Montana Invasive Species Council

Meeting Materials Packet for March 2, 2022

Contents

Note: Documents are hyperlinked for ease of navigation

MONTANA INVASIVE SPECIES COUNCIL

AGENDA

Note: Agenda is subject to change and item times are approximate. Actual times may vary by up to one hour.

Montana Capitol, Room 472, Helena, MT. Hybrid meeting.

WEDNESDAY, March 2, 2022

9:00 a.m. – 9:40 a.m.	**INTRODUCTIONS** Chair Bryce Christiaens Roll call and MISC orientation presentation
9:40 a.m. – 10:50 a.m.	**ADMINISTRATIVE BUSINESS** *ACTION: December 9, 2021 meeting minutes AIS Grant Cycle-1 Report and Cycle-2 applications *ACTION: PNWER Conference in Calgary - MISC representation Amy Gannon and Jill Hautaniemi, DNRC, Science Advisory Committee Update *ACTION: Invasive Species Information Document
10:50 a.m. – 11:00 a.m.	**BREAK**
11:00 a.m. – 12:00 p.m.	**INVASIVE SPECIES SUMMIT** Update on planning and deliverables
12:00 p.m. – 12:30 p.m.	**LUNCH BREAK (Provided)**
12:30 p.m. – 1:00 p.m.	**MISC WEBINARS** Update on planning, topics and schedule
1:00 p.m. – 1:30 p.m.	**FERAL SWINE UPDATES** Dr. Tahnee Szymanski, Dept. of Livestock (invited)
1:30 p.m. – 2:00 .m.	**GRANT RECIPIENT PRESENTATIONS** FLATHEAD LAKE BIOLOGICAL STATION Phil Matson, Leif Howard & Gordon Luikart (invited)
2:00 p.m. – 2:10 p.m.	**BREAK**
2:10 p.m. – 3:15 p.m..	**2022 AGENCY & PARTNER UPDATES** Round robin of season updates
3:15 p.m. – 3:30 p.m.	**WRAP UP AND ADJORN** Location for June and September meetings Final discussion *Public Comment

This meeting is open to the public. The most current meeting information including meeting materials are available on the MISC website at: https://invasivespecies.mt.gov/misc/meetings-schedule.

Members of the public who wish to participate via Zoom may do so by emailing a request with your name to emoran@mt.gov. Instructions for joining and participating will be sent by 5 p.m. the day before the meeting.

*Public comment will be available during times the Council acts on items as indicated on the agenda and during the end of the meeting. To provide public comment, participants may "raise their hand" and participate after being recognized by the presiding officer or Zoom manager. Comments will be taken in order. Written public comment may be sent via email in advance of the meeting to emoran@mt.gov and will be provided to council members.

Any oral or written public comment provided to the committee is a public record that is recorded and archived.

The Montana Department of Natural Resources and Conservation will make reasonable accommodations for persons with disabilities who wish to participate in this public meeting. For questions about accessibility or to request accommodations, please contact Emily Moran at 406-444-2613 or emoran@mt.gov as soon as possible before the meeting date.

MEETING MINUTES

These abbreviated summary minutes will become the official adopted minutes at the next Montana Invasive Species Council meeting when they will be approved. Until then, they are considered a draft.

Meeting/ Project Name:	**MISC**		
Date of Meeting:	**December 9, 2021**	**Time:**	9:00 AM
Minutes Prepared By:	**Emily Moran and Liz Lodman**	**Location:**	Billings Northern Hotel, and virtual via Zoom

Attendees

MISC Voting Members: Bryce Christiaens (Missoula County Weed District – Chair), Steve Wanderaas (Conservation Districts—Vice Chair), Tom Woolf (FWP- Vice Chair), Amy Gannon (DNRC representative), Andy Welch (Hydropower Representative), Bob Gilbert (Private Landowner Representative), Leigh Greenwood (The Nature Conservancy), Jane Mangold (MSU-Ext.), Jan Stoddard (DOC representative), Bob Cloninger (MDT Representative), Martin Charlo (Salish and Kootenai Tribes), Charles Headdress (Fort Peck Tribes Representative), Dennis Longknife (Fort Belknap Representative), Steve Tyrrel (Agriculture), Paul Rossignol (Wildlife Organization Representative), Michael Bias (Fishing Outfitter Representative)

MISC Tribal, State, and Federal Partners: Michelle Cox (USFS), Jessica Zarate (USFWS), Monica Pokorny (NRCS), Philip Holmes (CBP), Gary Adams (APHIS) Wendy Velman (BLM), Ian Foley (MDA), Beth Eiring (MDA), Jason Allen (MDT), Liz Lodman (FWP), Stephanie Criswell (DNRC), Kate Wilson (DNRC- UC3), Jill Hautaniemi (DNRC), Shawn Cobell (Blackfeet Tribe), Shantell Frame-Martin (MSU), Jeff Littlefield (MSU), Jennifer Birdsall (MSU), Nathan Luke (Australia Department of Agriculture),

Other Attendees: Torrey Ritter (FWP), Wendy Jones (CEMIST) Bryan Wilson (MCC), Bryce Maxell (MNHP), Phil Matson (FLBS), Sara Owen (UM), Juli Thurston (MSU), Molly Yurdana (MSU), Molly Masters (MSU), Amber Skillman (APHIS)

Agenda and Notes, Decisions, Issues

Topic	Discussion
December 8th	**Tour of Lake Elmo State Park Invasive Clam Eradication and Improvement Project** Craig McLane, FWP AIS Specialist. Mike Ruggles, FWP Region 5 Supervisor Terri Walter, FWP State Park Manager Shannon Blackburn, FWP Region 5 Fisheries Biologist Bob Gibson, FWP Region 5 Information & Education Manager
Welcome & Roll call	Bryce opened the meeting at 9:00 a.m. conducted roll call and confirmed quorum. Mike Bias, the fishing outfitters association representative for MISC was introduced. **Action Item: Approval of June 2nd, 2021, Meeting Minutes.** Motion: Jane Mangold moved to approve June 2nd meeting minutes with the revision of "Ryan Brook's" name on page 5. Second: Martin Charlo Discussion: None Public comment: None Action on motion: Motion passed unanimously.
2021 Invasive Species Program Updates	**Bryce Maxell, Montana Natural Heritage Program, Montana State University** • Montana Natural Heritage Program (MNHP) Databases have added 25 new non-native species within this past year. • Added 210,000 observation records this past year. • MNHP has created 'risk of invasion' models for 172 species

- Risk Modeling efforts have been captured in a PowerPoint that will be released at the end of the year or beginning of 2022.

