2017 Project Abstract
For the Period Ending June 30, 2020

PROJECT TITLE: Chemicals of emerging concern in subsistence species used by Minnesota Chippewa
PROJECT MANAGER: Seth Moore
AFFILIATION: Grand Portage Band of Lake Superior Chippewa
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FUNDING SOURCE: Environment and Natural Resources Trust Fund
LEGAL CITATION: $400,000 the first year is from the trust fund to the commissioner of natural resources for an agreement with the Grand Portage Band of Lake Superior Chippewa to identify chemicals of emerging concern and metals in fish, water, and sediments from approximately 30 water bodies in northeastern Minnesota used for subsistence harvest and recreation. This appropriation is available until June 30, 2020, by which time the project must be completed and final products delivered.

APPROPRIATION AMOUNT: $400,000
AMOUNT SPENT: $ 388,028
AMOUNT REMAINING: $ 11,576

Sound bite of Project Outcomes and Results
This is the first study to comprehensively analyze Chemicals of Emerging Concern (CECs) within fish tissues, waters, and sediments in 28 Minnesota lakes and in Lake Superior. We found 117 CECs with 101 found in water samples, 67 in sediments, and 35 in fish tissues.

Overall Project Outcome and Results
This is the first study to comprehensively analyze a large suite of Chemicals of Emerging Concern (CEC's) across the three media of fish tissues, water, and sediment in a broad geographic extent in northern Minnesota. CEC's are pharmaceuticals and personal care products that can linger in the environment and have been shown to affect behavioral and reproductive health of aquatic life. This study was focused on fish species and water bodies used for subsistence by the Grand Portage Band of Chippewa and recreational users of northeastern Minnesota. We described the spatial distribution of CEC occurrences in 25 Minnesota lakes and in Lake Superior. We consider our most important findings to be the number of detections and the classes of CEC's that were detected. We found 117 CECs across all media types with 101 found in water samples, 67 in sediments, and 35 in fish tissues. The pharmaceutical classes that were most frequently detected included hormones (100% of sites), DEET insect repellent (100% of sites), antidepressants (80% of sites), and antimicrobials (80% of sites). These results were derived from surface water samples, sediment samples, and fish samples of walleye/yellow perch in inland lakes and lake trout/cisco from Lake Superior. We also related measures of fish health and parasite loading to CEC's and land use. Our findings are consistent with early literature on CEC's in Minnesota lakes that studied water samples only. We used Aquatic Toxicity Profiles (ATP's) to identify those chemicals that may pose risks to aquatic life. ATPS provide an overview of chemical-specific information such as acute toxicity, endocrine activity, physicochemical properties, and occurrence information in the aquatic environment. We found that even in undeveloped sites that had a fewer number of total contaminants, they often had a high percentage of high priority contaminants. More work is needed to determine the effects of CEC's on aquatic life.

Project Results Use and Dissemination
The following products have been developed from this project.
Video:
https://www.youtube.com/watch?v=FIxVQU2gSBA&list=PLnC4yTjRBxQeDnfIW1Oxd2BlHnBKc4_bY&index=5

Radio: There were three radio broadcasts on this work, WTIP, Grand Marais and Minnesota Public Radio (MPR).
WTIP
https://www.wtip.org/lsproject-second-round-testing-completed-chemicals-emerging-concern-area-lakes
https://www.wtip.org/dr-seth-moore-partnership-university-minnesota-looks-grand-portage-ecosystem-health

MPR
https://www.mprnews.org/story/2020/06/14/study-finds-pharmaceuticals-in-remote-minnesota-lakes

Online and paper media outlets:
The Circle
http://thecirclenews.org/environment/scientists-find-117-chemicals-in-grand-portage-fish-and-lake-bottom/
http://thecirclenews.org/environment/snowflakes-raindrops-and-dust-bring-cecs-to-lakes-and-fish/

MN Daily
https://www.mndaily.com/article/2020/05/n-umn-researcher-discovers-concerning-chemicals-in-grand-portage-tribal-waters

