M.L. 2014, Chp. 226, Sec. 2, Subd. 03j Project Abstract For the Period Ending June 30, 2017

PROJECT TITLE: Identifying Causes of Exceptionally High Mercury in Fish PROJECT MANAGER: Bruce Monson AFFILIATION: Minnesota Pollution Control Agency MAILING ADDRESS: 520 Lafayette Road North CITY/STATE/ZIP: Saint Paul, MN 55155 PHONE: (651) 757-2579 E-MAIL: bruce.monson@state.mn.us WEBSITE: www.pca.state.mn.us FUNDING SOURCE: Environment and Natural Resources Trust Fund LEGAL CITATION: M.L. 2014, Chp. 226, Sec. 2, Subd. 03j

APPROPRIATION AMOUNT: \$ 743,000 AMOUNT SPENT: \$ 671,258 AMOUNT REMAINING: \$ 71,742

Overall Project Outcomes and Results

This study investigated probable causes of higher mercury levels in fish in certain mercury-impaired Minnesota rivers. We hypothesized that these high fish-mercury concentrations are caused by increased efficiency of mercury transmission to fish. To evaluate this, we measured mercury flow through the watershed, conversion of mercury to methylmercury, and accumulation of methylmercury in the riverine food web. The first two processes determine mercury availability to the food web, while the structure of the food web also determines mercury in fish.

The project focused on the Roseau River, in the Red River Basin, with comparison measurements in six other rivers. Also in the Red River Basin, Thief River was chosen for its similarity and proximity and the Mustinka River because of its relatively low fish-mercury levels. The other rivers had high mercury levels in fish, similar to Roseau, but differed in watershed land cover and water chemistry. Data collection for this project is complete, but data analysis will continue.

Mustinka had the lowest methylmercury levels, and the lowest rates of conversion of inorganic mercury to methylmercury (methylation). Roseau and Thief Rivers had the highest methylmercury levels in water and biota, showing increasing methylmercury in the food web from upstream to downstream locations.

Although analysis of results will continue over the next several years, the preliminary results seem to indicate the dominant cause of high mercury in the food webs differ among the rivers and even within rivers. Organic carbon has a predominant role in transporting mercury, but inhibiting uptake by the food web. For instance, methylmercury levels in Vermilion River fish were similar to Roseau River, but levels in the water were much lower in Vermilion. Lake Vermilion appears to retain mercury before water flows to the Vermilion River; however, low organic carbon in the river allows more methylmercury available for uptake by the biota.

Project Results Use and Dissemination

Six presentations were completed by June 30, 2017, two poster presentations at the International Conference on Mercury as a Global Pollutant and four presentations at various scientific conferences. Three manuscripts are proposed for publication. When the data are compiled, analyzed, and reported, completed publications will be shared with LCCMR.

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Environment and Natural Resources Trust Fund (ENRTF) M.L. 2014 Work Plan

Date of Report:	September28, 2017
Date of Next Status Update Report:	none
Date of Work Plan Approval:	June 4, 2014
Project Completion Date:	June 30, 2017
Does this submission include an ame	endment request? <u>NO</u>

PROJECT TITLE: Identifying Causes of Exceptionally High Mercury in Fish

Project Manager:	Bruce Monson
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Location: Northwestern (Red River of the North watershed) and northeastern (Vermilion, St. Louis, and Kettle watersheds)

Total ENRTF Project Budget:	ENRTF Appropriation:	\$743,000	
	Amount Spent:	\$671,258	
	Balance:	\$71,142	

Legal Citation: M.L. 2014, Chp. 226, Sec. 2, Subd. 03j

Appropriation Language:

\$743,000 the second year is from the trust fund to the commissioner of the Pollution Control Agency to quantify the probable causes of high mercury levels in fish within the Roseau River and two tributaries of the Red River of the North, by comparing mercury movements within watersheds to understand the drivers of mercury biomagnification in the food web of rivers with similarly high mercury levels, and to guide further mercury reduction initiatives. This appropriation is available until June 30, 2017, by which time the project must be completed and final products delivered.

I. PROJECT TITLE: Identifying Causes of Exceptionally High Mercury in Fish

II. PROJECT STATEMENT:

Mercury is toxic to the nervous system and eating fish is the primary route of exposure to humans and wildlife. More than two-thirds of Minnesota lakes and streams evaluated by the Minnesota Pollution Control Agency (MPCA) are "impaired" because of mercury in fish. Roughly one in ten of these impaired waters have exceptionally high mercury in fish, such that they do not qualify for inclusion in Minnesota Statewide Mercury TMDL. Less than one percent of the mercury entering Minnesota's lakes and rivers comes from wastewater discharges. The primary source of mercury in Minnesota waters is the burning of coal and other compounds, which deposit on the watersheds from the atmosphere. Mercury in the environment can convert to a form that accumulates in the food chain, increasing in concentration as it moves from microscopic plants, to tiny animals, to fish, and and eventually to humans and wildlife (see graphic).

The first step in solving the problem of mercury in fish is reducing the amount of mercury entering lakes, streams and wetlands. Toward this end, the MPCA is implementing a plan to reduce the mercury released from Minnesota smokestacks by 93 percent and continue to reduce mercury from wastewater discharges. This large reduction in mercury releases in Minnesota, coupled with global reductions, will benefit all waters of the state and fully restore about 90% of Minnesota's water bodies from the effects of mercury pollution. For the remaining 10% of water bodies it is necessary to go beyond mercury source reductions, addressing the mobilization and bioavailability of mercury in the watersheds.

This proposal focuses on the ecosystem processes that control mercury into fish. These processes include mercury delivery from subwatersheds, production and destruction of methylmercury (the bioavailable form), food web feeding relationships, and the fish growth rates. Scientists understand some of the factors that cause enhanced mercury accumulation, but not well enough to know the relative importance of each factor and what actions could reduce the enhanced mercury accumulation. The goal of this project is to achieve sufficient knowledge about the causes of exceptionally high mercury levels to inform additional efforts to reduce mercury in fish.

In our original proposal, MPCA partnered with the Minnesota Department of Natural Resources (DNR) and the United States Geological Survey (USGS). Each agency committed to specific matching funds for the original proposal request. Given the LCCMR's recommendation to fund the project at one quarter of what MPCA requested, the scope has been scaled down accordingly and the commitments from the partnering agencies are no longer effective. Nevertheless, the MPCA expects the DNR and USGS will continue as partners in this research.

III. PROJECT STATUS UPDATES:

Project Status as of January 1, 2015:

In general, the first six months of the project were spent on refining logistics for the project and negotiating work plans for contracts. There were no expenditures as of December 21, 2014, other than time of MPCA staff (which is not reimbursed by ENRTF). The project manager discussed contract needs with an MPCA contract specialist, selected load monitoring sites in Red River of the North Basin, and discussed work plan and logistics with potential contractors for Activity 2 and Activity 3. A contract was amended to conduct sampling for Activity 1 beginning in May 2015.

Project Status as of July 1, 2015:

Water sampling for load monitoring began in late March for Roseau, Thief, and Mustinka rivers. A contract was signed and finalized with University of Minnesota Duluth on June 3 to conduct the methylation studies and biota collections (Activities 2 and 3). The field work will begin this summer in the same rivers where load monitoring is currently in place. No invoices have yet been received from the contractors.

Project Status as of January 1, 2016:

Considerable field work and sample collections were achieved in the second half of 2015. Water sampling in the Roseau, Thief, Mustinka, and Wild River rivers for Activity 1 continued throughout the summer and ended around November 1, 2015. Water sampling at these sites will continue after ice-out in 2016. Field work for Activities 2 and 3 began with reconnaissance visits to Roseau, Thief, and Mustinka rivers by the Activity 2 and Activity 3 teams to establish sample sites and meet with local resource managers. Each team completed several subsequent trips to the three rivers throughout the summer to collect water, sediment, periphyton, seston, and fish. Activity 2 team includes Nate Johnson, University of Minnesota Duluth, Jeff Jeremiason, Gustavus-Adolphus, Carl Mitchell, University of Toronto, and their students. Activity 3 team is led by Mark Sandheinrich, University of Wisconsin – La Crosse.