Tom Woolf, Montana Fish, Wildlife & Parks AIS Coordinator, MISC Vice Chair- AIS Program Update

- Tiber is in the process of being delisted. Official designation will be announced after the public comment period closes; expected in February.
- Mussel detections are expanding nationally, but not in the west. Mussel populations in the west have stayed static.
- Federal funding is available through the Army Corps of Engineers to fund Watercraft Inspection Stations.
- Watercraft inspections have decreased this year, most inspections, and interviews have been with local traffic.
- There have been improved training and quality control and assurance by working closely with partners to increase trained staffed.
- In 2021 there have been 300 citations and warnings issued due to focused enforcement and increased signage.
- Over 110,819 boats have been inspected in 2021.
- Hardin station piloted night stations leading up to the July 4th. Data found stations operated during daylight intercept 90% of boater traffic. Night stations bring safety and staffing challenges.
- Watercraft inspection/decontamination station's locations will be reviewed. Hope to demobilize stations around Tiber after it has been delisted. Southeastern locations will be funded by Montana Fish, Wildlife & Parks, Powder River Conservation District, and Big Horn Conservation District. Next year, Tongue River and the Fresno stations will be closed; no high-risk boats have been seen, nor are they anticipated.
- 61 Mussel fowled boats have been intercepted in 2021, up from 35 in 2020. Many are recently purchased boats from midwestern states. Looking to create partnerships within the Midwest to promote education and outreach. Regional coordination is key.
- FWP worked closely with UC3 and Big Sky Watershed members on outreach to local businesses across the state to promote AIS awareness.
- FWP is working to partner with four new Conservation Districts. Local involvement and interest improves quality control.
- Rapid Response Planning exercise simulated a mussel detection at Fort Peck Reservoir. This exercise included US Army Corps, USFWS, Pacific States Marine Fisheries Commission, conservation districts across the state, North and South Dakota invasive species coordinators. The exercise summary now available at westernAIS.org
- Lab processed a record number of early detection samples. No mussel detections were found in Montana. Labs are located in Helena and Kalispell.
- New detections AIS include:
    - A new crayfish species in Miles City
    - New Zealand Mudsnails in three western Montana locations.
    - Red Rim Melenia Snail (warm water aquarium species) found in two Southwestern locations.
    - Curly-leaf pondweed was found in two new South-Central locations.
    - Eurasian Water Milfoil was found in Nilan Reservoir and was treated with herbicide
- Final "New Detections" report will be released early 2022.
- Moss Balls imported from Ukraine were found to have zebra/quagga mussels and other Ukrainian invertebrates living inside them. This discovery was made by a pet shop employee who notified authorities. Moss ball importation is governed by APHIS, while USFWS holds the authority over zebra/quagga mussels. The 'Moss Ball Crisis' was a great learning experience and exercise of authority.
- FWP AIS program and contracted inspection stations are funded through the

legislature, federal, and local funding.

- Targeted outreach is continuing the 'Clean.Drain.Dry', "Don't Let It Loose', and 'The cr-A-y Team' uniform messages.
- FWP AIS program plans to expand partnerships, improve communication and coordination, and acknowledge success in 2022.

Discussion:
The main reasons boaters do not stop at inspection/decontamination stations are that it is either a new concept for the boaters, or they did not see the signs.

The Southern Plains Crayfish is the only non-native species or crayfish known in Montana. It was found in the Miles City hatchery and is not known to be invasive.

How does the delisting of Tiber allow FWP to redirect their resources?

- Historically Tiber management has utilized $300,000 a year; after delisting, these funds can go to contracting more stations with different conservation districts.

Can FWP partner with MDT to install cameras in highly trafficked boating corridors to monitor boating traffic? For example, Lolo Pass.

- MDT does not have live video cameras installed, currently the cameras capture photos at a certain rate of time. Identification information captured from these cameras cannot be used to cite or ticket offenders. This topic will need to be investigated further.

Amy Gannon, Forest Pest Management Program Manager, DNRC- Tree and Forest Pest Update

- There are 20 species of pests that put our forest resources at risk.
  - Currently Established:
    - Balsam woolly adelgid
      - Uncertain about impacts in MT. Additional distribution surveys are scheduled for 2022 and will be funded from the USFS Forest Health Monitoring Program.
    - White pine blister rust
    - Dutch elm disease
    - Larch casebearer
  - On the Horizon:
    - *Lymantria dispar*
      - Statewide multi-agency trapping program in place. Egg mass survey scheduled for January 2022. Delimitation survey scheduled for the summer of 2022.
    - Emerald ash borer
      - The federal quarantine has been cancelled; Montana has an external quarantine on ash coming into the state. Montana DNRC Urban and Community Forestry Program received a grant from USFS to replace ash in Montana communities.
    - Pine shoot beetle
    - Asian long horned beetle
    - Sirex woodwasp
    - Bark and ambrosia beetles
    - The pests we don't know are typically the most concerning.
- USFS Forest Health Monitoring Funds
  - Don't Move Firewood campaign, successful partnership with The Nature Conservancy and collaboration between neighboring states.
    - Printed ads in the hunting and fishing regulations, RV and travel magazines, and physically printed and posted at camp kiosks, entry stations, shower facilities, rest areas, etc.
  - Have $25,000 remaining in the Emerald Ash Borer (EAB) funds that expire in 2025. Original amount was $27,933.
    - Funds will be used for outreach efforts that deter the transport of out-of-state firewood into Montana. Such as, purchasing ad space, printing posters, implementation of recommendation from the Firewood Science

	Advisory Panel. ▪ Amy is open to ideas from the council or public. • USFS EAB Sampling Project o Partnered with Laurie Kerzicnik, Montana State University, to conduct destructive branch sampling in four cities (Bozeman, Helena, Billings, Missoula). The objective was for detection and education/outreach. **Discussion:** Is the $25,000 funding for the Emerald Ash Borer funds adequate to reach your goals? It is a good amount of money for purchasing ad space, it is not the only funding the program has available. Potential ideas for spreading 'Don't Move Firewood" message: poster should be hung in local sporting goods stores. Creating a lesson plan for school age kids. How is the EAB external quarantine being enforce and has there been pushback or need to utilize it? o Industry members are familiar with the requirements. Amy and The Nature Conservancy's education and outreach efforts have made the industry aware of the situation. It is very important that Montana enforces their own external quarantines, antidotal evidence shows states without an external quarantine receive mass amounts of untreated, mixed, hardwood firewood, which, may contain EAB. Implementation of an external quarantine has been variable between states. For questions about the 'Don't Move Firewood' or any firewood outreach reach out to Leigh Greenwood (Lgreenwood@tnc.org). The renaming of Lymantria *dispar* will be finalized and announced January or February of 2022. Amy is currently working with the tree pest education and outreach group to coordinate information dissemination efforts. First quarter meeting in 2022 should focus on feral hogs and terrestrial plants updates.
Noxious Weed Education/Outreach Campaign	**Shantell Frame-Martin, Montana Noxious Weed Education Campaign (MNWEC) Project Coordinator- MSU** • The Montana Statewide Noxious Weed and Education Campaign was started in 1995. In 2012 the campaign was rebranded to what we know today, Montana Noxious Weed Education Campaign. • MNWEC strives to provide county weed coordinators, state, federal and tribal land managers with the materials they need to meet their-area specific educational goals. It also strives to build and work to strengthen a concise, cohesive statewide noxious weed education campaign message. • MNWEC has focused on targeting small acreage landowners, real estate professionals/ developers, and recreationalists coming into Montana. • MNWEC project highlights include: o published educational campaigns in the 2021 Hunting and Fishing regulations. o Partnered with MISC to create an "all campaigns" poster. o Successful outdoor and television advertisements. o Created two Montana Noxious Weeds Education Program and Montana Invasive Species Education curriculums for students k-9. o Create MNWEP trainings for Real Estate Professionals. o Recently completed the 'Noxious vs. Native' and 'Friend vs. Foe' video series. o Drought & Hay Facebook advertising campaign. o Partnered with Montana Weed Control Association to create a survey for promotional items • Adopt a Trailhead Program- Montana o Similar to the Adopt a Highway program, volunteers/civic group adopt a trailhead and required to do trail maintenance and noxious weed pulls once a year. ▪ Signs and kiosks are installed at adopted trailhead.