UMN products
https://vetmed.umn.edu/news/researchers-measure-contaminants-emerging-concern-aquatic-systems

Medium
https://medium.com/@carolynbernhardt/thanks-to-climate-change-and-your-pee-contaminants-are-in-the-water-and-theyre-spreading-cf9fd91abe23

City Pages
http://www.citypages.com/news/study-so-theres-cocaine-some-of-minnesotas-water/571350071

Grand Rapids Herald Review
https://www.grandrapidsmn.com/free_press/study-finds-pharmaceuticals-other-chemicals-in-remote-minnesota-lakes/article_a7d05eb4-af15-11ea-b6e3-a325516440c3.html

Environment and Natural Resources Trust Fund (ENRTF)
M.L. 2017 LCCMR Work Plan Final Report

Date of Submission: January 31, 2020

Final Report

Date of Work Plan Approval: 06/07/17

Project Completion Date: January 2020

PROJECT TITLE: **Chemicals of emerging concern in subsistence species used by Minnesota Chippewa**

Project Manager: Seth Moore, PhD

Organization: Grand Portage Band of Lake Superior Chippewa

Mailing Address: 27 Store Rd.

City/State/Zip Code: Grand Portage, MN 55605

Telephone Number: (218) 370-9310

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Location: This project will take place in the 1854 Ceded Territory of Minnesota, in Cook and Lake counties.

Total ENRTF Project Budget:	**ENRTF Appropriation:**	**$400,000**
	Amount Spent:	**$388,424**
	Balance:	**$ 11,576**

Legal Citation: M.L. 2017, Chp. 96, Sec. 2, Subd. 04g

Appropriation Language:
$400,000 the first year is from the trust fund to the commissioner of natural resources for an agreement with the Grand Portage Band of Lake Superior Chippewa to identify chemicals of emerging concern and metals in fish, water, and sediments from approximately 30 water bodies in northeastern Minnesota used for subsistence harvest and recreation. This appropriation is available until June 30, 2020, by which time the project must be completed and final products delivered.

I. PROJECT TITLE: Chemicals of emerging concern in subsistence species used by Minnesota Chippewa

II. PROJECT STATEMENT:

We propose to evaluate levels of unregulated and emerging contaminants within key fish species, waters, and sediments in Northeastern Minnesota within the 1854 Ceded Territory. Specifically, we will assess the threat of low concentration but potentially toxic "micropollutants" within species of fish used for subsistence and their environment as part of a long-term ecosystem health program. Further, the proposed project will be the first of its kind in regard to contaminant surveillance in animals and the environment simultaneously in Minnesota, providing new methodologies for natural resource managers. This project directly addresses the second priority item in the Water Resources strategic guidance of the LCCMR 2017 RFP to determine the environmental fate and ecological effects of emerging and unregulated contaminants.

Tribal communities in Minnesota rely heavily on fish species in the Lake Superior watershed and the 1854 Ceded territory for subsistence; however, concerns for the impact of endocrine-active or toxic chemicals in the environment on the health of fish populations and humans that consume them raises questions about the safety and security of subsistence foods. This has potential long-term implications for the Minnesota recreational industry as well. To address these concerns, the Grand Portage Band of Chippewa is collaborating with the University of Minnesota, Minnesota Pollution Control Agency, and Environmental Protection Agency to assess concentrations of such contaminants in the Lake Superior watershed and describe potential environmental exposure pathways.

