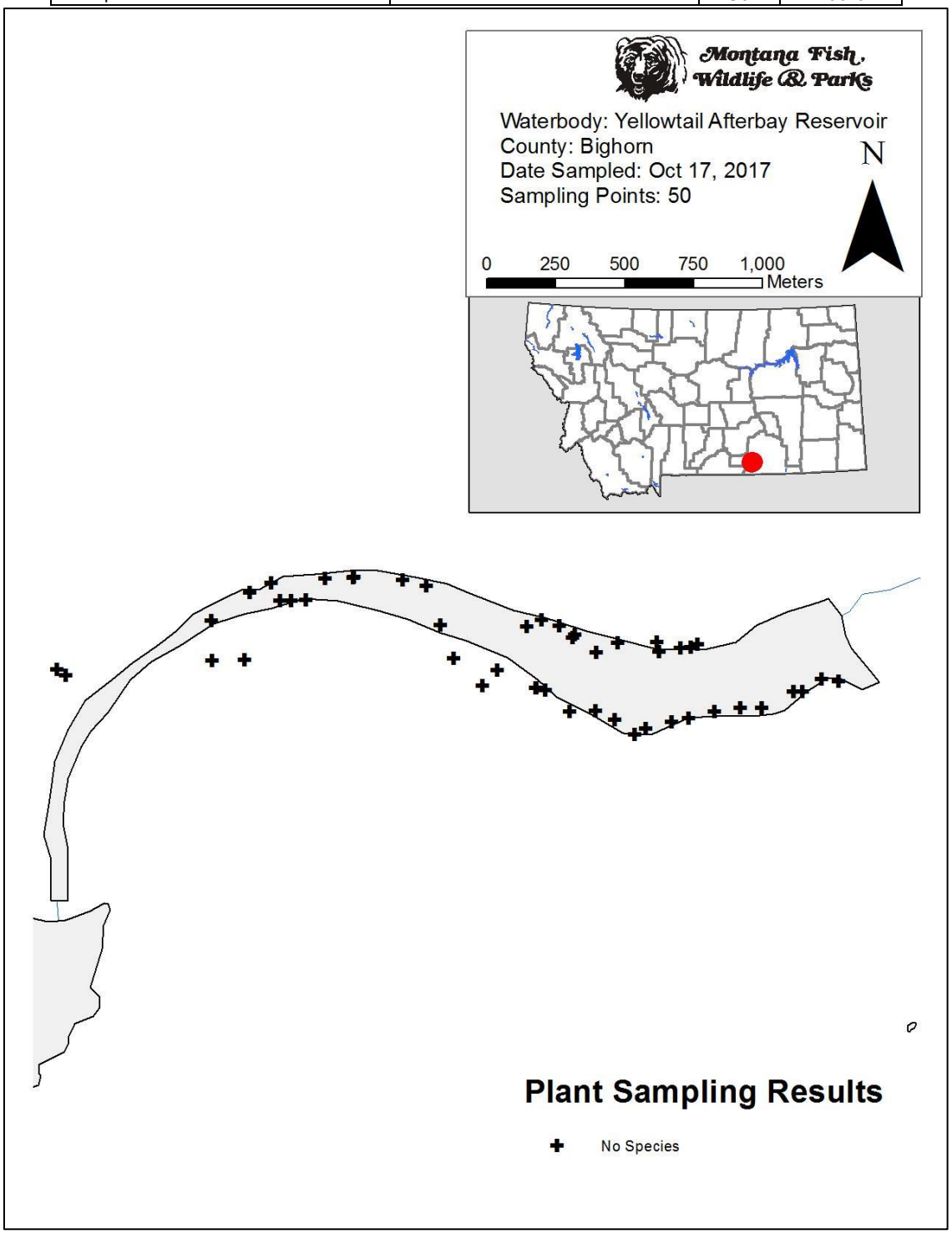
15. Yellowstone River (Downstream of Glendive)

Yellowstone River	n=26		
Common Name	**Scientific Name**	**Count**	**Frequency**
No species	-	26	100%