- So far, 73 kiosks have been installed.
    - Kiosks and signs incorporate the 'Play.Clean.Go.' messaging and use national uniform 'Adopt a Trailhead' messaging.
- Looking to incorporate the 'Pull Your Share' program (PYS), will have further discussions on logistics.

- Future Projects:
    - Reprinting and updating 'A Guide to Montana's Freshwater Plants', funded through DNRC
    - Creating a guide for landowner/homeowners and the green industry to identify noxious weeds called "Plant This Not That".
    - Looking to incorporate the 'Pull Your Share' (PYS) program with 'Adopt a Trailhead'. PYS focuses on school age children, while Adopt a Trailhead focuses on older civic/volunteer groups.
- Noxious Weed Survey funded by the Noxious Weed Trust fund, in partnership with the MSU HELPS lab.
    - 2019 survey was modeled off the 1994 survey to sample Montanan's knowledge of Noxious Weeds education.
        - 40% of respondents believe their knowledge has increased in the last five years.
        - The comparable percent of respondents saying they "know little or nothing" about noxious weeds has dropped from 67% to 48% in the last five years
        - Survey identified that 18-29 age group is the demographic that is the most underserved, or less knowledgeable of Noxious Weeds.
        - These results show the MNWEC education and outreach have been successful and where the program needs to target education and outreach.
    - 5,000 surveys mailed, with a 18% response rate.
    - Results have been presented at the MSU Extension Conference, MWCA Conference, and WSSA/WSWS Conference
    - Report and manuscript accepted on 11/5/21 for inclusion in the Journal of Invasive Plant Science & Management.
    - If interested, please contact Shantell Frame-Martin for Noxious Weed Survey reports and results.

Discussion:

Did the noxious weed survey provide other areas of focus?

- The survey was broken down by the state's MWCA areas; each area has their own information and score.

Are there other opportunities the council or partners can support MNWEC?

- There are more possible partnering opportunities between MISC and MNWEC, further discussions will be needed.

Shantell posed the following questions to MISC, please contact Shantell with further ideas and recommendations:

- As representative from different noxious weed/land management agencies, do you have suggestions or ideas for new noxious weed educational materials, presentations, brochures, or other ideas for projects?
    - Adopt a Trailhead (AAT) and Pull Your Share (PYS) should be incorporated, should think about expanding into fishing access sites, state parks, and FFA leaders.
        - AAT has kiosks installed in two state parks.
    - Fort Belknap would like to look into integrating Pull Your Share into their community.
- What are some partnering opportunities between MISC and MNWEC?
- What do you think the MNWEC is doing well and what do you think needs improved upon?

Science Advisory Panel Updates	**Bryce Christiaens, Missoula County Weed/AIS Manager, MISC Chair** ***Mogulones crucifer* Science Advisory Panel, 2019 Update:** • The petition for *Mogulones borraginis* (weevil that attacks houndstongue) has been submitted and is going through the approval process. Process can take anywhere from three to five years. • The petition for *Mogulones crucifer* is slated to be submitted within a year. More research has been done in Montana and Idaho. **Eastern Heath (*Xerolenta obvia*) Snail Advisory Panel, 2020 Updates:** • Eastern Health Snail economic impact study was kicked off in October; Tino Sonora from the University of Montana is leading the research. There is currently not much information available for this species. • APHIS science and technology group spearheaded additional research for methods and practices of *Xerolenta obvia*. Found regulatory action will not occur unless significant impact is found. **Discussion:** Producers are interested in learning more information about this species. MISC has a one page fact sheet that can be accessed on the website; this document will need updates and branding. **Next Science Advisory Panel Topic Discussion:** Timeline: 1. Typically, a subcommittee is formed and identifies the guiding questions and scope by the end of January. The panel of experts will be selected shortly after. 2. The Science Advisory Workshop is typically held at the beginning of May. 3. Need to determine which members are interested in participating in the subcommittee group and potential scope, as soon as possible. **Discussion:** Questions currently focus on researching firewood as a pathway, which is different than the motion approved in June. The Science Advisory Panel should include specific questions and experts focused on Emerald Ash Borer: • What would the impact of Emerald Ash Borer be to Urban Canopy in Montana? • Jane will coordinate with Amy on further questions • Science Advisory Panels are a tool that are used as a broad review of what information is known, Science Advisory Panelists are not required to be experts Kick-off meeting to formalize questions will take place Jan. 13th. Emily will send out an email surveying interested council members.

Budget Update	**Stephanie Criswell, Conservation Districts Bureau Chief, DNRC, previous MISC Coordinator**
	
	Budget Update:
	
	Handout: MISC Budget- FY 22 Budget Worksheet
	• The current biennium and fiscal year end June 30, 2022. Total remaining amount is $47,812.47
	  ○ $220 has been expended on Council Member NAISMA registration, which will be refunded.
	  ○ No money has been expended on the 2022 summit, or Science Advisory Panels.
	  ○ $13,034.47 remaining to spend on education and outreach.
	
	Action Item: Approval of FY22 MISC Budget
	Motion: Bob Gilbert moved to approve the FY22 MISC Budget
	Second: Jane Mangold
	Discussion: None
	Public comment: None
	Action on motion: Motion passed unanimously.

	Handout: USDA Feral Swine Proposed Summary Budget
	• Western Invasive Species Council (WISC) agreed to formulate a Transboundary Feral Swine Working group with the goal to work with Canada to manage and prevent the spread of Feral Swine.
	  ○ Workgroup created a set of recommendations that can be found on the MISC website.
	  ○ Transboundary Feral Swine Work group met at the 2021 NAISMA to discuss implementation of the recommendations, USDA approached workgroup with funding opportunities.
	• Budget has been approved and is in the process of contracting.
	• Total request from the USDA is $167,559.82. Deliverables include:
	  ○ Salaries and Benefits for MISC and WISC Coordinators.
	  ○ Funding to host two transboundary summits and other relevant meetings, including travel.
	  ▪ First summit will be incorporated in annual PNWER meeting in Calgary, Canada.
	  ▪ Second summit will be in 2023 in the United States.
	  ▪ Meeting between U.S. and Canadian Border Patrol.
	  ▪ Workshop to include decision makers, such as legislators and the ministries of agriculture and environment.
	  ○ WISC to roll out 'Squeal on Pigs!' campaign. MISC to create "Squeal on Pigs!" Website
	  ▪ Needs to obtain licensing and will create branding and usage guidelines. 'Squeal on Pigs!' mobile app will be created.
	  ○ MISC to host Science Advisory Panel.
	• $49,644.88 will be available to DNRC and MISC.
	
	Discussion:
	
	Marnie Zimmer has taken Stephanie's position as co-chair on the Transboundary Feral Swine Workgroup; she works for the Canadian Wildlife Health Cooperative.
	
	The Department of Livestock has assumed an advisory role in the USDA funding. Tahnee Szymanski is a part of the Transboundary Feral Swine Work Group.
	
	Currently, MISC's funding comes exclusively from the Aquatic Invasive Species funding. With the delisting of Tiber Reservoir and increased council involvement in terrestrial work, it would be beneficial to broaden the MISC funding source. The executive committee will set a time up with DNRC's Director Kaster and Mark Bostrom to discuss funding.
	
	MISC should also consider if standard biennium funding amount is sufficient for the future.