A pilot study on fish and water samples on the Grand Portage Indian Reservation conducted in 2015 revealed numerous micropollutants in water and fish tissues from locations in Lake Superior and in remote wilderness sites without an obvious source of contamination. Our team screened for over 100 CECs in four sample locations on reservation lands, which resulted in the detection of five compounds in water (androstenedione, androsterone, cotinine, hydrocodone, and metformin), seven compounds in fish tissue (betamethasone, venlafaxine, triclosan, clotrimazole, hydrocortisone, iopamidol, and triclocarbon), and DEET in both water and fish tissues. Iopamidol was ubiquitous in fish tissue across sites and DEET was ubiquitous in water and fish tissues. Research has found that aquatic exposures to some estrogen-mimics (e.g., ethinyl estradiol, a chemical used for birth control) can render fish sterile and thus threaten the sustainability of those populations. However, little is known about the potential for micropollutants to accumulate in fish and wildlife tissues, thus potentially exposing higher-level organisms and humans that consume them. Baseline levels of these contaminants in the Minnesota ecosystem have not been determined, thus no long-term chemical management strategies exist.

This study will characterize the occurrence of micropollutants within fish from northeastern Minnesota lakes and streams and guide further initiatives to investigate effects on fish populations and risk to human health. Furthermore, this study, *the first of its kind*, will provide new data and new models and tools for risk assessment and surveillance useful for a variety of resource management agencies.

Preliminary data show that there are measurable levels of CECs in this pristine environment that need to be further characterized. Baseline levels of many CECs in the Great Lakes ecosystem have not been determined; coupled with a lack of water quality standards or effects benchmarks for most of these contaminants, no long-term chemical management strategies exist. This study will 1) characterize the baseline occurrence of CECs and trace metals in these waters and in important subsistence species upon which the people of GPIR rely; 2) help prioritize risk management research efforts based upon our results. Ultimately, we will use this information to guide further initiatives to investigate biological effects on wildlife populations. Finally, this study will help evaluate and validate new tools for science-based management of these emerging issues by providing new data and spatial analysis for risk assessment and prediction, and offering information to assist systematic surveillance useful for a variety of resource management agencies.

The goals of this research are to:
1) Collect baseline data on the presence of chemicals of emerging concern (CECs) and trace metals in fish from areas used by tribal members for the harvest of subsistence species within the Minnesota Lake Superior watershed.
2) Analyze detected contaminants to identify how they may be related to human landuse characteristics, natural landscape dynamics, and measures of fish health.

III. OVERALL PROJECT STATUS UPDATES:

Project Status as of *[January 2018]***:**
We collected water, sediment, and fish tissue samples during July to September, 2017 from nineteen waterbodies within GPIR and the 1854 Ceded Territory in northeastern Minnesota. Lakes were chosen based on classification of human development, geographic distribution, accessibility, and presence of target subsistence fish species. We sampled from seven waterbodies that were classified as 1) watersheds in remote wilderness without significant human development, and six waterbodies each 2) from highly developed waterbodies that include lakes with significant numbers of human residences, and 3) from areas downstream of wastewater treatment outfalls. Two key subsistence fish species, walleye and yellow perch from inland lakes and lake trout and cisco from Lake Superior locations, were collected from each location with the exception of two lakes that did not contain yellow perch. We measured basic water chemistry at all waterbodies, and water, sediment, and fish tissue samples were sent to labs for analysis of Pharmaceuticals and Personal Care Products (PPCPs) and heavy metals. We evaluated fish health in seventeen waterbodies using a fish health index that we refined for this study. The aim of this preliminary analysis is to quantitatively compare the health of fish sampled in three environments that differ in the degree of anthropogenic activity; undeveloped, developed, and discharge related areas. Natural Resources Department Staff from the Grand Portage Band of Lake Superior Chippewa and 1854 Treaty Authority collected fish, water, and sediment samples. Faculty and students from the University of Minnesota analyzed fish health.

Project Status as of *[July 2018]***:**
This project is currently ahead of schedule by an estimated 6 months. Activity 1, which is the field component of the work is largely complete. Project leads decided to complete all field sampling during the first sampling season to reduce the likelihood of interannual variability in chemical loadings in fish tissues and water (sediments would likely not vary greatly). Furthermore, due to the extensive logistical arrangements among project partners to accomplish field sampling, it made sense to wrap up all sampling in the first season to reduce that logistical burden for the subsequent year. All data from sample media of fish tissue, water, and sediment has been processed, sent to detection laboratories, and results have been provided back to project leads. All sample data has been entered into appropriate databases for analysis, proofed, and summary statistical analysis has been performed as detailed below. Two doctoral students have been hired to perform the statistical and spatial modeling and those projects are well underway.