Amendment Request (01/01/2016):

An amendment is requested to shift funds between activities. The activities remain unchanged.

For Activity 1—Transport of Mercury—MPCA staff are available to collect water samples at no cost to the project and existing contracts for pollutant load monitoring can be amended to add mercury sample collection and cost the project only for the additional time to collect the mercury sample set. Therefore, we are leveraging existing staff availability and contracts to greatly reduce the expected cost of sample collection from \$135,000 to \$25,000. Much of that savings (\$73,000) will be used to increase the number of sites (an additional site on the Wild Rice River) and sample collections per site (from budgeted 32 collections per site in two years to 50). The remaining \$37,000 savings is transferred to Activity 2.

For Activity 2—Methylmercury Production & Destruction—the budget is increased from \$128,000 to \$289,500 because the original budget had underestimated analytical costs and the scope was expanded to include monitoring groundwater movement to the streams. Groundwater monitoring requires multiple well installations in each of the watersheds, which requires increased time and materials costs.

For Activity 3—Food Web Relationships—the subcontractor, University of Wisconsin – La Crosse, is able to perform the laboratory analysis of mercury and methylmercury, in addition to the sample collection; therefore, \$138,800 laboratory budget is transferred to the contract with the University of Minnesota for the subcontractor, University of Wisconsin. To supplement the budget for Activity 2, \$56,200 of the laboratory budget is transferred to Activity 2. The remaining \$15,000 for Activity 3 laboratory budget is intended for stable isotope analysis using an existing contract with the University of Minnesota.

For Activity 4—Database and Statistics—MPCA staff can provide much of the GIS and statistics activity, and most of the travel for the project manager can be covered by MPCA's general travel funds; therefore, \$65,000 is transferred from the contract budget for Activity 4 to Activity 2 and \$3,300 from the travel budget is transferred to Activity 2.

Amendment approved by LCCMR 2-22-16.

Project Status as of July 1, 2016:

Load monitoring (Activity 1) continued in the Roseau, Thief, Mustinka, and Wild Rice Rivers for the second year. It was an early spring, with sampling beginning March 7, 2016. Water and sediment sampling (Activity 2) continued in the Roseau, Thief, and Mustinka Rivers, and sampling began in the Vermilion and Kettle Rivers. Biota sampling (Activity 3) began in the Vermilion, Kettle, and St. Louis Rivers. The team met in St. Paul in the late winter to discuss data collected in 2015 and made plans for the 2016 sampling.

Project Status as of January 1, 2017:

Load monitoring (Activity 1) for the second and final year was completed in the Roseau, Thief, Mustinka, and Wild Rice rivers. Water and sediment sampling (Activity 2) was completed in the Roseau, Thief, Mustinka, Vermilion, and Kettle Rivers. Biota sampling (Activity 3) was completed in the Vermilion, Kettle, and St. Louis Rivers.

Overall Project Outcomes and Results:

This study investigated probable causes of higher mercury levels in fish in certain mercury-impaired Minnesota rivers. We hypothesized that these high fish-mercury concentrations are caused by increased efficiency of mercury transmission to fish. To evaluate this, we measured mercury flow through the watershed, conversion of mercury to methylmercury, and accumulation of methylmercury in the riverine food web. The first two processes determine mercury availability to the food web, while the structure of the food web also determines mercury in fish.

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Mustinka had the lowest methylmercury levels, and the lowest rates of conversion of inorganic mercury to methylmercury (methylation). Roseau and Thief Rivers had the highest methylmercury levels in water and biota, showing increasing methylmercury in the food web from upstream to downstream locations.

Although analysis of results will continue over the next several years, the preliminary results seem to indicate the dominant cause of high mercury in the food webs differ among the rivers and even within rivers. Organic carbon has a predominant role in transporting mercury, but inhibiting uptake by the food web. For instance, methylmercury levels in Vermilion River fish were similar to Roseau River, but levels in the water were much lower in Vermilion. Lake Vermilion appears to retain mercury before water flows to the Vermilion River; however, low organic carbon in the river allows more methylmercury available for uptake by the biota.

IV. PROJECT ACTIVITIES AND OUTCOMES:

ACTIVITY 1: Transport of Mercury and Methylmercury

Description:

Fundamental to understanding mercury availability is quantifying how much mercury and methylmercury is transported through the river. Concentrations of thirteen analytes (Table 1) will be measured 32 times over two years at the gaging station in the Roseau River and two other tributaries of the Red River of the North. Water samples will be collected as grab samples below the water surface in the main flow the stream (i.e., thalweg). Mercury and methylmercury samples will be collected using the "clean hands – dirty hands" technique (EPA Method 1669). In addition, field measurements will include pH, temperature, specific conductivity, and dissolved oxygen. Water flows are continuously measured at these sites, which are in the cooperative flow gaging network supported by the DNR and USGS (http://www.dnr.state.mn.us/waters/csg/index.html). Flow and concentration data will be used in the FLUX32 model to calculate annual mass loads at each of the sites.

Final selection of the two tributaries for comparison to the Roseau will be based on an examination of land cover and stream flow characteristics. The likely candidates are the Red Lake River, Buffalo River, and Marsh River.

All sample collection and laboratory analysis will be contractual. Sample collection will most likely be done by the USGS or DNR under contract with the MPCA. Laboratory analysis methods and costs are based on Minnesota Department of Health's Environmental Laboratory.

Analytes	Method	Reporting Limit	Units	Holding Time (days)
Total Organic Carbon	SM 5310C	1	mg/L	28
Dissolved Organic Carbon	SM 5310C	1	mg/L	28
Sulfate	EPA 300.1	1.00	mg/L	28
Phosphorus, Total	EPA 365.1	0.01	mg/L	28
Iron Dissolved, Low Level	EPA 200.7	14.0	ug/L	180
UV Absorbance @ 254 nm	SM 5910B	0.0050	Abs units	2
Solids, Suspended	SM 2540D	1.0	mg/L	7
Suspended Solids, Volatile	EPA 160.4	10	mg/L	7
Solids, Total Dissolved	SM 2540C	10	mg/L	7
Low Level Mercury, Total	EPA 1631	0.400	ng/L	90
Low Level Mercury, Dissolved	EPA 1631	0.400	ng/L	90
Methyl Mercury, Total	EPA 1630	0.050	ng/L	180
Methylmercury, Dissolved	EPA 1630	0.050	ng/L	180

Summary Budget Information for Activity 1:

ENRTF Budget:	\$ 173,000
Amount Spent:	\$ 124,436
Balance:	\$ 48,564

Activity Completion Date: March 30, 2017

Outcome	Completion Date	Budget
1. Water quality sampling	October 30, 2016	\$ 25,000
2. Laboratory Analysis	March 30, 2017	\$ 148,000
3. Flux (mass load) calculations	March 30, 2017	MPCA IN-KIND

Activity Status as of January 1, 2015:

The activity description refers to sampling of the Roseau River and two tributaries in the Red River of the North Basin (RRB). The two tributaries selected for the project are the Thief River and the Mustinka River. All three rivers have comparable watershed areas. The Thief River watershed borders part of the Roseau River basin and has a similar land use/cover distribution. The Mustinka River is farther south, in the headwaters of the RRB and is more dominated by agriculture. Mercury concentrations in fish from the Thief River were almost as high as in the Roseau and the river is impaired for mercury in fish tissue; whereas, mercury concentrations of fish from the Mustinka River were below the impairment threshold.

MPCA has a contract with the International Water Institute (IWI) to collect water samples and calculate pollutant loads for multiple sites within the Red River of the North Basin. The contract was amended to include sample collections for this project.