County Weed District Survey	**Bryce Christiaens, Missoula County Weed/AIS Manager, MISC Chair**
	
	In the summer 2021, a weed coordinator inquired about a task identified in the control portion of the Montana Invasive Species Framework, specifically building the capacity to effectively manage invasive species in Montana.
	• Tasks reads: "Hire full-time county weed coordinators or combined county coordinators and provide these staff with training on grant writing and property weed assessments."
	• A subcommittee is working on a survey that will be sent out to state weed coordinators to identify their needs and capacity to complete this task.
	  ○ Framework was written in 2016 with outdated priorities, goals, objectives, and tasks.
	  ○ Would the council like to update and reevaluate this document, and is the task listed above a priority of the Council?
	Discussion:
	The framework should be reviewed, and the Councils' priorities could be reflective of MISC's funding sources.
	
	Reaching out to the weed coordinators would be beneficial. It would also be beneficial to look into funding tribal weed coordinator positions through MSU Extension.

	Consensus was to go forward with County Weed District Survey, no action item taken.
Framework Discussion	**Bryce Christiaens, Missoula County Weed/AIS Manager, MISC Chair** Montana Invasive Species Framework was last published in 2016, the document needs to be updated; A work plan also needs to be created for the council. The rework of the Framework and creation of a work plan will be the theme of the 2022 MISC Summit. Tasks within the Framework were originally created to identify needs in the state, not necessarily items MISC members needed to complete. Subcommittees will be created to review and revise each Framework sections; the results from the subcommittee will be shared with relevant stakeholders for further input. This activity may identify new potential funding sources and will be valuable for the next legislative session. Stephanie Criswell will send a document that tracks the progress that has been accomplished from the Framework.
AIS Grant Program Update	**Emily Moran, MISC Council Assistant, DNRC** **AIS Grant Program Updates** • DNRC/MISC first grant cycle closed November 3rd • The total funds available for fiscal year 2022 are $250,720. • Grant Review Committee will meet December 15th to review and score applications. • Grant hearing is scheduled for January 12th and will be a hybrid meeting. • Total Request amount from cycle one is $191,858. • A second DNRC/MISC grant cycle will open after funds have been awarded. **Discussion:** None.
Grant Recipient Presentations	**Wendy Jones, Lower Musselshell Conservation District Administrator, Regional Coordinator of CEMIST** Handout: Scope of Work: Central and Eastern Montana Invasive Species Team AIS Education & Outreach and Monitoring MISC viewed the CEMIST Story Map • CEMIST stands for Central Easter Montana Invasive Species Team and was founded in 2017 in response to the discovery of zebra/quagga mussels in Tiber and Canyon Ferry. • CEMIST's goal is to share unified invasive species messaging through education and outreach. ○ CEMIST partners with state agencies, Conservation Districts, Big Sky Watershed members, tradeshows, and private industries. • CEMIST received a DNRC/MISC grant and has been using the awarded grant money to attend conferences and tradeshows to provide invasive species education and outreach, coordinate monitoring efforts, social media campaigns, and collaborating with North and South Dakota to build up their invasive species management response plans. **Discussion:** CEMIST is broken into 6 regions, covering the central and eastern Montana areas, UC3 covers the western region of Montana. **Torrey Ritter, Region 2 Nongame Wildlife Biologist, Montana Fish, Wildlife & Parks** Handout: Scope of Work: Mapping and Control of Non-native Frogs and Turtles • Montana FWP, in coordination with Montana Conservation Corps (MCC), and Confederated Salish and Kootenai Tribes (CSKT) was awarded a MISC/DNRC grant in 2021 to monitor

	and control Snapping Turtles, American bullfrogs, and Pond Sliders in the FWP regions 1 and 2. The main question of the project was "What are the current extent and severity of the infestations, and can they be controlled or at least contained?" ○ Snapping Turtles are native east of the Continental Divide but are invasive and non-native west of the Continental Divide. ○ American bullfrogs are a likely transmission vector for diseases, out compete native species, and once established, complete removal of the species is unlikely. ○ Pond sliders out-compete the native painted turtles and are typically reported as released pets. During the project, there were not enough Pond Sliders reports to be considered significant. • Project goals: ○ Use surveys to collect data on the extent of the current invasive herptile infestations in western Montana (FWP Regions 1 and 2). ○ Use control efforts to eliminate invasive species herptiles from key wetlands and dispersal pinch-points to reduce their spread and provide native herptile refugia. ○ Use outreach to educate the public about these invasive herptile species and how citizens can help control their spread and impact. • Project hired five MCC members to complete the goals above • Results: ○ Snapping Turtles: ▪ 78 traps set ▪ 40 different wetlands ▪ 593 trap-nights ▪ 11 turtles caught (10 in Flathead County, R1 and one in Missoula Valley, R2) ▪ 1 nest with 75 eggs in Stevensville ○ Bullfrogs: ▪ 110 nocturnal calling surveys ▪ 28/106 locations bullfrogs detected ▪ 118 bullfrogs removed in 11 nights of gigging • Conclusions: ○ R1 Snapping Turtles= high numbers and lots of active breeding. Need future concentration efforts. ○ R2 Snapping Turtles= low numbers and limited evidence of successful breeding. ○ No infestation. Continued community outreach and targeted trapping moving forward. ○ R1 Bullfrogs= Widespread in Flathead River. Recent expansion into the Mission Valley requires immediate attentions. Overlap with the Northern Leopard Frog introduction. ○ R2 Bullfrogs= Range similar to previous surveys. No bullfrogs upstream of Hellgate Canyon with buffer of frog-free wetlands around the mouth. Critical monitoring in the future. **Discussion:** Are there reports of Snapping Turtles east of the Continental Divide? • Yes, they are a species of concern and are native east of the Continental Divide, which is a good thing. Please report any Snapping Turtle or Northern Leopard frog sightings to Bryce Maxell with the Natural Heritage Program. Can the program use eDNA to search for invasives? • It is a great idea, and the program anticipates using the technology in the future.
Submittable Training	**Emily Moran, MISC Council Assistant, DNRC** **Mark Bostrom, DNRC Conservation and Resource Development Division Administrator** DNRC has switched to submittable for the grant management system. Current DNRC grant cycles can be found here: https://grants.dnrc.mt.gov/submit Please contact Emily Moran or Liz Lodman for help, or use the following resources for Submittable help:

	Getting Started with Submittable Submittable Help Center

Wrap-up Adjourn	**Final Discussion:**
	• MISC should think about how the council and state defines native vs. non-native species, as well as habitat alterations and range expansions. This is especially relevant with the FWP/MCC Snapping Turtle project featuring the Continental Divide boundary. Examples also include Sandhill Cranes and inclusion of game fish.
	  ○ This discussion can be incorporated while updating of the Framework.
	  ○ Jane will reach out to author as a potential presenter at the summit.
	• Bryce and Emily will send out emails to coordinate future meeting dates and locations
	  ○ Potentially in Flathead?
	
	Public Comment:
	None
	
	Motion: Steve Wanderaas moved to adjourn the meeting.
	Second: Tom Woolf
	Discussion: None
	Public Comment None
	Action on motion: Motion passed unanimously
	Meeting adjourned: 3:12 pm

Emerald Ash Borer Compilation of Resources

General:

EAB information network This is a website with a lot of good information administered through Michigan State University.

2013 Review Article Herms, D.A and McCullough, D.G. 2013. Emerald Ash Borer Invasion of North America: History, Biology, Ecology, Impacts, and Management. Annual Review of Entomology 59:13-30.

APHIS EAB program manual Animal and Plant Health Inspection Service. 2020. Emerald Ash Borer Program Manual: 2nd Ed. USDA- APHIS Publication.

Where EAB is and when it was detected there- part of EAB Information Network site

Identification and Monitoring:

Signs and Symptoms Wilson, M.; Rebek, E. 2005. Signs and Symptoms of the Emerald Ash Borer. Michigan State University Extension Bulletin E-2938.