Project Status as of *[January 2019]***:**
This project is making good progress towards meeting the timeline and objectives described in the workplan. Activity 1 is now complete and Activity 2 is well underway. Our team has accomplished all data collection, sample analysis, data compilation, and summary analysis. We are working towards the accomplishment of the spatial analysis and environmental gradient analysis outlined in activity two. We continue to conduct scientific and public outreach on the results of our work, and those outputs are described below under the specific activity reports. We look forward to timely completion of this project and sharing our results with the LCCMR.

Amendment request as of January 2019
We request to include the $16,465 budgeted towards UMN travel into the UMN contract subheading to enable reimbursement per UMN policies. This will ease some of the reimbursement challenges faced with meeting

requirements of DNR passthrough rules. The requested changes are identified in the attached budget spreadsheet.
Amendment Approved 2/13/19

Project Status as of *[July 2019]***:**
We are currently performing the statistical analyses listed under Activity 2 to estimate the potential contribution of point and non-point sources to contaminant levels across the environment. Statistical analyses, such as Pearson correlation coefficients and Redundancy Analysis, are being done to determine the effect of anthropogenic activity on presence of contaminants. Data were collected for a variety of factors, and the following were found to be suitable for further analyses: catchment units, impervious land, businesses, wastewater treatment, building footprints, and population. We will identify areas of risk where human consumption of contaminated subsistence species warrants further investigation.

Project Status as of *[January 2020]***:**
We have submitted the first manuscript that will result from this project. The manuscript is currently under review at Science of the Total Environment. We have continued statistical analyses listed under Activity 2. As a result of Pearson correlation coefficients and Redundancy Analysis, we have begun identifying important predictor variables to include in regression models. Regression models are currently being built and results assessed. To prioritize contaminants, Aquatic Toxicity Profiles have been completed. Additionally, we are currently assessing the rFHI using common measures of validity, sensitivity and specificity, using histopathological indices as a gold standard. Histopathological indices have been created and lesions of particular interest are currently being stained for further investigations of etiology.

Overall Project Outcomes and Results:

IV. PROJECT ACTIVITIES AND OUTCOMES:
This study will 1) characterize the baseline occurrence of CECs and trace metals in these waters and in important subsistence species upon which the people of GPIR rely; 2) help prioritize risk management strategies and 3) guide further initiatives to investigate biological effects on wildlife populations, as well as risks to human health. Finally, this study will help evaluate and validate new tools for science-based management of these emerging issues by providing new data and models for risk assessment and prediction, and systematic surveillance useful for a variety of resource management agencies.

ACTIVITY 1: Collect data on chemicals of emerging concern from 18 waterbodies by sampling fish, water, and sediments.

Description:
Eighteen eco-hydrologically relevant sites will be chosen within GPIR and the 1854 Ceded Territory for contaminant surveillance. Sites will include inland lakes and rivers as well as locations along the Lake Superior coast. Site selection will follow a multicriteria selection process that includes fish assemblage information, human land use, and watershed characteristics including proximity to potential point sources of contaminant release (e.g. downstream of wastewater effluence or mining activities, as well as several control sites); presence of important subsistence species; and value as a fish harvesting location for tribal members (implying human and wildlife exposure risk). Each site will be sampled once. For comparative purposes, efforts will be made to sample all locations in the same season to control for seasonal fluctuations associated with environmental conditions.
Table of Experimental Design