Activity Status as of July 1, 2015:

The contractor for water sampling, International Water Institute, began collecting samples from the Roseau, Thief, and Mustinka rivers in March and sampling has continued without any problems in the field. The laboratory, Minnesota Department of Health, has had staffing problems that have delayed the analysis of samples for mercury and methylmercury. Fortunately, the holding times for those, analytes is six months and some of the initial results were submitted to the MPCA in June. No invoices have yet been received by the contractor.

Activity Status as of January 1, 2016:

Water sampling continued through until the end of October 2015, by the IWI crews and MPCA staff collected water samples from the Wild Rice River. At least 19 visits were made to each site, which exceeded the goal of 16 visits per site. Sampling will begin again after ice-out in the spring of 2016 for the second and final year of load monitoring for this activity.

Activity Status as of July 1, 2016:

The second year of load monitoring began in early March 2016 at six stations in the Red River tributaries— Roseau, Thief, Mustinka, and Wild Rice. Load monitoring will continue through October 2016. Sample results from 2015 were compiled in a spreadsheet, along with ancillary analytes provided by the Minnesota Pollutant Load Monitoring Network program. A total of 18 to 25 samples were collected at each of the six river collection sites. Annual loads for mercury, methylmercury, total organic carbon, and sulfate were calculated for each of the stations using MPCA's FLUX32 application.

Activity Status as of January 1, 2017:

The second year of load monitoring was completed in 2016 at six stations in the Red River tributaries—Roseau, Thief, Mustinka, and Wild Rice. Load monitoring began in the Vermilion and Kettle rivers—two stations in each river. Sample results from 2016 were compiled in a spreadsheet, along with ancillary analytes provided by the Minnesota Pollutant Load Monitoring Network program. Annual loads for mercury, methylmercury, total organic carbon, and sulfate were calculated for each of the stations using MPCA's FLUX32 application.

Final Report Summary:

All load monitoring was completed in the rivers by June 30, 2017, although data analysis and preparation of manuscripts will continue through 2018. The eastern and western rivers show distinct difference in land cover. Mustinka river is distinctly dominated by agriculture (84%), while the Thief and Roseau rivers are approximately one-third agriculture land cover (36% and 32%, respectively). While Thief and Roseau have abundant wetland coverage, they have only small forested land cover, in contrast to the eastern rivers, which are clearly forested and wetland covered watersheds.

Percent Wetland and Forest



Figure 1 Percent land cover for the rivers; St. Louis River (SLR-Scanlon) is shown for comparison, although loads were not monitored as part of this project

Table 2 Load monitoring sites and number of sample collection events for each site

					N COLL	ECTED
RIVER	SITE_ID	DESCRIPTION	LAT_DD	LONG_DD	2015	2016
MUSTINKA	S000-681	MUSTINKA R AT BR CSAH-9 AT WHEATON	45.81386	-96.5089	20	17
MUSTINKA	S002-001	MUSTINKA R AT CSAH-9 BRIDGE, 1.3 MI NW OF NORCROSS	45.88750	-96.2133	19	16
ROSEAU	S000-115	ROSEAU RIVER CR-53 AT CARIBOU	48.98306	-96.4494	18	15
THIEF	S002-088	THIEF R ON CSAH-7 BRG IN AGASSIZ NWR, 6 MI E OF HOLT	48.30045	-96.0708	19	22
THIEF	S002-079	THIEF R, 1 MI E CSAH-32, HILLYER BRG, 4 MI N THIEF R FALLS	48.18720	-96.1734	19	24
WILD RICE	S002-102	WILD RICE R AT CR-25, 0.8 MI E OF HENDRUM	47.26685	-96.7970	25	24
KETTLE	S007-954	KETTLE R AT LONG LAKE RD, 1.25 MI SW OF WILLOW RIVER, MN	46.30631	-92.8616		9
KETTLE	S000-121	KETTLE R BRIDGE ON MN-48, 4.5 MI E OF HINCKLEY	46.01086	-92.8398		10
VERMILLION	S005-088	VERMILLION R AT FR-491, 3.5 MI W OF CRANE LK	48.26480	-92.5658		22
VERMILLION	S006-505	VERMILLION RIVER AT CSAH-24 / CRANE LK RD,15 MI NE OF ORR, MN.	48.12375	-92.5231		21



Figure 2 Examples of sample collection, showing stream discharge and sample periods during the 2015 sample period

Mercury (Hg) and methylmercury (MeHg) load and yield (load divided by watershed area) showed a strong positive relationship to organic carbon ($R^2 = 0.85$ and 0.84). There were no significant relationships between Hg, MeHg, sulfate, and total suspended solids. Yields of organic carbon and Hg were lower in the Red River Basin sites (Roseau, Thief, Wild Rice, and Mustinka) compared to the eastern sites (Vermilion, Kettle, and St. Louis).

Table 3 Results of annual loading as yield (load divided by drainage area) for total suspended solids (TSS), organic carbon (OC), sulfate (SO4), mercury (Hg), and methylmercury (MeHg)

			DRAINAGE	TSS	OC	SO4		MeHg
RIVER	SITE_ID	STATION NAME	AREA (km2)	(kg/km2)	(kg/km2)	(kg/km2)	Hg (g/km2)	(g/km2)
ROSEAU	S000-115	Roseau	3,678	1,593	3,035	1,766	0.41	0.056
THIEF	S002-088	Thief-Holt	1,647	2,891	2,229	6,715	0.33	0.058
THIEF	S002-079	Thief-Thief R Falls	2,551	3,076	1,899	6,622	0.35	0.055
WILD RICE	S002-102	Wild Rice	4,040	10,569	507	2,706	0.55	0.010
MUSTINKA	S000-681	Mustinka-Wheaton	2,045	5,954	460	28,750	0.38	0.011
MUSTINKA	S002-001	Mustinka-Norcross	490	9,252	603	37,948	0.41	0.007
KETTLE	S000-121	Kettle-Sandstone	2,248	7,605	10,144	608	3.52	0.127
KETTLE	S007-954	Kettle-Willow River	1,684	6,769	12,816	550	3.40	0.146
VERMILLION	S005-088	Vermilion-Crane	2,344	1,210	5,050	1,954	1.04	0.042
VERMILLION	S006-505	Vermilion-Buyck	1,544	1,378	4,242	2,465	0.89	0.043
TSS: total suspended solid; OC: organic carbon; SO4: sulfate; Hg: mercury; MeHg: methylmercury								

Hg annual yields were similar among the four Red River Basin rivers, except for Wild Rice having a higher Hg yield because of higher total suspended solids (TSS) load. MeHg yields were relatively low in the Mustinka and Wild Rice Rivers, while Roseau and Thief were higher (and similar to the eastern rivers) and associated with the higher organic carbon (OC) loads. More OC is expected in Roseau and Thief watersheds because they are slightly more than half forest and wetlands (54% and 52%), while Wild Rice and Mustinka are predominantly agricultural with forest and wetlands comprising 32% and 5%, respectively in the two watersheds.



Figure 3 Comparison of yields for the Red River Basin rivers: (a) Hg, (b) MeHg, (c) TSS, and (d) OC

ACTIVITY 2: Methylmercury Production and Destruction Description:

Mercury methylation and demethylation will be measured by the stable isotopes. In situ rates of methylation activity is measured by adding inorganic mercury isotope (e.g., ²⁰¹Hg²⁺) to each sample and monitoring formation of the labelled methylmercury (e.g., Me²⁰¹Hg⁺). Similarly, for demethylation, the same sample is spiked with another stable-isotope labelled methylmercury (e.g., Me¹⁹⁹Hg⁺) and it is monitored for a decrease in concentration. The ratio of methylation rate/demethylation rate gives the net methylation rate potential. The methylation-demethylation study will be done at nine sites, with at least three samples per site visit, and each site will be visited three times to capture seasonal differences of the rates. This study will begin in the second year of this project because sites will be selected based on the first year of water quality monitoring.