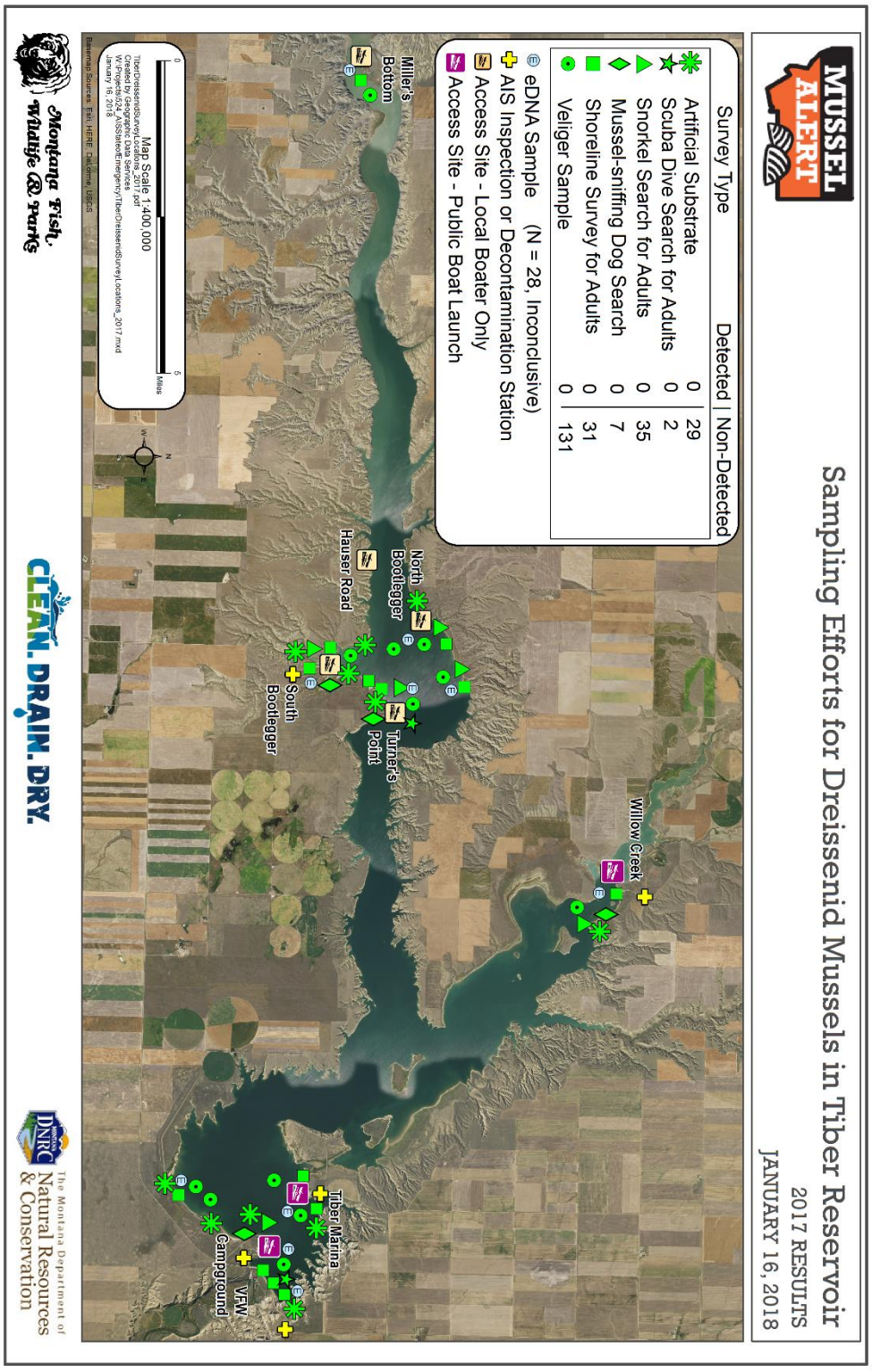


16. Yellowtail Afterbay Reservoir

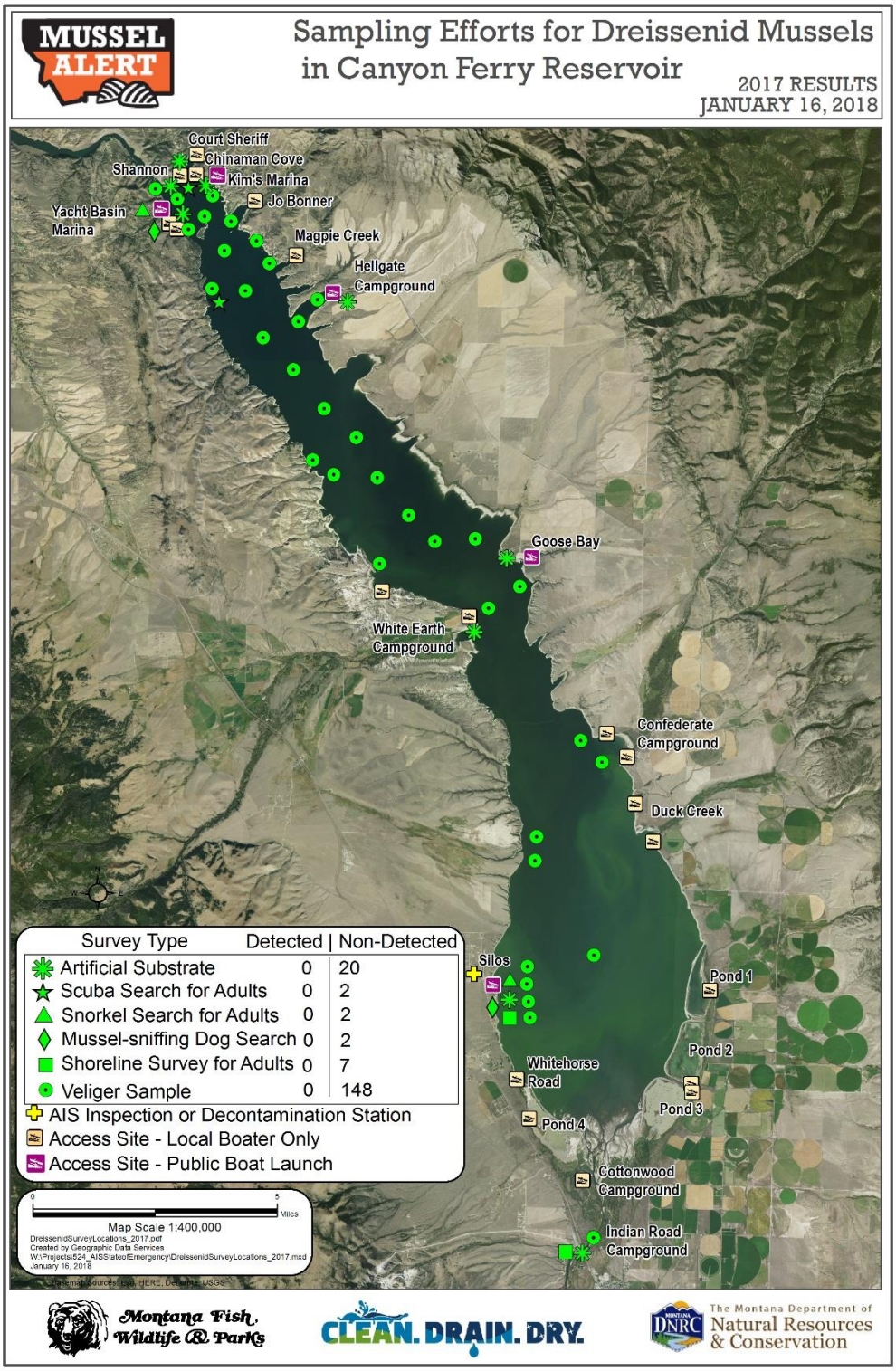
Yellowtail Afterbay Reservoir	n=50		
Common Name	**Scientific Name**	**Count**	**Frequency**
No species	-	50	100%