Landuse	Waterbodies	Tissue	Water	Sediment	Species	Species of interest
Undeveloped watersheds/systems	6	12	6	6	2	Inland lakes
Wastewater effluences	6	12	6	6	2	Walleye
Highly developed watersheds	6	12	6	6	2	Perch
		36	18	18		
						Lake Superior
Note: Each waterbody gets two pooled tissue samples, each comprised of a single species used for subsistence consumption						Lake trout
						Lake Herring
		Total Samples		72		

Sampling for chemical surveillance will include water, sediment, and fish tissue in order to understand the ecohydrology of the phenomena systemically. For water sampling, POSIS samplers, will be used to collect a time-integrated water sample that will be more representative (than by comparison, a grab sample) of what aquatic organisms might be exposed to over a longer time period such as a season. A single sediment sample will be collected from each site for chemical screening and to detect chemicals that partition to particulate. At the same time that water and sediment samples are obtained, fish will be collected by standard field methods (e.g. electroshock, gill netting, etc.) and sacrificed according to an IACUC-approved protocol for chemical analysis. Following collection, fish will be weighed, measured by length and logged by species. Tissues from all species caught at a site will be pooled in equal proportions to obtain a representative measure of CECs in fish tissue for the site. This method has been utilized in our preliminary investigations and will be followed for consistency and comparability. However, we are also interested in understanding chemical exposures by species. Thus, in addition to an overall pooled sample, we will also pool tissue samples by species for CEC and metal analysis. Two key subsistence species will be captured from each location (e.g. walleye/yellow perch from inland lakes, and lake trout/cisco from Lake Superior locations) and be prioritized for species-stratified chemical sampling. We expect that lake trout/cisco and walleye/yellow will be consistently collected from Lake Superior and inland water bodies, respectively; therefore, in our efforts to assess contaminant exposure by species, we will prioritize pooling tissues among these four species for CEC testing. In addition, a piece of liver (a traditional sampling tissue for trace metal analysis) will be sampled (prior to tissue pooling for CEC analysis, which will include remaining liver among all other tissues) from all fish and pooled in the same manner for site and species-specific trace metal analysis. As with the stratified CEC analysis, we will prioritize pooling of liver samples from two key subsistence species for species-stratified analyses, specifically targeting walleye and lake trout.

Following collection of fish, water, and sediment samples, they will be processed and shipped for screening of more than 100 chemicals of emerging concern.

Summary Budget Information for Activity 1:

ENRTF Budget: $ 316,489
Amount Spent: $ 311,573
Balance: $ 4,916

Outcome	Completion Date
1. *18 sites sampled for fish, water, and sediment*	November 2018
2. *Analyze sampling data with UMN ecohealth researchers and state and federal toxicologists*	January 2019

Activity 1 Status as of *[January 2018]***:**

Activity 1. Part 1: 18 sites sampled for fish, water, and sediment

Study Area

Water, sediment, and fish samples were collected from nineteen locations in northeastern Minnesota, in Cook, Lake, and St. Louis Counties (Fig. 1) during July to September of 2017. The lakes ranged in surface area from 64 to 20,288,000 acres with maximum depths of 5 to 406 m (Table 1). We sampled from seven waterbodies that were 1) watersheds in remote wilderness without significant human development, and six waterbodies each from 2) highly developed waterbodies that include lakes with significant numbers of human residences, and 3) areas downstream of wastewater treatment outfalls (Table 2). Lakes were chosen based on classification of human development, geographic distribution, accessibility, and presence of target subsistence fish species.