Photodemethylation is considered the most important removal process of methylmercury in lakes, but it has been studied much in rivers; therefore, photodemethylation rates will be measured at the same nine sites as the methylation-demethylation rate study. The method for measuring photodemethylation of mercury is to suspend clear ("light") and opaque ("dark") bottles, in situ, with sufficient replicates to measure methylmercury concentrations before and after incubation. Ultraviolet and photosynthetically active radiation will be measured continuously during the incubations. The sites for the photodemethylation study will be downstream of the nine

methylation-demethylation sites. The methylation-demethylation sites are expected to be wetlands hydrologically connected to the rivers and the photodemethylation sites will be downstream sites unobstructed by tree canopy.

The quantity and quality of dissolved organic matter (DOM) strongly influences mercury methylation and demethylation. Therefore, the DOM from the selected river sites will be quantified as total organic carbon (TOC) and dissolved organic carbon (DOC) as part of Activity 1 and the quality of the DOM will be assessed using several state-of-the-art techniques. The characterization of DOM will show if the DOM sources differ among the rivers, which could determine how they affect methylation and demethylation.

Summary Budget Information for Activity 2:	ENRTF Budget:	\$ 28	9,500
	Amount Spent:	\$ 28	9,185
	Balance:	\$	315

Activity Completion Date: January 1, 2017

Outcome	Completion Date	Budget
1. Methylation potential & riparian inflow	January 1, 2017	\$ 289,700
2. Photodemethylation	January 1, 2017	\$
3. DOC characterization	January 1, 2017	\$

Activity Status as of January 1, 2015:

The Project Manager has been discussing logistics to develop a work plan and budget with a team of researchers lead by Dr. Nathan Johnson at the University of Minnesota – Duluth. The MPCA will contract for this work through a State of Minnesota Professional Services Contract with the University of Minnesota. The UMD research team has worked together on similar mercury process research in the St. Louis River watershed under a contract with the Minnesota Department of Natural Resources.

Activity Status as of July 1, 2015:

The contract with Dr. Nathan Johnson at University of Minnesota – Duluth was completed on June 3, 2015. Field work for this activity is planned for July in the Roseau, Thief, and Mustinka Rivers. Field work was planned for June but was postponed because of high river flows. In addition, reconnaissance trips are planned for Kettle and Vermilion Rivers.

Activity Status as of January 1, 2016:

A team of researchers led by Dr. Johnson from UMD, in consultation with local resource management agencies, chose sites in three Red River of the North tributaries: Roseau, Thief, and Mustinka. Most sites were located on DNR Wildlife Management Areas to facilitate ease of access. The team traveled to the sites and began collecting environmental samples to help assess rates of methylmercury (MeHg) production and transport. Supplies were purchased to install small, shallow groundwater piezometers adjacent to stream banks at two sites each in the three western watersheds. Installation occurred during summer 2015 and water level sensors were left to continuously monitor changes in groundwater level through October 2015. Samples from surface water, groundwater, and soil were collected in summer and fall 2015 and preserved for the analysis of total mercury (THg), MeHg, as well as the speciation of carbon, sulfur, and iron to provide the geochemical context for mercury processes at the sites. Additionally, rates of mercury transformation including mercury methylation and MeHg demethylation were measured using enriched stable mercury isotopes in both groundwater adjacent to the streams as well as stream waters. Much of the analytical work had been completed as of December 31, 2015, but some samples are still waiting to be analyzed at partner labs.

Activity Status as of July 1, 2016:

Between January 1 and June 30, 2016, students and staff from UMD and partner institutions (Gustavus Adolphus College and University of Toronto Scarborough) participated in a meeting at MPCA in St. Paul to discuss the progress of the project with partners from MPCA and the University of Wisconsin LaCrosse. UMD students and staff also finished laboratory work analyzing sediment and water samples collected during the fall of 2015. In preparation for the 2016 field season, they worked with partner institutions to install shallow groundwater wells at two sites each in the Vermilion and Kettle River watersheds soon after ice out. Water and sediment samples were subsequently collected from 10 sites (2 in each of the 5 watersheds) and preserved for laboratory analysis of mercury and related chemistries. A second sampling trip at the end of June gathered only water samples from wells and surface water. A total of 321 solid phase samples and 228 water (surface water and porewater) samples have been collected in 2016. Some of these samples have been analyzed in the lab, but most are preserved and awaiting analysis at UMD and partner labs.

Activity Status as of January 1, 2017:

Sample collection continued through the summer and fall season at each of the 10 sites (2 per watershed). Water was collected from streams and groundwater wells approximately monthly through October and soil samples were collected in early August and late September. All samples were preserved and sent to partner institutions for analysis. Solid-phase MeHg is being analyzed at University of Toronto Scarborough and waterphase MeHg is being analyzed at Gustavus Adolphus College. Water level sensors were removed from all sites and surveys of water level piezometers were recorded in October and November 2016. As of Jan 1, 2017, about 65 % of the (over 400) water and soil samples have been analyzed at partner labs and data is being entered into spreadsheets for analysis. Water level measurements have been downloaded from loggers and are being consolidated and synthesized with streamflow gauging data.

Final Report Summary:

Water sampling, water level measurements, and methylation/demethylation rate studies were completed by June 30, 2017, although data analysis and preparation of manuscripts for publication will continue through 2018. Sites were chosen for methylation/demethylation measurements in each watershed considering stream morphology, soil type, topography, and surrounding land use (Table 4 and Figure 4). Sites were also chosen in the context of load monitoring and biological monitoring locations in each of the watersheds and, where possible, occurred in identical places in the streams (see table and map below). Each site had three sub-sites: A: in-stream; B: stream bank; C: uplands. Samples were collected in four periods over two years.

SiteID	Watershed	Description	LatDD	LonDD
RR-WMA	Roseau River	End of 190th Ave (& 360th St)	48.92481	96.20619
RR-MH	Roseau River	Off Morehouse Road, above Hayes Lake	48.57840	95.46321
THI-MR	Thief River	Downstream from Moose River WMA; past parking area	48.43195	95.44521
THI-EKV	Thief River	Eckvoll WMA on Mud River; end of 330th Ave (& ~310th St)	48.32749	95.76138
MUS-260	Mustinka River	outlet of Mustinka WMA @260th St; NE side; A well: 45.99308 -96.05119	45.99304	96.05064
MUS-BB	Mustinka River	Mustinka River near Lake Traverse; north end of 620th Ave; NW side	45.77425	96.59386
VER-BYK	Vermilion River	Hwy 24 Crane Lake Road, ~3 mi south from Buyck; E side by small trib	48.08672	92.49389
VER-GLD	Vermilion River	Landing on Gold Mine Road (4538)	48.23839	92.57951

Table 4 Methylation/demethylation and groundwater/surface water monitoring sites



Figure 4 Map of methylation/demethylation sites

Figure 5 shows water level monitoring at the Lower Roseau site during 2016, with subplots MeHg concentrations in surface water and groundwater during each sampling event. There was not a clear correlation between groundwater water level and MeHg concentrations, which is what we had posited we would see. Surface water MeHg was elevated under warm, high flow conditions. Surface water MeHg concentrations were lower than porewater concentrations except during high flow conditions. Porewater in stream sediments had elevated MeHg concentrations relative to groundwater concentrations. The groundwater gradient was consistently away from the river under high flow and only towards the river under very low flow.



Figure 5 Water depths at the Lower Roseau station in 2016; subplots show MeHg concentrations during the four sampling events

There was considerable variability in measured concentrations and rates among sites and by season that obscured obvious patterns. MeHg accumulated in stream and riparian soils even in sulfate-poor systems. Organic carbon appears to be the most important determining factor across sites for determining the absolute quantity of MeHg in soils and groundwater. Mustinka watershed soils are outliers in having very high sulfur (sulfate and sulfide) concentrations and low groundwater MeHg. Mustinka was also unusual in having higher MeHg concentration in the deeper groundwater.