More in-depth identification guide Parsons, G.L. 2008. Emerald Ash Borer *Agrilus planipennis* Fairmaire (Coleoptera:Buprestidae) A guide to identification and comparison to similar species. Department of Entomology, Michigan State University, November, 2008.

Life Cycle US Forest Service/Michigan State University. 2009. Unwanted!: Emerald Ash Borer. USDA Bulletin E-3004.

USDA Survey Guidelines "USDA APHIS PPQ Emerald Ash Borer Survey Guidelines"

How to Conduct a Tree Inventory "Tree Inventories." *Wisconsin Department of Natural Resources*,

Phenology Network EAB Forecast Tool "Emerald Ash Borer Forecast." *USA National Phenology Network.* This site uses national weather trends to predict occurrence of life stages of EAB around the country as if the insect was present.

Prism Traps "Emerald Ash Borer Trapping Program." *Bioforest.*

Multifunnel vs prism traps Crook et al, 2014. Improving detection tools for emerald ash borer: comparison of multifunnel traps, prism traps, and lure types at varying population densities. Journal of Economic Entomology 107:1496-1501

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Attachment A - Scope of Work
Dreissenid eDNA qPCR Methods and Reporting Standardization

In addition to the scope of work described below, supporting documents and attachments submitted with the grant application are incorporated herein by this reference.

Project Scope of Work
The goal of this project is to increase manager-researcher communication, understanding, and confidence in the use of eDNA technology for early detection of Dreissenids. Sponsor will provide standardized field and lab methods to improve regional efforts for early detection of Dreissenids using quantitative PCR (qPCR).

This study is intended to bridge the gap between research & management and serve as template for future efforts to standardize eDNA sample analysis for early detection, monitoring, and reporting of AIS detections to help protect Montana's waterways and regional economies.

Project Objectives
1: quantify between-lab repeatability of eDNA results using 3 USGS-standardized laboratory qPCR assays on two field sampling methods: small-volume filtered grab samples and large-volume net samples. This project will standardize, validate, and quantify repeatability of the large-volume tow net method in 2 different labs (USGS & FLBS).

2: test the hypothesis that mussel eDNA is concentrated near the thermocline.

3: test the hypothesis that veliger number is correlated with eDNA copy number and that eDNA tests will be more sensitive and repeatable than veliger microscopy, using tow nets.

4. outline reporting standards & methods with researchers and managers. We will analyze reporting decision-trees to help standardize communication methods, language, and formatting for reporting eDNA test results.

Project Tasks

Task 1: Zebra mussel eDNA sampling (3 negative lakes)
- Sites likely are Flathead Lake, Canyon Ferry and Tiber Reservoir
- 5 tow and 5 filtered samples will be collected for a total of 10 samples per site

Task 2: Zebra mussel eDNA sampling (3 positive lakes)
- Sites likely are Lake Bemidji, North Star Lake, Apache Lake (low density mussel populations)
- 10 shallow tows, 10 deep tows and 10 filtered samples will be collected for a total of 30 per site

Task 3: Lab processing and standardization (negative samples)
- Samples from Tibor Reservoir will be extracted by 2 labs, with 2 qPCR assays
- Total of 30 samples to be analyzed

Task 4: Lab processing and standardization (positive samples)
- Samples from Lake Bemidji, Lake X and Apache Lake will be extracted by 2 labs and analyzed using 2 qPCR assays.
- Total of 90 samples to be analyzed

Task 5: Travel
- Travel costs are associated with sampling trips to Lake Bemidji and North Star Lake X (MN)

This study will evaluate the 3 highest-quality Dreissenid mussel qPCR assays (a genus-specific, zebra-specific, and quagga specific assay) with proven high-repeatability between 4 USGS labs when used on grab-filter samples (Sepulveda et al. 2020). To strengthen, extend, and further validate the Sepulveda et al. qPCR methods, and to address MISC Science Advisory Panel outcomes, Sponsor will employ collaborative eDNA research between the USGS and Flathead Lake Biological Station (FLBS). Sponsor will compare the sensitivity and

between-lab repeatability of the USGS standard qPCR protocol of the same 3 assays for grab-filter samples (Sepulveda et al. 2020) on large-volume tow net samples. FLBS has optimized the 3 qPCR assays for tow net samples by using a special protocol (buffer/master-mix) that is resistant to PCR-inhibition common in large-volume samples with large quantities of material (plant material, DNA) (Gingera et al. 2017; Sepulveda et al. 2019; Schabacker et al. 200; Miller et al. in prep.). A key difference between the FLBS tow-net vs. USGS grab-filter qPCR protocol is FLBS's is optimized for higher sensitivity and specificity using plankton tow net samples, which contain massive amounts of material that often inhibits nonoptimized assays (Sepulveda et al. 2019; Schabacker et al. 2020; Miller et al. in prep).

This project will make the Sepulveda et al. (2020) grab-sample qPCR protocol standardized and replicable in more labs (non- USGS labs) and extend qPCR protocol standardized for use on large-volume tow net samples for improved Dreissenid eDNA detection. To achieve the desired objectives of standardization between labs (USGS, FLBS), Sponsor will qPCR analyze the same paired (filter vs. tow net) field samples and perform independent between-assay and between-collection technique comparisons. Results will then be compared between labs to measure the consistency of between lab replication of results. A main goal of this study is to increase the understanding and confidence of managers that results from eDNA sample collection and qPCR analysis for the early detection of Dreissenids can be reliably replicated between labs.

Additionally, the reporting standards and methods between labs will be compared to help standardize the communication methods, language, and format for eDNA qPCR dreissenid results. This part of the study will specifically address how labs report results to managers, what vocabulary is used (e.g., positive amplification vs. positive sample etc.), definitions, and LOD's (limits of detection), and eDNA copy numbers reported for positive and negative controls.

This work and deliverables will translate into useful information for managers by informing them in detail about the strengths and challenges of eDNA sampling approaches, qPCR laboratory methods, detection probabilities for tow nets, and data interpretation. The data and results will be interpreted and discussed with managers and stakeholders to standardize field sampling and facilitate eDNA data use. Sponsor will also produce a training/educational video, with input from managers and field and lab personnel, on how to collect a high-volume tow net sample, and how to interpret qPCR results, emphasizing vocabulary and definitions along with causes and prevention of false positives and negatives.

Deliverables and Outcomes

Sponsor will provide a document and peer-review publication detailing the best practices for communication of eDNA results along with R scripts for converting results (e.g., DNA copy number) directly into standardized figures for reporting to managers. Sponsor will also provide a document on standardizing sample volume, collection methods, and sample preservation, along with an educational training video.