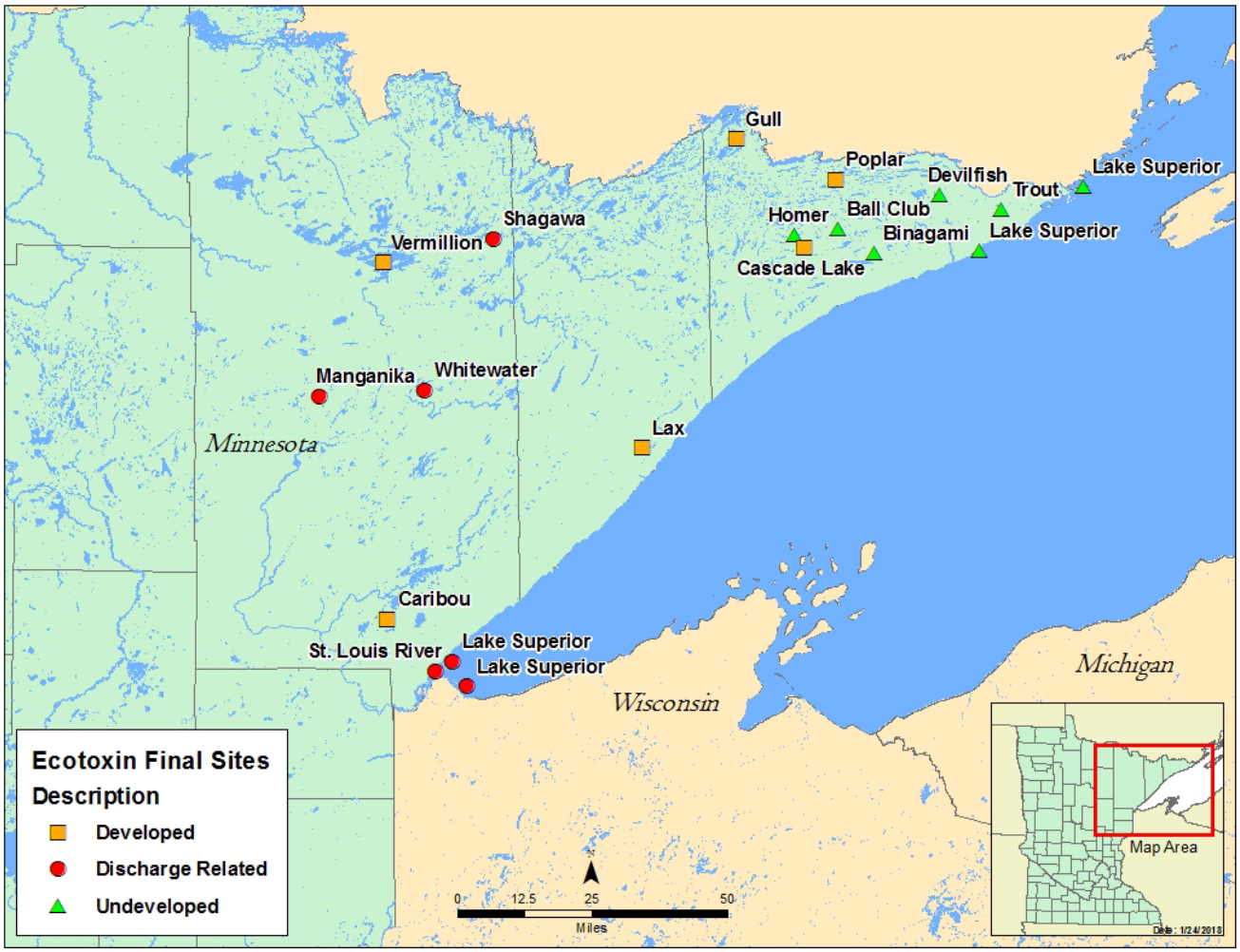

Figure 1. Map of collection locations for 2017 LCCMR study in northeastern Minnesota.

Water Collection

We deployed Polar Organic Chemical Integrative Samplers (POCIS) to collect water samples for analysis of PPCPs. By deploying POCIS, we were able to collect a time-integrated water sample over thirty days that will be more representative, than by comparison, a grab sample, of what aquatic organisms might be exposed to over a longer time period such as a season. We deployed three POCIS disks per waterbody for a minimum of thirty days during July 10 - August 31, 2017. POCIS were placed in a canister and carrier provided by EST Labs (St. Joseph, MO) and remained submerged under a minimum of twelve inches of water throughout the entire deployment period. Before deployment and after retrieval, POCIS were stored in carriers in airtight metal containers. They were frozen immediately after collection and remained frozen until shipment to SGS AXYS Analytical (Sydney, British Columbia, Canada) for analysis. We deployed one field blank POCIS at Poplar Lake. When the field-deployed POCIS in Poplar Lake was exposed to air, the lid to the field blank container was also opened, thereby exposing it to air for the same duration as the field-deployed POCIS for comparison. The field blank was stored frozen in sealed metal cans.