Methylation rates (K_{meth}) for four sampling periods by site and the within-site locations shows K_{meth} in the instream sediment was significantly different across many study sites, but differences depended upon season (Figure 6). At the Mustinka site, MUS260 – the site with the highest sulfate and organic carbon – K_{meth} was consistently lower than most sites in May and August 2016. In contrast, THIEKV showed consistently greater K_{meth} , particularly in 2015. Across most watersheds (Thief, Kettle, Roseau and Vermillion), K_{meth} at the in-stream plot was significantly greater than at the near-stream or outer riparian edge. This was observed in both Kettle watershed sites in August and October 2016, in both Roseau watershed sites in 2015 and August 2016, Vermillion watershed sites in October 2016, and Thief watershed sites in October 2016. The only watershed in which K_{meth} at the in-stream plot was not significantly elevated relative to the upland plots was the Mustinka watershed where no significant within-site variability was observed.



Figure 6 Methylation rate potentials at each site during four sampling events; Sub-sites: A - river, B - stream bank, C - upland

In addition to seasonal variability, there is the variability between methylation rates K_{meth} and demethylation rates K_{demeth} , as see in the May 2016 sample period (Figure 7). K_{demeth} typically decreased with distance away from the river channel, but there were sites that were an exception to that pattern (in Thief, Kettle, and Vermilion rivers).



Figure 7 Methylation rates (K_{meth}) and demethylation rates (K_{demeth}) during the May 2016 sampling event

ACTIVITY 3: Food Web Relationships Description:

Aquatic food webs in five rivers will provide a comparison of bioaccumulation rates and possible differences in growth rates of top predator fish. Food web collections will include seston, benthic macro invertebrates, forage fish, and predator fish. Isotopic ratios of naturally-occurring stable isotopes for nitrogen (N) are carbon (C) will be measured in the biota samples to identify feeding relationships in the food webs and carbon sources. Food web collection and sample analysis will follow the procedures described in Scudder et al., 2008. This USGS publication describes the procedures for collection and processing of biota samples for mercury, methylmercury, and stable isotope analyses. Bioaccumulation of mercury in the food webs will be measured as the linear regression slope of log-transformed methylmercury concentration versus the N-isotope ratios (delta-15 N) in the biota samples. Growth rates in top predator fish will be calculated based on the aging and length data. USGS procedures include collection of otoliths from top predator fish for age determination.

Because of the natural variability in mercury and isotope levels in biota, meaningful comparisons require multiple sites and adequate samples of biota at each site. A total of 63 biota samples at each of 17 sites (1071 samples) was used to estimate the budget for the food web comparison.

Summary Budget Information for Activity 3:	ENRTF Budget:	\$ 274,800
	Amount Spent:	\$ 257,503
	Balance:	\$ 17,297
Activity Completion Date: March 30, 2017		

Outcome	Completion Date	Budget
1. Biota collection	January 1, 2017	\$ 129,000
2. Fish age determination and growth analysis	January 1, 2017	\$ 7,000

Activity Status as of January 1, 2015:

The current plan is to work with a UMD and their subcontractor to collect the biota samples. These subcontractors will also analyze the samples for mercury and methylmercury. Stable isotopes of carbon and nitrogen in the biota will be analyzed by the University of Minnesota under a separate state government master contract for environmental isotopes.

Activity Status as of July 1, 2015:

The contract with the University of Minnesota – Duluth, which was completed on June 3, 2015, includes a subcontract with the University of Wisconsin – La Crosse. An experienced team of biologists and chemists at UW-La Crosse have conducted biota collections for mercury for the National Park Service in five parks throughout the Great Lakes, as well as, previous studies in Voyageurs National Park. They managed to mobilize and collect fish, invertebrates, and water samples in June, despite the high flows, from Roseau and Thief rivers. They will continue to collect samples in July from additional sites in Thief and Mustinka rivers. They reported successful collections at multiple sites in each river. They will be processing the samples and analyzing them for mercury and methylmercury. No invoices have yet been received from the contractors.

Activity Status as of January 1, 2016:

During June and July 2015, water, seston, aquatic invertebrates, and prey fish were collected for analysis of methylmercury (MeHg) and total mercury (THg) from fourteen sites on the Mustinka River, Roseau River, and Thief River. To date, 74 filtered and unfiltered water samples have been analyzed for MeHg and THg; 28 seston samples have been analyzed for MeHg and 11 have been analyzed for THg. Fifty samples of periphyton have been lyophilized and homogenized and are currently being analyzed for MeHg and THg. Aquatic invertebrates from each site are in the process of being sorted, identified, lyophilized and homogenized for mercury analysis. More than 400 prey fish have been identified, measured, weighed, lyophilized and homogenized. A subset of these fish will be analyzed in the near future for MeHg and THg.

Activity Status as of July 1, 2016:

For the period January 1-June 30, 2016, a total of 24 seston (suspended particles), 44 periphyton (attached algae), and 45 water samples were processed and analyzed for total mercury (THg) and methylmercury (MeHg) content. During June 2016, water, seston, aquatic invertebrates, and prey fish were collected for analysis of THg and MeHg from 18 sites on the Vermilion River, Kettle River and St. Louis River; water and seston were collected from one additional site on the St. Louis River. More than 700 prey fish collected from these three rivers have been identified, measured, weighed, lyophilized and are currently being homogenized; a subset of these fish will be analyzed for MeHg and THg. Aquatic invertebrates collected from each site on the Vermilion River, Kettle River and St. Louis River are in the process of being sorted, identified, lyophilized and homogenized for mercury analysis. We are currently processing and analyzing for MeHg and THg several hundred benthic invertebrates collected in 2015 from the Thief and Mustinka Rivers, as well as analyzing water samples from the Vermilion, St. Louis, and Kettle Rivers for MeHg and THg.

Activity Status as of January 1, 2017:

After two field seasons, all samples from seven watersheds have been collected. Since August 2016, the University of Wisconsin – La Crosse lab has analyzed the following number of samples for total mercury (THg) and methylmercury (MeHg) content: Fish: 259 THg, 70 MeHg; Periphyton: 57 THg, 77 MeHg; Benthic Invertebrates: 297 THg, 247 MeHg. Mercury analyses are now completed for periphyton, water, and seston. Analyses are ongoing for fish and benthic invertebrates. Freeze-dried tissue samples will be sent for 15-N and 13-C stable isotope analysis.

Final Report Summary:

Food webs were sampled at 33 sites among six rivers (Table 5 and Figure 8). Data analysis is ongoing and preparation of manuscripts for publication are expected to continue through 2018. Samples included water, suspended particulate matter (seston), attached algae (periphyton), benthic macroinvertebrates, and prey fish. We analyzed 141 water samples for total Hg and 145 for MeHg; 337 macroinvertebrates of 33 different taxa from four of the six streams; 508 fish of 29 different species from the six streams.

In addition to analysis for mercury and methylmercury, carbon and nitrogen stable isotopes were measured in the biota; temperature, pH, conductivity, sulfate and organic carbon were measured in water. Mustinka had the lowest methylmercury levels in waters and prey fish (34 ng/g). Roseau and Thief Rivers had extremely high methylmercury levels in water and biota at the sites downstream of areas subject to flooding and periodic flushing. Methylmercury in prey fish from the Vermilion River (60 ng/g) were similar to Roseau River (61 n/g), but the methylmercury levels in the water were much lower than in Roseau or Thief Rivers. Lake Vermilion probably acts as a sink for mercury and methylmercury.

The percent of total mercury as methylmercury (%MeHg) was low in the seston, averaging 4.5% (0.5 - 9.7%) and higher in the periphyton, which averaged 14% (2 - 67.8%). Benthic invertebrates had average %MeHg of 75% (3 - 100%). The higher %MeHg in periphyton and benthic invertebrates indicates food webs dependent on benthos are likely to have higher mercury levels than those dependent on plankton. From all fish samples, the average %MeHg was 89%.