Document 1: Plankton tow (vs filter sampling) standardized protocol & presentation to WPR (Tasks 1, 2, 3, 4). Fall 2020

Document 2: Lab standardization and lab test results table (Tasks 3 & 4). Spring 2021

Document 3: Draft of MT-FWP decision tree, communications and vocabulary guidelines presented to Western Regional Panel (All Tasks). April/May 2021

Document 4: Draft publication with sampling variance results (between labs) and qPCR test variation (within and between labs) including decision tree, communications and vocabulary guidelines, and reporting standards recommendations (Tasks 3 & 4). June 2021

Document 5: Plankton tow instructional video (not part of the scope of work of this contract). July 2020

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The specific measurable objectives of this study are:

1. Achieve a 90% detection rate of zebra mussel (and quagga mussel) eDNA in lakes with low-density zebra mussels, and 90% repeatability between two labs (USGS and FLBS); a 3rd laboratory (Yale Passamanek, BOR) will also independently replicate qPCRs
2. Quantify how tow nets compare to filter in terms of sensitivity, repeatability between labs, and uncertainty within labs (variance among technical qPCR replicates) using the USGS-filter (Sepulveda et al. 2020) and FLBS tow-net optimized qPCR assays (Miller et al. in prep.)
3. Test if zebra and quagga mussel eDNA (cells, veligers, pseudofeces) is concentrated near the thermal cline in lakes with low-density (recently invaded) zebra mussels. We will also test for veligers & quantify repeatability by microscopy repeated in two independent labs.
4. Quantify how inter-lab variation is affected by the qPCR mastermix, annealing temperature, and qPCR assay (zebra, quagga, genus-specific) used in each lab?
5. Describe the best way to communicate positive qPCR tests, “positive samples”, false positive risks, limit of detection (LOD), and implementation of possible management strategies based on end-user tolerance for mistakes such as false positive and false negative (for both veliger microscopy and eDNA qPCR)(Sepulveda et al. 2020b).

Project Schedule

August – October 2020	Planning, coordination, and supply procurement
September 2020	qPCR assay distribution and re-optimization: 3 standardized USGS assays, the same 3 assays were standardized (and optimized) for tow nets (buffer & extraction protocol)
September	Quarterly report
September – December 2020	Sample Procurement: August summer 2020 (**for positives:** 2 Minnesota lakes, 1 Arizona lake; **for negatives**: 2 MT lakes). Extraction, qPCR and analysis, veliger microscopy. We sample in Minnesota because low-density zebra mussel populations exist. We sample in Arizona because a low-density quagga population exists
December 2020	Quarterly report
December 2020 – Feb. 2021	Comparative analysis between labs and between tow vs filter samples
January – February 2021	Results reporting, outline vocabulary, definitions & communication strategies
March – June 2021	Development of deliverables, reports submitted, write publication
March 2021	Quarterly report
August 2021	Final report and project close

Project Coordination and Management

This project will be coordinated and managed by the MCGL (Montana Conservation Genomics Laboratory) located at UMT, Missoula Campus, and Flathead Lake Biological Station located in Polson, MT. It will be administered by Gordon Luikart and Stephen Amish at the UM lab and Adam Sepulveda at the USGS.

All supplies with be procured from University of Montana preferred vendors. Throughout the project, there will be regular communication and coordination with MT Fish, Wildlife & Parks (e.g., Tom Wolf), the Western Regional Panel on AIS, and the Bureau of Reclamation (Yale Passamaneck, Colorado).

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Monitoring Reporting
All survey data will be submitted through MT FWP's Mobile data collection app Survey123. Work with MT FWP to obtain access to that application prior to beginning survey efforts.

ANY positive survey results WILL BE REPORTED WITHIN THREE DAYS to Craig McLane, MTFWP (CMcLane@mt.gov, 406-444-1224) and Stephanie Criswell, DNRC (Scriswell@mt.gov, 406-444-0547). Reporting survey results to the press will be a joint effort between FWP and the contractor, which will typically be accomplished via a joint press release.

Grantee agrees to follow FWP's monitoring protocols located at:
http://cleandraindry.mt.gov/Resources

For plankton sampling, grantees are required to obtain a scientific collector's permit from FWP at:
http://fwp.mt.gov/fishing/license/applications.html

Contact FWP's fisheries office for information at 406-444-2449.

Branding Coordination Clause
To ensure effectiveness, consistency, and accuracy in messaging, Sponsor agrees to coordinate with Fish, Wildlife & Parks on the narratives and graphic identity of education and outreach materials produced through this grant. Branding resources are located at: http://cleandraindry.mt.gov/Resources.

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Attachment A - Scope of Work
Project Title

In addition to the scope of work described below, supporting documents and attachments submitted with the grant application are incorporated herein by this reference.

Project Scope of Work

The Flathead Lake Biological Station (FLBS) will conduct dreissenid mussel early detection through plankton tow sampling on Flathead Lake for microscopy and eDNA analysis during the 2021 field season. Each round will consist of 31 sites resulting in 774 total samples, using both open water and shoreline plankton tow techniques following FLBS Protocol, based on the Western Regional Panel on Nuisance Aquatic Species' dreissenid mussels sampling and monitoring protocol. Additionally, Flathead Lake sampling includes visual inspection of sampled shoreline sites for taxa listed on MT Fish, Wildlife & Park's (FWP) list of priority invasive or introduced species and send photos of any suspicious-looking specimen to FWP AIS personnel for identification/verification. We will also archive a subset of the eDNA samples for potential use in future research.

FLBS will also conduct two rounds of dreissenid plankton tow sampling for microscopy and eDNA analysis during the 2021 summer and fall seasons on Lake Elwell (herein known as Tiber Reservoir). Sampling locations will be situated around the Tiber Dam wall in discrete locations gleaned from prior investigations using the underwater rover (ROV) during the September 2020 sampling event. A total of 200 samples will be collected, including field blanks, from both open water and shoreline sites using various plankton tow techniques (including boat, shoreline, and ROV) following the same protocols described above.

Project report will include in-depth information about the use of the ROV sampling method in addition to results and how its contributing to eDNA research regarding efficacy and consistency as a tool for early detection. Information will include the advantages and disadvantages of the method, why it is used with other methods, and if/how it addresses gaps.

The FLBS field crew will provide outreach. Using FWP supplied merchandise and the Clean Drain Dry concept, outreach will occur opportunistically as field crews converse with the public.

Project Tasks, Deliverables and Objectives:

Row	Task Name & Description	Task Deliverables
Task 1	Flathead Lake sampling. To begin after sub-surface water temperatures warm to >55° F through surrounding peak (60°F to 65°F) and residual spawning temperatures. A 64-micron plankton mesh net will be used to collect plankton samples at 19 shoreline and 12 boat sites per round of sampling. At each sampling site, one field blank and two 50-ml duplicate samples (A and B samples) will be collected.	Dreissenid presence/absence data and surveillance of priority AIS at Flathead Lake. A second deliverable will be the support of local watershed groups. Another deliverable will be to build capacity for future microscopy or eDNA research. Education and outreach will be delivered to inquisitive members of the public as we engage with them. Replicate samples that do not get immediately analyzed will be stored in an FLBS freezer for use by future researchers or agencies.
Task 2	Tiber Reservoir sampling will begin after sub-surface water temperatures warm to >55° F and will continue surrounding peak (60°F to 65°F) and residual spawning temperatures. A typical 64-micron plankton mesh net (50-cm opening tapered to 6 cm, 1.5 meters long) will be used to collect plankton samples at 15 sites at Tiber Reservoir per round of sampling. At each site a replicate sample (A and B samples) will be taken, and field blanks as needed.	The main deliverable expected is expanded surveillance for dreissenid mussels at Tiber Reservoir. Other deliverables include the ROV video, ADP data, and water quality data. Another deliverable will be an archived set of samples to potentially be used for future microscopy or eDNA research relevant to MISC AIS priorities (i.e. multi-species eDNA detection). Education and outreach will be accomplished using the consistent message to Protect Our Waters and Clean, Drain, and Dry.

Project Schedule

	Apr-May 2021	June 2021	July-Aug 2021	Sept-Nov 2021	# Days
Task 1 - Flathead Lake Sampling		X	X	X	30
Task 2 - Tiber Reservoir Sampling			X	X	11
Project Administration	X	X	X	X	14
Project Reporting	• Quarterly status reports are due by the last day of the month in June, September, December, and March. • A final report, data, and deliverables are due 90 days after the term of the contract.				