We collected basic water chemistry measurements including conductivity, pH, dissolved oxygen, and temperature by using water chemistry meters at the nineteen study locations during July 10 - September 6, 2017. Water chemistry measurements were collected three times, during deployment and retrieval of POCIS and during fish collection. Grab water samples were collected at all waterbodies and sent to Pace Analytical (Duluth, MN) for measurement of dissolved organic carbon, chloride, and hardness. Samples were collected using containers provided by Pace Analytical. A 50ml aliquot grab water sample per waterbody was collected and sent

to Michigan State University's Diagnostic Center for Population and Animal Health to be analyzed for trace metals, which included aluminum, antimony, arsenic, barium, cadmium, chromium, copper, iron, lead, manganese, mercury, phosphorus, selenium, sodium, sulfur, thallium, boron, and zinc. Collectors used a plastic container and wore latex gloves. All water samples were stored on ice in coolers and then refrigerated until shipment within two days of collection.

Sediment Collection

We collected three grab samples of sediment per waterbody for analysis of PPCPs, heavy metals, and total organic carbon (TOC) during August 17 - September 6, 2017. All materials used to collect sediment were washed with detergent and tap water and then rinsed with distilled water. We collected sediment samples in 250 mL plastic containers from the top 10 cm of sediment using a ponar dredge or sediment siphon. Samples were stored on ice immediately after collection. Samples to be analyzed for PPCPs were frozen until overnight shipment to AXYS Analytical Labs. Samples to be analyzed for heavy metal and TOC were stored in a refrigerator and shipped within two days of collection. Pace Analytical analyzed sediment for TOC and provided containers for collection. Sediment for trace metal analysis was collected in 250 mL plastic containers and sent to Michigan State University's Diagnostic Center for Population and Animal Health for analysis of aluminum, antimony, arsenic, barium, cadmium, chromium, copper, iron, lead, manganese, mercury, phosphorus, selenium, sodium, sulfur, thallium, boron, and zinc.

Fish Collection and Processing

Two key subsistence species were collected from each location, walleye (*Sander vitreus*) and yellow perch (*Perca flavescens*) from inland lakes and lake trout (*Salvelinus namaycush*) and cisco (*Coregonus artedii*) from Lake Superior locations. Two waterbodies did not contain yellow perch, so walleye were collected at Binagami Lake and walleye and black crappie (*Pomoxis nigromaculatus*) were collected at Manganika Lake (Table 2). A minimum of three individuals of each species per waterbody was collected for PPCP analysis. We attempted to collect a minimum of twenty individuals of each species per waterbody for fish health analysis, but constraints due to small lake size or small fish population limited the sample sizes collected.

Fish were collected from July 25 - September 21, 2017 by standard field methods using gill and fyke nets, electrofishing, and bottom trawls. Efforts varied by field gear used and lake size. Gill nets were strategically placed in locations with overlapping thermal and foraging habitats of targeted species to maximize catch per effort. Lake Superior fish were caught using 732-m-long by 1.8-m-high bottom set gill nets made of 210/2 multifilament nylon webbing with stretch mesh sizes of 51, 64, 76, 89, 102, and 114 mm, fished at depths between 46 - 91 m for 24 hours. Fish in inland lakes were caught using 1.8-m-high bottom set gill nets made of 210/2 multifilament nylon webbing at two sizes: 76-m-long with stretch mesh sizes of 76, 102, 127, 152, and 178 mm and 46-m-long with stretch mesh sizes of 38, 51, 64, 76, 89, and 102 mm, fished at depths between 1 to 8 m for 24 hours. Electrofishing was performed at night and day along shorelines using a boom shocking boat operated at 250-500 V and 3-4 A with pulsed DC at 60 pulses per second. All equipment was rinsed in water from the waterbody being sampled prior to fish collection. Researchers wore latex or nitrile powder-free gloves when handling fish.