River	Site Code	Description	Collection Date	Latitude	Longitude	Comment
Mustinka	MUS-1	Upstream wetland	7/14/15	46° 9'6.72"N	96° 5'2.01"W	
	MUS-2	Mustinka Flowage	7/16/15	45°52'27.56"N	96° 7'0.39"W	
	MUS-3	Mouth, 12 Mile Creek	7/15/15	45°51'37.58"N	96°21'32.17"W	
	MUS-4	Wheaton, MN	7/15/15	45°48'50.35"N	96°30'30.09"W	
Roseau	ROS-1	Canadian border	6/15/15	48°55'27.86"N	96°12'26.00"W	
	ROS-2	Below Roseau, MN	6/15/15	48°54'19.51"N	95°49'56.60"W	
	ROS-3	Malung, MN	6/16/15	48°47'0.37"N	95°44'16.88"W	
	ROS-4	Wannaska, MN	6/1/15	48°37'38.03"N	95°44'55.53"W	
	ROS-5	Below Hayes Lake	6/17/15	48°38'29.76"N	95°35'47.56"W	
Thief	THF-1	Outflow, Thief Lake	6/17/15	48°29'18.90"N	95°56'49.06"W	
	THF-2	Mouth, Moose R.	6/18/15	48°29'14.34"N	95°49'6.15"W	
	THF-3	Mud R.	6/18/15	48°19'34.94"N	95°45'54.93"W	
	THF-4	Thief River	7/16/15	48°18'1.30"N	96° 4'14.58"W	
	THF-5	Thief River Falls, MN	6/19/15	48°11'14.40"N	96°10'25.05"W	Water and seston only
Vermilion	VRM-1	Mouth, Pike R.	6/1/16	47°47'13.09"N	92°22'45.50"W	
	VRM-2	Vermilion R. Dam	5/30/16	47°57'50.79"N	92°28'29.79"W	
	VRM-3	Above Buyck, MN	5/31/16	48° 4'20.19"N	92°29'8.13"W	
	VRM-4	Vermilion R.	5/31/16	48°14'30.88"N	92°36'12.74"W	
Kettle	KTL-1	Upper Kettle R.	6/2/16	46°32'58.62"N	92°56'12.15"W	

Table 5 Biota collection sites

KTL-2 Upper Moose Horn R. 6/2/16 46°33'31.67"N 92°44'27.23"W KTL-3 Lower Moose Horn R. 6/4/16 46°22'57.14"N 92°50'27.55"W KTL-4 Split Rock R. 6/4/16 46°26'48.34"N 92°50'27.55"W KTL-5 Banning State Park 6/3/16 46° 26'48.34"N 92°50'4.71"W KTL-6 St. Croix State Park 6/3/16 45°56'41.23"N 92°46'36.24"W St Louis STL-1 Below Skibo, MN 6/1/16 47°28'51.14"N 92° 2'25.10"W StLouis STL-2 Meadowlands, MN 6/5/16 46°59'46.12"N 92°48'31.48"W Water and seston only STL-3 Whiteface R. Hwy 53 6/5/16 47°11'8.03"N 92°29'12.23"W Water and seston only STL-4 Mouth, Cloquet R. 6/7/16 46°42'13.18"N 92°25'6.12"W STL-5 Scanlon, MN 6/7/16 46°39'33.81"N 92°16'50.44"W STL-5 Scanlon, MN 6/6/16 STL-5 Scanlon, MN 6/6/16 46°39'6.01"N 92°12'11.13"W STL-7 STL-6 Fond du Lac, MN 6/6/16 46°39'6.01"N 92°12'11.13"W ST							
KTL-4 Split Rock R. 6/4/16 46°26'48.34"N 92°56'55.56"W KTL-5 Banning State Park 6/3/16 46°9'51.51"N 92°50'4.71"W KTL-6 St. Croix State Park 6/3/16 45°56'41.23"N 92°46'36.24"W St Louis STL-1 Below Skibo, MN 6/1/16 47°28'51.14"N 92°2'25.10"W STL-2 Meadowlands, MN 6/5/16 46°59'46.12"N 92°20'12.23"W Water and seston only STL-3 Whiteface R. Hwy 53 6/5/16 47°11'8.03"N 92°25'0.12"W Mater and seston only STL-4 Mouth, Cloquet R. 6/7/16 46°42'13.18"N 92°25'6.12"W STL-5 Scanlon, MN 6/6/16 46°39'33.81"N 92°16'50.44"W STL-6 Fond du Lac, MN 6/6/16 46°39'6.01"N 92°12'11.13"W STL-7 Oliver, WI 6/6/16 46°43'7.02"N 92° 8'39.20"W		KTL-2	Upper Moose Horn R.	6/2/16	46°33'31.67"N	92°44'27.23"W	
KTL-5 Banning State Park 6/3/16 46° 9'51.51"N 92°50'4.71"W KTL-6 St. Croix State Park 6/3/16 45°56'41.23"N 92°46'36.24"W St Louis STL-1 Below Skibo, MN 6/1/16 47°28'51.14"N 92° 2'25.10"W STL-2 Meadowlands, MN 6/5/16 46°59'46.12"N 92°48'31.48"W Water and seston only STL-3 Whiteface R. Hwy 53 6/5/16 47°11'8.03"N 92°29'12.23"W STL-4 Mouth, Cloquet R. 6/7/16 46°53'46.65"N 92°30'53.04"W STL-5 Scanlon, MN 6/7/16 46°42'13.18"N 92°25'6.12"W STL-6 Fond du Lac, MN 6/6/16 46°39'33.81"N 92°16'50.44"W STL-7 Oliver, WI 6/6/16 46°39'6.01"N 92°12'11.13"W STL-8 Belknap, Superior, WI 6/6/16 46°43'7.02"N 92° 8'39.20"W		KTL-3	Lower Moose Horn R.	6/4/16	46°22'57.14"N	92°50'27.55"W	
KTL-6 St. Croix State Park 6/3/16 45°56'41.23"N 92°46'36.24"W St Louis STL-1 Below Skibo, MN 6/1/16 47°28'51.14"N 92°2'25.10"W STL-2 Meadowlands, MN 6/5/16 46°59'46.12"N 92°48'31.48"W Water and seston only STL-3 Whiteface R. Hwy 53 6/5/16 47°11'8.03"N 92°29'12.23"W Vater and seston only STL-4 Mouth, Cloquet R. 6/7/16 46°53'46.65"N 92°30'53.04"W STL-5 STL-5 Scanlon, MN 6/7/16 46°39'33.81"N 92°16'50.44"W STL-6 STL-6 Fond du Lac, MN 6/6/16 46°39'6.01"N 92°12'11.13"W STL-7 STL-8 Belknap, Superior, WI 6/6/16 46°43'7.02"N 92° 8'39.20"W STL-8		KTL-4	Split Rock R.	6/4/16	46°26'48.34"N	92°56'55.56"W	
St Louis STL-1 Below Skibo, MN 6/1/16 47°28'51.14"N 92° 2'25.10"W STL-2 Meadowlands, MN 6/5/16 46°59'46.12"N 92°48'31.48"W Water and seston only STL-3 Whiteface R. Hwy 53 6/5/16 47°11'8.03"N 92°29'12.23"W Water and seston only STL-4 Mouth, Cloquet R. 6/7/16 46°53'46.65"N 92°30'53.04"W STL-5 Scanlon, MN 6/7/16 46°39'33.81"N 92°25'6.12"W STL-6 Fond du Lac, MN 6/6/16 46°39'33.81"N 92°16'50.44"W STL-7 Oliver, WI 6/6/16 46°39'6.01"N 92°12'11.13"W STL-8 Belknap, Superior, WI 6/6/16 46°43'7.02"N 92° 8'39.20"W		KTL-5	Banning State Park	6/3/16	46° 9'51.51"N	92°50'4.71"W	
STL-2 Meadowlands, MN 6/5/16 46°59'46.12"N 92°48'31.48"W Water and seston only STL-3 Whiteface R. Hwy 53 6/5/16 47°11'8.03"N 92°29'12.23"W 40°53'46.65"N 92°30'53.04"W STL-4 Mouth, Cloquet R. 6/7/16 46°53'46.65"N 92°30'53.04"W 40°53'46.65"N 92°25'6.12"W STL-5 Scanlon, MN 6/6/16 46°39'33.81"N 92°16'50.44"W 40°53'46.65"N 92°12'11.13"W STL-6 Fond du Lac, MN 6/6/16 46°39'6.01"N 92°12'11.13"W 40°12'11.13"W STL-8 Belknap, Superior, WI 6/6/16 46°43'7.02"N 92° 8'39.20"W		KTL-6	St. Croix State Park	6/3/16	45°56'41.23"N	92°46'36.24"W	
STL-3 Whiteface R. Hwy 53 6/5/16 47°11'8.03"N 92°29'12.23"W STL-4 Mouth, Cloquet R. 6/7/16 46°53'46.65"N 92°30'53.04"W STL-5 Scanlon, MN 6/7/16 46°42'13.18"N 92°25'6.12"W STL-6 Fond du Lac, MN 6/6/16 46°39'33.81"N 92°16'50.44"W STL-7 Oliver, WI 6/6/16 46°39'6.01"N 92°12'11.13"W STL-8 Belknap, Superior, WI 6/6/16 46°43'7.02"N 92° 8'39.20"W	St Louis	STL-1	Below Skibo, MN	6/1/16	47°28'51.14"N	92° 2'25.10"W	
STL-4 Mouth, Cloquet R. $6/7/16$ $46^{\circ}53'46.65"N$ $92^{\circ}30'53.04"W$ STL-5 Scanlon, MN $6/7/16$ $46^{\circ}42'13.18"N$ $92^{\circ}25'6.12"W$ STL-6 Fond du Lac, MN $6/6/16$ $46^{\circ}39'33.81"N$ $92^{\circ}16'50.44"W$ STL-7 Oliver, WI $6/6/16$ $46^{\circ}39'6.01"N$ $92^{\circ}12'11.13"W$ STL-8 Belknap, Superior, WI $6/6/16$ $46^{\circ}43'7.02"N$ $92^{\circ}8'39.20"W$		STL-2	Meadowlands, MN	6/5/16	46°59'46.12"N	92°48'31.48"W	Water and seston only
STL-5Scanlon, MN6/7/1646°42'13.18"N92°25'6.12"WSTL-6Fond du Lac, MN6/6/1646°39'33.81"N92°16'50.44"WSTL-7Oliver, WI6/6/1646°39'6.01"N92°12'11.13"WSTL-8Belknap, Superior, WI6/6/1646°43'7.02"N92° 8'39.20"W		STL-3	Whiteface R. Hwy 53	6/5/16	47°11'8.03"N	92°29'12.23"W	
STL-6 Fond du Lac, MN 6/6/16 46°39'33.81"N 92°16'50.44"W STL-7 Oliver, WI 6/6/16 46°39'6.01"N 92°12'11.13"W STL-8 Belknap, Superior, WI 6/6/16 46°43'7.02"N 92° 8'39.20"W		STL-4	Mouth, Cloquet R.	6/7/16	46°53'46.65"N	92°30'53.04"W	
STL-7 Oliver, WI 6/6/16 46°39'6.01"N 92°12'11.13"W STL-8 Belknap, Superior, WI 6/6/16 46°43'7.02"N 92° 8'39.20"W		STL-5	Scanlon, MN	6/7/16	46°42'13.18"N	92°25'6.12"W	
STL-8 Belknap, Superior, WI 6/6/16 46°43'7.02"N 92° 8'39.20"W		STL-6	Fond du Lac, MN	6/6/16	46°39'33.81"N	92°16'50.44"W	
		STL-7	Oliver, WI	6/6/16	46°39'6.01"N	92°12'11.13"W	
STL-9 Brookston, MN 6/7/16 46°52'10.75"N 92°36'4.73"W		STL-8	Belknap, Superior, WI	6/6/16	46°43'7.02"N	92° 8'39.20"W	
		STL-9	Brookston, MN	6/7/16	46°52'10.75"N	92°36'4.73"W	