Project Coordination and Management
This project will be administered by FLBS as an eligible government entity associated with the Montana University System. FLBS and UM have extensive experience in the administration of extramural grants and contracts and have a strong history of compliance with the requirements of a wide range of funders. For administrative purposes, FLBS follows UM's procurement policies that are set by the Board of Regents and are in compliance with Montana State requirements. James Elser (PI) will work closely with FLBS research scientist Phil Matson (Co-PI) in implementing the project. Elser serves as FLBS Director and has over 30 years of experience in project management, fieldwork, event coordination, plankton monitoring, and educational outreach.

Day to day work on the project will come from Phil Matson who coordinates the AIS sampling and monitoring efforts for the FLBS. Phil has over 19 years of monitoring and project management experience.

FLBS will coordinate with the Confederated Salish and Kootenai Tribe, the Bureau of Reclamation, and FWP.

Watercraft Inspection Reporting Clause Reporting
All survey data will be submitted through MT FWP's Mobile data collection app Survey123. Work with MT FWP to obtain access to that application prior to beginning survey efforts.

ANY positive survey results WILL BE REPORTED WITHIN THREE DAYS to Craig McLane, MTFWP (CMcLane@mt.gov, 406-444-1224) and Stephanie Criswell, DNRC (Scriswell@mt.gov, 406-444-0547). Reporting survey results to the press will be a joint effort between FWP and the contractor, which will typically be accomplished via a joint press release.

Grantee agrees to follow FWP's monitoring protocols located at:
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For plankton sampling, grantees are required to obtain a scientific collector's permit from FWP at:
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Contact FWP's fisheries office for information at 406-444-2449.

Branding Coordination Clause
To ensure effectiveness, consistency, and accuracy in messaging, Sponsor agrees to coordinate with Fish, Wildlife & Parks on the narratives and graphic identity of education and outreach materials produced through this grant. Branding resources are located at: http://cleandraindry.mt.gov/Resources.

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Attachment A - Scope of Work
eDNA Research on Decontamination Preservation and Storage

In addition to the scope of work described below, supporting documents and attachments submitted with the grant application are incorporated herein by this reference.

Project background
eDNA as an early detection and monitoring tool has been widely tested and evaluated by scientists and agency managers and is becoming more popular and advantageous with each day (Goldberg et al., 2015). Advantages include the ability to detect rare or endangered species without the need to handle or visually document them, as well as the ability to analyze archived samples as technology improves or funding allows. Some caveats to the technology, however, involve the need to decontaminate equipment to avoid cross-contamination, the need to preserve the sample before the DNA degrades (ex., EtOH, Longmire's buffer, sodium acetate), and the uncertainty of sample viability following extended storage times. Each of these caveats come with their own crucial considerations which could render collected samples useless and funding wasted.

Following a literature review of peer-reviewed journal articles and accepted agency plankton tow protocols, Flathead Lake Biological Stations (FLBS) researchers concluded this project is essential for increasing confidence for eDNA results reported for plankton tow net samples because:

1. Most protocols are developed for veliger decontamination, preservation and storage.
2. Bleach concentration recommendations vary between studies
3. Preservation concentrations are developed mainly for veliger preservation not for eDNA
4. Other preservatives like Longmire's buffer or grain alcohol could be used to eliminate some concerns of preservation and availability
5. Not much work has been done on the effect of time and storage temperature for high-volume eDNA samples (most studies focus on filters)

Since these crucial concerns have not specifically been addressed for standardized eDNA tow protocols, and because these crucial steps have the potential to lead to false analyses and wasted resources, the purpose of this project is to conduct research to identify the best decontamination and preservation methods. The overarching goal of this project is to eliminate these remaining uncertainties around decontamination, sample preservation and sample storage which could impact sensitivity for detecting AIS using our high-volume plankton tow sampling approach. Results will directly translate into usable information to improve the early detection of Dreissenidae and other AIS threatening MT and will be presented and disseminated to the invasive mussel early detection community.

Project objectives
1) Test the effect of bleach concentration, contact time and rinsing method/soak time on the removal of residual bleach.
2) Test the effect of bleach concentration, contact time and rinsing method/soak time on the removal of contamination from previous sampling.
3) Test the effect of preservative (EtOH) concentration on copy number and viability of eDNA samples.
4) Test the effect of preservative choice on eDNA copy number.
5) Test the effect of time and storage temperature on the copy number for archived eDNA samples.
6) Test the effect of storage time at 8C before extraction on the copy number for eDNA samples.

Project scope of work
FLBS staff (Phil/Leif) will conduct a 3-part study to assess the effect of bleach concentration, preservative concentration and choice and the effect of storage time and temperature on the viability of eDNA plankton tow samples collected for AIS monitoring. This project will begin in May of 2021. Fieldwork will be conducted at FLBS and lab analysis will take place at the University of Montana's Genetics Lab. Task 1 in the following table describes the methods for the

bleach concentration facet of this study. The completion of this first part of the study will provide 30 samples for analysis by titration and 30 samples for qPCR analysis. Task 2 listed in the following table describes the methods for the preservation concentration and choice facet of this study. The completion of this portion of the study will yield 30 samples for qPCR analysis. Task 3 in the following table describes the methods for the storage temperature and time facet of this study. The completion of this portion of the project will yield 40 samples for qPCR analysis.

Leif Howard and Phil Matson will complete extraction and qPCR analysis for all collected samples (as described in task 4 below). A total of 110 samples will be extracted and analyzed in triplicate using the UMCG optimized qPCR protocol for quantification of copy numbers of chum mackerel (Scomber japonicus) from water samples. They will also test the bleach concentration of the designated samples using the titration method. A total of 30 samples will be analyzed, and results will be reported in ppm.

A final report will include a statistical comparison between the treatments for each study and a discussion of what our findings mean and how they can be applied. Findings will be made available to the wider AIS management community by creating a manuscript to be submitted to a peer-reviewed journal and presenting our available findings at the 2021 NAISMA conference (pending abstract acceptance).

Task 1: Bleach Decontamination

Test the effects of bleach concentration on the efficacy of removing DNA from plankton tow nets. To do this FLBS will create a slurry of 5 gallons of DI water and pureed Kroger brand canned chum mackerel (Scomber japonicus). The bleach concentrations to be tested are: 5%, 10% and 30%. For each bleach treatment, FLBS will submerge and agitate 5 assembled tow nets (all parts labeled) in the mackerel slurry. A field blank consisting of DI water will be collected prior to submerging each net. Each net will be rinsed off with water from a local source, disassembled and then placed into the respective bleach treatment. Soak time for nets will differ between bleach concentrations and will follow recommended soak times from existing peer reviewed studies. Then a sample using DI water will be collected. FLBS will also test the effect of bleach concentration and rinsing method (Rinse with hose, Soak, and Soak and Rinse) on the ability to remove bleach residue following decontamination. This will be done for 30% bleach concentration, the highest concentration from the previous portion of this study. The rinsing treatments are: Rinse with hose only; soak and agitate only; soak and agitate and rinse with hose. To complete this task, for each net a DI field blank will be collected. FLBS will then soak 5 nets for the recommended time in the bleach solution. The nets will then be removed from the bleach bin and be rinsed according to the rinsing treatment to be applied. Following the rinsing treatment, FLBS will collect a DI sample by pouring 1 L of DI through the net and decanting until a 50 mL sample is achieved.