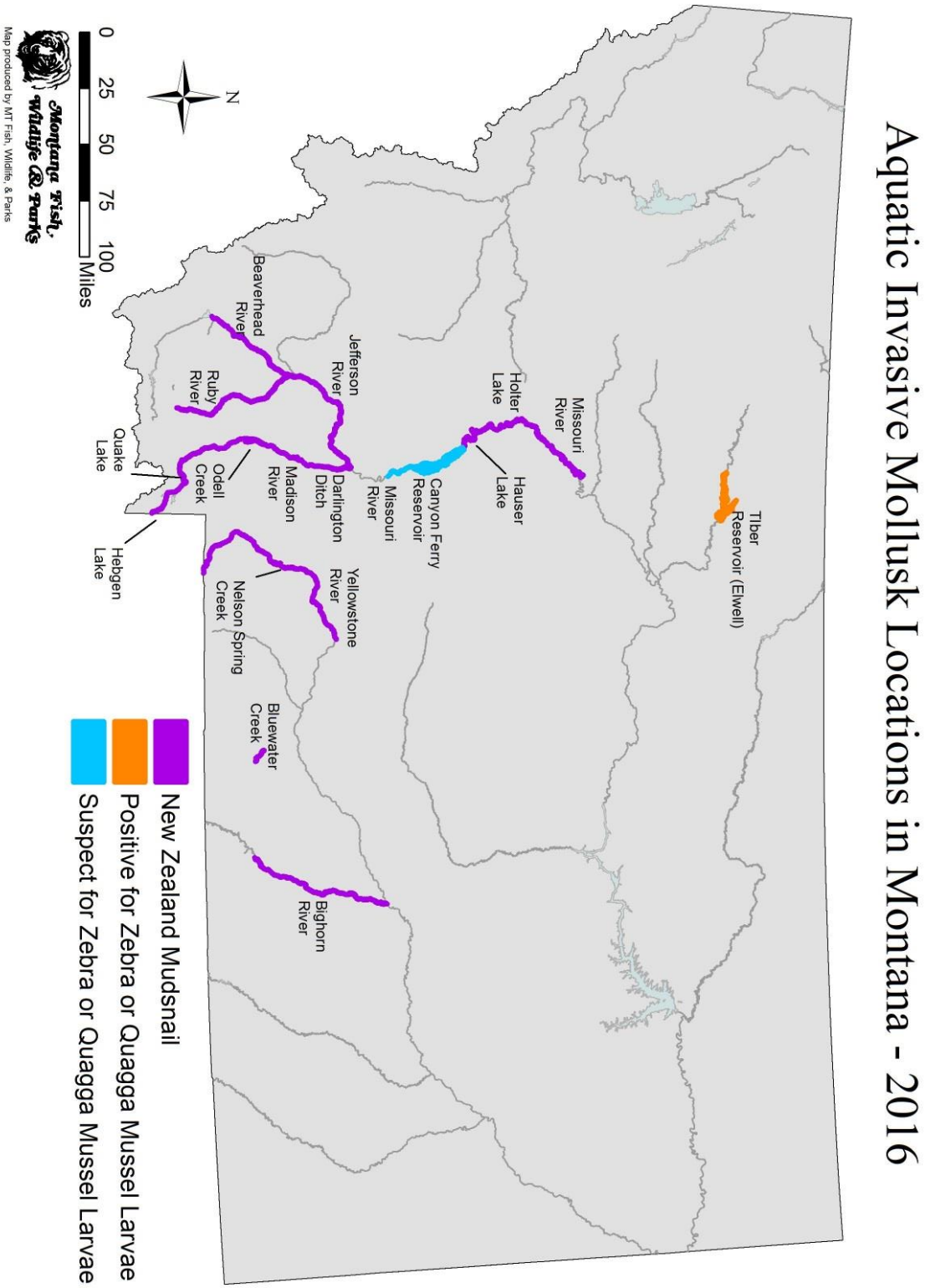
Appendix C. Mussel response sampling events on Tiber Reservoir