Figure 8 Map of biota collection sites within the project wetlands

Mustinka had the lowest Hg in prey fish among the rivers (Figure 9). Roseau has similar fish-Hg concentrations to St. Louis and Vermilion, but the MeHg in water from the latter two river is very low compared to Roseau, Thief, and Mustinka. Roseau River has a wide range of MeHg concentrations in water and periphyton.



Figure 9 Box-whisker plots of Hg in Fish, MeHg in Periphyton, MeHg in Water, and MeHg in Seston; colored box is the interquartile range (25th to 75th percentiles); midline is median and "X" is arithmetic average

MeHg concentrations in water and seston were highest at the downstream site (ROS1) and moving upstream the concentrations decreased (Figure 10). Periphyton MeHg levels in the two lower sites were nearly twenty times higher than the other three upstream sites. The pattern of MeHg in fish among the five sites is similar to what was seen in the lower trophic levels, but they do not provide insight into the highest MeHg concentrations in invertebrates and fish were in the middle ROS3 site.



Figure 10 MeHg concentrations from the five Roseau biota monitoring sites

A useful tool for identifying the similarity of food webs among the rivers is with carbon and nitrogen stable isotope ratios. Figure 11 shows the distribution of the d15N and d15C for all fish species (trophic level 3) in each of the rivers. All of the rivers, except Mustinka overlap, indicating similar food webs. The high d15N in Mustinka could be caused by the dominance of agriculture in that watershed. Some Vermilion species are skewed to the right on the d13C scale, indicating a stronger dependence on benthic carbon (i.e., organisms growing in or near the river bottom sediment).



Figure 11 Bivariate plot of the stable isotope ratios in fish, used to distinguish food web dynamics within and among the rivers

ACTIVITY 4: Database and Model Development

Description:

The purpose of this activity is to geo-reference all data in a single database, along with land cover summaries for each watershed. The compiled data will be statistically analyzed with as yet unspecified tests. Likely statistical tools are spatial statistics/plotting, principal components analysis, and mixed effects modeling. These statistical tools can provide a measure of the relative importance of the multiple variables and processes measured in this study. From the statistical analysis, an empirically-based conceptual model will be developed for the important drivers of mercury accumulation in the riverine food webs. This activity includes a travel budget for the project manager to visit sites, assist with sampling, and to attend in-state meetings.

Summary Budget Information for Activity 4:	ENRTF Budget: Amount Spent: Balance:	\$ 134
Activity Completion Date: June 30, 2017		
Outcome	Completion Date	Budget
1. Database development	March 30, 2017	MPCA IN-KIND
2. Statistical Analysis & Reporting	March 30, 2017	\$ 5,000
3. Travel to assist field work and attend meetings	June 30, 2017	\$ 700

Activity Status as of January 1, 2015:

There was no activity for Activity 4.

Activity Status as of July 1, 2015:

There was no activity for Activity 4.

Activity Status as of January 1, 2016:

Project Manager, Bruce Monson, traveled from Saint Paul to Superior, Wisconsin, for the St. Louis River Summit to share information about the project with other researchers and to learn about other research projects that could potentially lead to collaborations.

Activity Status as of July 1, 2016:

There was no activity for Activity 4.

Activity Status as of January 1, 2017:

There was no activity for Activity 4.

Final Report Summary:

This task could not be completed. Because data collection was not initiated until June 2015, it continued until the end of the project on June 30, 2017; consequently there was no time to prepare the database. We will continue to analyze the data and prepare manuscripts for publication. We expect to have manuscripts ready for submittal in fall of 2018. Published papers will be provided to LCCMR when they become available.

V. DISSEMINATION:

Description:

The research findings will be initially disseminated as reports to LCCMR. From these reports, the project manager and other primary researchers in this project will prepare manuscripts for scientific peer-reviewed publications and present the results at conferences. Major results will undoubtedly be of interest to the news media, because of the public's interest in mercury and consuming fish. The results of this research should be useful for developing total maximum daily load studies for mercury in fish in these waters that do not qualify for the Statewide Mercury TMDL because of exceptionally high mercury levels. The mercury results for fish will be added to the fish contaminant database maintained by Minnesota's interagency Fish Contaminant Monitoring Program.