> Deliverables and Objectives: The objectives of task 1 are to determine the best bleach concentration and rinse method to both eliminate cross-contamination and remove bleach residue.
>
> This will result in the collection of 30 samples for extraction and qPCR analysis. 50% of these samples will be extracted and analyzed and 50% will be stored at -18C for analysis if necessary. Additionally, task 1 will yield 15 samples with 15 corresponding field blanks (N = 30). To test for bleach residue in the collected samples a titration method for detecting chlorine in ppm will be used.

Task 2: Preservation Study

The first portion of this task will test the efficacy of 3 concentrations of EtOH on the ability to preserve eDNA for qPCR analysis. FLBS will first create a slurry of chum mackerel and DI water (weight of mackerel and water volume will be recorded.) 5 x 10 mL subsamples of the mackerel slurry will be collected and extracted immediately. The mean copy number from qPCR analysis of these initial 5 samples will serve as the baseline for measuring the efficacy of the preservation methods. From the same slurry and on the same day, FLBS will create 5 replicates by adding 10 mL of the slurry to 15 tubes. Each replicate will consist of 3 identical 10 mL samples. For each replicate, EtOH will be added to create concentrations of 70%, 80%, 90%, respectively. Differences in copy number will be assessed between the qPCR results for the initial samples collected from the mackerel slurry at the outset of this task. The copy number for replicates will also be compared between the different concentration treatments to test for differences. The second portion of this task will test the ability of other preservatives to be used for DNA preservation. FLBA will create another 15 replicate 10 mL samples from the same mackerel slurry from the first portion of this task. To 5 of the replicates 100% EtOH will be added to create

an 80% concentration. To another of the 5 replicates, high-Proof food grade alcohol will be added to create a concentration of 80% and to the remaining 5 replicates, 3.33 mL of Longmire's buffer will be added to the 10 mL of sample material in each tube.

Deliverables and objectives: The objectives of task 2 are to determine the effect of preservative choice and concentration on eDNA copy number.

The first part of task 2 will result in 15 samples to extract and analyze with qPCR as described below in Task 4. The second portion of Task 2 will result in the 5 initial baseline samples plus 15 samples to extract and analyze with qPCR as described below in Task 4.

Task 3: Archive Viability

To assess the effect of time on high-volume samples stored at 8C before transfer to -18C, FLBS will create 5 replicate samples per the method described in task 2. Each sample will be split into 3 aliquots and preserved with 100% EtOH. Storage treatments at 8C will be 0 days; 2 days; 1 week; 4 weeks. 1 of the aliquots from each of replicates will be subjected to 1 of the storage treatments. Following the time treatments, samples will be extracted and copy numbers will be assessed using qPCR as described below. The second facet of Task 3 will assess the effect of time on the copy number for samples that have been stored at -18C for 4 months and 1 year by re-running the qPCR analysis and comparing to results from the first run. Using the same methodology as described for the first portion of this task, FLBS will create 5 replicate samples and split each sample into 3 aliquots to which EtOH will be added to a concentration of 80%. 1 aliquot from each replicate will then be subjected to 1 of the 3 storage treatments: 0 months; 6 months; 1 year. Following the allotted time spent in storage, the samples will be extracted and analyzed using qPCR as described in Task 4.

Deliverables and objectives: The objectives of task 3 are to measure the effect of storage temperature and time on eDNA copy number for recently collected and archived eDNA samples.

The first part of task 3 will result in 20 samples for extraction and qPCR analysis. The second portion of task 3 will result in 15 samples plus 5 field blanks to be extracted and analyzed with qPCR.

Task 4: Lab Work

This task consists of the analysis of 30 samples from Task 1 by titration and of the extraction and analysis of all DNA-based samples. FLBS will extract 50% of each sample and reserve 50% for a second extraction if required. The 100 samples collected during the previous 3 tasks will be extracted using the MCGL high-volume Sera-Mag bead extraction protocol. Samples will be extracted in groups of 15 with 1 extraction negative control per group for Tasks 1 and 2. Samples from Task 4 will be extracted in groups of 20 with 1 extraction negative control per group. Each extraction group will solely consist of samples from a single Task subsection (i.e. only samples from Task 2b will be extracted together). Additionally, the 5 samples of mackerel from Task 2 will be extracted as a separate group the day the slurry is created. The total number of samples to be extracted, including the extraction negative controls, is 110. qPCR analysis will be done in triplicate for each sample. FLBS will follow the MCGL qPCR protocol developed for quantifying copy numbers for chum mackerel. This protocol has been validated by previous results provided to retail clients. Each qPCR plate will include 3 internal positive control replicates and 3 qPCR negative control replicates to measure the success of the reactions and control for contamination during the qPCR process respectively. qPCR analysis will provide estimates of copy number for the 110 samples in triplicate. FLBS will then use the mean from all amplifications above the Limit of Detection from the 3 replicates for each sample as our estimate of copy number for the specific sample.

Deliverables and objectives: The objective of task 4 is to provide results for the previous 3 tasks and for the creation of deliverables.

Task 4 will result in titration data for 30 samples and estimated copy numbers for 110 samples.

Task 5: Analysis, Writing, and Presentation of Results

Analysis, development of deliverables and translation of the results from our study into usable information to be presented to MISC and WRP states.

Deliverables and objectives: The objective of task 5 is to develop our final report and to present our findings to MISC and the wider AIS management community.

FLBS will provide a deliverable in the form of a comprehensive publishable document on our findings on the effect of decontamination, preservation and storage methods on the rate of false positives and false negatives for Dreissenidae and other AIS. Results will be reported during the 2021 NAISMA conference (providing acceptance of abstract) close to the completion of this project and present our training video (TBD).

Project Schedule

TASK	May - Sept 2021	Oct 2021 - Mar 2022	April - Sept 2022
Task 1: Decon Test	X		
Task 2: Preservative Concentration	X		
Task 3: Archive Viability	X	X	
Task 4: Lab work	X	X	X
Task 5: Deliverables			X
Project Admin	X	X	X

Project Management and Coordination

FLBS will provide the administrative support for Tasks 1-5, the research facilities for Tasks 1-3, and the conference center for Task 5. Task 4 will be conducted at the Montana Conservation Genomics Lab (UMCG).

James Elser (PI) will work closely with FLBS research scientist Phil Matson (Co-PI) in implementing the project. Elser serves as FLBS Director and has over 30 years of experience in project management, fieldwork, event coordination, plankton monitoring, and educational outreach. Phil Matson, FLBS Research Coordinator, will coordinate and manage the project. Matson is a contributing member of the WRP eDNA working group and has over 20 years of monitoring and project management experience.

Phil Matson will be assisted by Leif Howard, who has 4 years of experience working with AIS prevention and monitoring (2 years watercraft inspection/ 2 years field experience sampling and handling of invasive species). Leif Howard is also a trained member of the UMCG staff. Samples will be collected by our field staff who have been trained and have prior experience with all sampling gear.

Samples will be transferred to Montana Conservation Genomics Lab (UMCG) for extraction and qPCR analysis. UMCGL has provided qPCR results from eDNA samples for six state, tribal and federal agencies and retail customers for many years. They have highly trained staff and receive samples from entities inside and outside of the state of Montana. As one of those employees, Leif Howard (with the assistance of Phil Matson) will extract and provide qPCR results for all samples collected for this project.

FLBS will also coordinate and consult with MT Fish, Wildlife & Parks and the United States Bureau of Reclamation through the project.