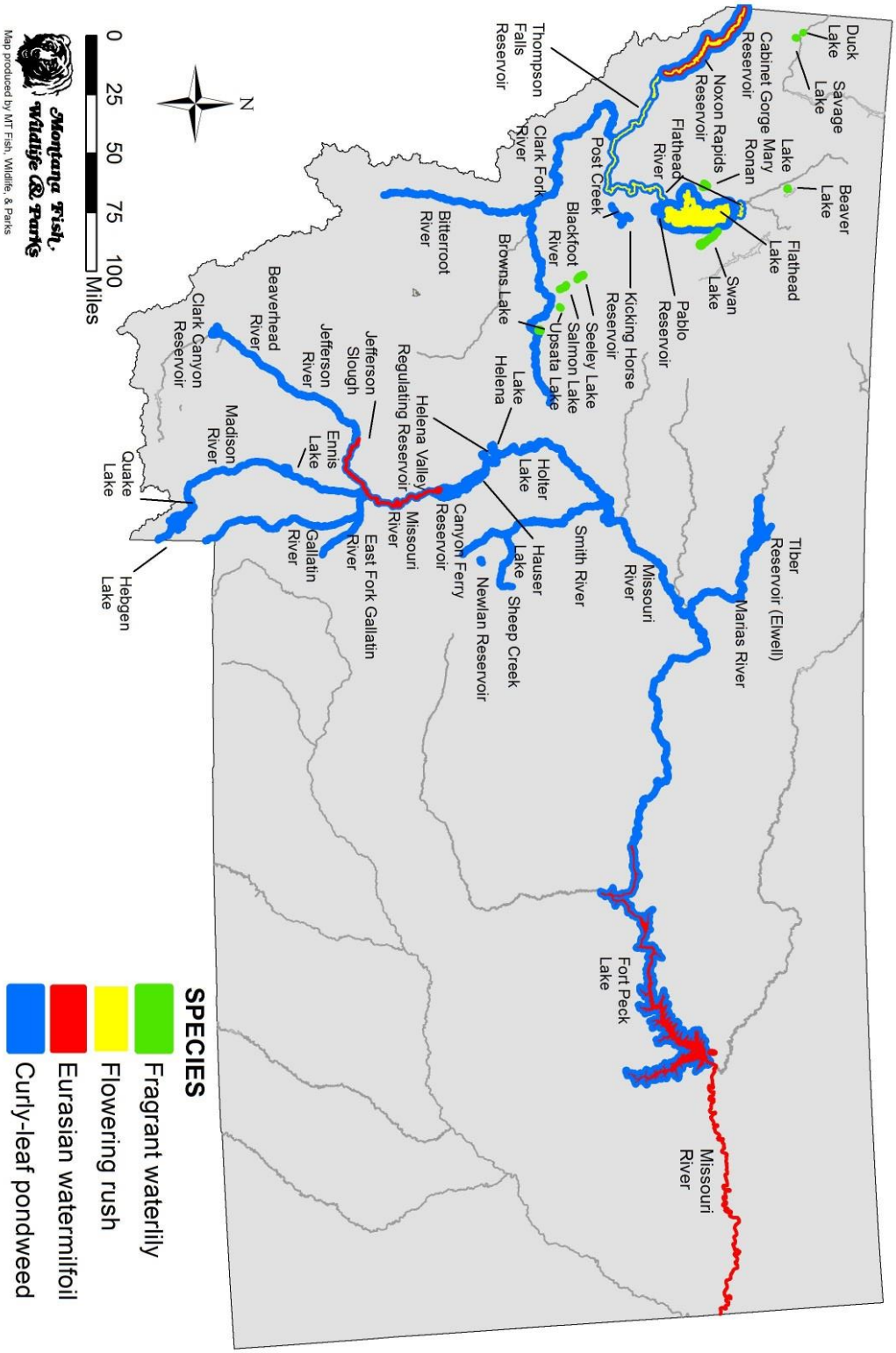
Appendix D. Mussel response sampling events on Canyon Ferry Reservoir



Appendix E. Map of invasive mollusks in Montana



Appendix F. Map of invasive plants in Montana



Appendix G: 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 H: 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 I: 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

ID	MinValue	Max Value	Parameter	Score
34	Low use <25% quartile		Angler Days	1
35	Medium Low 26- 50% quartile		Angler Days	2
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

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

- 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