Status as of January 1, 2015:

There was no activity for dissemination.

Status as of July 1, 2015:

There was no activity for dissemination.

Status as of January 1, 2016:

There was no activity for dissemination.

Status as of July 1, 2016:

There was no activity for dissemination.

Status as of January 1, 2017:

There was no activity for dissemination.

Final Report Summary:

As listed below, some communications were completed by June 30, 2017, and some manuscripts are proposed for publication (see end of list below). When the data are compiled, analyzed, and reported, completed publications will be shared with LCCMR.

Poster presentations at the International Conference on Mercury as a Global Pollutant, July16-21, 2017

MERCURY METHYLATION AND DEMETHYLATION ACROSS DIFFERENT RIPARIAN LANDSCAPES NG, Kevin¹; JOHNSON, Nathan²; JEREMIASON, Jeff³; MITCHELL, Carl¹; (1) University of Toronto Scarborough, Toronto, Canada; (2) University of Minnesota Duluth, Duluth, MN, USA;

(3) Gustavus Adolphus College, Saint Peter, MN, USA

MERCURY AND METHYLMERCURY YIELDS FROM RIVERS IN NORTHERN MINNESOTA, USA

MONSON, Bruce¹; BUTZER, Andrew²; ASHIAMAH, Evelyn²; HALVORSON, Danni³; ULVEN, Andrew³; JAHNZ, James¹; TEDROW, O'Niell⁴;

(1) Minnesota Pollution Control Agency, Saint Paul, MN, USA; (2) Minnesota Pollution Control Agency, Detroit Lakes, MN, USA; (3) International Water Institute, Fargo, ND, USA; (4) Vermilion Community College, Ely, MN, USA

Presentations Given at Scientific Conferences

Despins M., Rogers P., Akindolire-King K., and Rolfhus K. (2017). Methylmercury Contamination in the Lower Food Web of At-Risk Minnesota Rivers. Viterbo University Seven Rivers Research Symposium (won best poster award), La Crosse, WI.

Rogers P. (2017). Methylmercury Contamination in the Lower Food Web of At-Risk Minnesota Rivers. National Conference on Undergraduate Research, Memphis, TN (oral).

Rogers P., Despins M., Akindolire-King K., and Rolfhus K. (2017). Methylmercury Contamination in the Lower Food Web of At-Risk Minnesota Rivers. Mississippi River Research Consortium, La Crosse, WI (poster).

Akindolire-King K. and Despins M. (2017). Methylmercury Contamination in the Lower Food Web of At-Risk Minnesota Rivers. 14th Annual University of Wisconsin System Research in the Rotunda, Madison, WI (poster).

Anticipated Manuscripts

Rolfhus K., Sandheinrich M., and Haro R. Spatial analysis and trophic transfer of methylmercury in the lower food webs of at-risk Minnesota Rivers. *Science of the Total Environment* expected submission summer/fall 2018.

Johnson, NJ et al. Riparian and sediment mercury methylation, hyporheic flows, and exchanges between riparian areas and surface water in northern Minnesota Rivers. Expected submission summer/fall 2018

Monson, BA et al. Relationships between methylmercury yields, mercury methylation rates, and trophic magnification factors in rivers with high fish-mercury levels. Expected submission summer/fall 2018.

VI. PROJECT BUDGET SUMMARY:

A. ENRTF Budget Overview:

Budget Category	\$ Amount	Explanation
Personnel:	\$0	Project manager salary is in-kind support and all
		other personnel costs included in contracts
Professional/Technical/Service Contracts:		All field sampling, experiments, and laboratory
Professional/Technical (Activities 1-4)	\$ 579,300	analysis will be completed under contracts with
Laboratory Services (Activities 1 and 3)	\$ 163,000	the MPCA; Where possible, MPCA will use
		existing master contracts. Requests for
		proposals will be issued by the MPCA in the
		third quarter of 2014 with the intent of signed
		contracts by January 1, 2015.
Travel Expenses in MN:	\$ 700	Meetings and field visits for project manager
TOTAL ENRTF BUDGET:	\$ 743,000	

Explanation of Use of Classified Staff: N/A

Explanation of Capital Expenditures Greater Than \$5,000: N/A

Number of Full-time Equivalents (FTE) Directly Funded with this ENRTF Appropriation: NONE

Number of Full-time Equivalents (FTE) Estimated to Be Funded through Contracts with this ENRTF Appropriation: TBD

B. Other Funds:

Source of Funds	\$ Amount Proposed	\$ Amount Spent	Use of Other Funds
Non-state			
	\$	\$	
State – (In-kind Support only)			
MPCA 0.5 FTE for 3 years	\$ 156,000	\$0	
TOTAL OTHER FUNDS:	\$	\$	

VII. PROJECT STRATEGY:

A. Project Partners: TBD (subject to revision for final work plan)

B. Project Impact and Long-term Strategy:

The long-term goal of this project is to protect public health by reducing mercury in fish. Understanding the relative importance of processes that cause high mercury levels in fish will inform future management activities. The eventual development of Total Maximum Daily Loads for these waters, funded by the Clean Water Legacy Fund, will benefit from the scientific results of this project.

C. Spending History:

Funding Source	M.L. 2008	M.L. 2009	M.L. 2010	M.L. 2011	M.L. 2013
	or	or	or	or	or

FY09	FY10	FY11	FY12-13	FY14

VIII. ACQUISITION/RESTORATION LIST: N/A

IX. VISUAL ELEMENT or MAP(S): See attached graphic.

X. ACQUISITION/RESTORATION REQUIREMENTS WORKSHEET: N/A

XI. RESEARCH ADDENDUM: See attached Research Addendum.

XII. REPORTING REQUIREMENTS:

Periodic work plan status update reports will be submitted no later than January 1, 2015, July 1, 2015, January 1, 2016, July 1, 2016, and January 1, 2017. A final report and associated products will be submitted between June 30 and August 15, 2017.

Project Title: Identifying Causes of Exceptionally High Mercury in Fish.

Legal Citation: M.L. 2014, Chp. 226, Sec. 2, Subd. 03j

Project Manager: Bruce Monson.

Organization: Minnesota Pollution Control Agency.

M.L. 2014 ENRTF Appropriation: \$ 743,000

Project Length and Completion Date: 3 Years, June 30, 2017

Date of Report: September 29, 2017

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET BUDGET ITEM	Revised 2- 22-16 Activity 1 Budget	Amount Spent	Activity 1 Balance	Revised 2- 22-16 Activity 2 Budget	Amount Spent ercury Proc	Activity 2 Balance	Budget	Amount Spent Veb Relatio	Balance	Revised 2- 22-16 Activity 4 Budget	Amount Spent	Activity 4 Balance		TOTAL BALANCE
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Professional/Technical/Service Contracts														
Water quality sample collection amended contracts with International Water Institute, Fargo ND	\$25,000	\$19,268	\$5,732										\$25,000	\$5,732
Biota collection; and methylation/demethylation studies contract with University of Minnesota and subcontracts to University of Wisconsin - La Crosse, Gustavus Adolphus College, and University of Toronto				\$289,500	\$289,185	\$315	\$259,800	\$247,389	\$12,411				\$549,300	\$12,726
Laboratory Services for water chemistry contract with Minnesota Department of Health Environmental Laboratory	\$148,000	\$105,168	\$42,832										\$148,000	\$42,832
Laboratory Services for stable isotope analysis contract with University of Minnesota							\$15,000	\$10,114	\$4,886				\$15,000	\$4,886
TBD: PT contract for statistics and data analysis										\$5,000		\$5,000	\$5,000	\$5,000
Travel expenses in Minnesota meetings and field visits for project manager.										\$700	\$134	\$566	\$700	\$566
COLUMN TOTAL	\$173,000	\$124,436	\$48,564	\$289,500	\$289,185	\$315	\$274,800	\$257,503	\$17,297	\$5,700	\$134	\$5,566	\$743,000	\$71,742