Following collection, fish were weighed, measured by length, and logged by species. Fish were sacrificed per an IACUC-approved protocol and stored in coolers on ice. Half of the liver from each fish was removed. Liver samples were pooled by species and waterbody and stored frozen until shipment to Michigan State University's Diagnostic Center for Population and Animal Health for analysis of heavy metals, including cobalt, copper, iron, manganese, molybdenum, selenium, zinc, arsenic, cadmium, lead, mercury, and thallium. Fish collected for PPCP analysis were wrapped in aluminum foil, placed into plastic bags, and frozen until processing.

Fish samples were homogenized using a stainless steel commercial meat grinder. Grinder and materials were cleaned with detergent and tap water, rinsed with deionized water, rinsed with methanol three times to remove chemical and other organic contamination, and then rinsed in acetone to dry. Whole fish bodies, including skin

and small bones, were homogenized. Fish were pooled together by species and waterbody and homogenized to obtain one sample per species and waterbody for PPCP analysis. After homogenization, we collected subsamples of tissue to total 100 g of fish tissue of each species and waterbody. Fish samples were stored frozen in plastic containers. A total of 37 samples were sent to AXYS Analytical for analysis of PPCPs (Table 2).

Fish Health Analysis

The aim of this preliminary analysis is to quantitatively compare the health of fish sampled in three environments that differ in the degree of anthropogenic activity; namely, undeveloped, developed, and discharge related areas. Fish were collected from 13 inland sites and 4 Lake Superior sites in Northeastern Minnesota (Table 3).

Fish health was evaluated by creating a refined fish health index (rFHI) (Table 4) adapted from the health assessment index described in Adams et al. (1993). This refined fish health index was modified by University of Minnesota faculty and graduate students. This index provides a measure of fish body condition and parasite presence. Fish were necropsied and parasites were identified in the field and anomalies observed in the tissues and organs were recorded. Variables included in the index were assigned a field code designation, which was then transcribed to a numerical value for statistical analyses. Substituted rFHI values range from 0 - 30, zero denoting normal condition and 30 denoting most severe abnormality (Table 4). The rFHI score was calculated for each individual fish by summing all the numerical values. Additionally, photographs were taken in the field of normal and abnormal tissues and organs. A total of 565 fish (182 from developed sites, 195 from undeveloped sites, and 188 from discharge related sites) were sampled from July 24 - August 31, 2017.

Data Analysis

We have received heavy metal data, TOC, water chemistry, and PPCP data for sediment and water. We have begun compiling and analyzing the data and are currently beginning to receive PPCP data from fish tissue. We have begun statistical analysis of fish health data and will continue as we receive PPCP data for fish tissue.

Table 1. Description of sample locations and fish species collected.

Lake	Undeveloped	Developed	Discharge Related	Surface Area (acre)	Max Depth (m)	Fish Species Collected
Trout	X			64	6.1	Walleye/Yellow Perch
Binagami	X			117	6.4	Walleye
Devilfish	X			405	12.2	Walleye/Yellow Perch
Homer	X			434	6.7	Walleye/Yellow Perch
Lake Superior	X			20,288,000	406	Cisco/Lake Trout
Lake Superior	X			20,288,000	406	Cisco/Lake Trout
Ball Club	X			206	7.6	Walleye/Yellow Perch
Gull		X		168	12.2	Walleye/Yellow Perch
Caribou		X		539	6.4	Walleye/Yellow Perch
Poplar		X		764	22.3	Walleye/Yellow Perch
Vermillion		X		39272	23.2	Walleye/Yellow Perch
Lax		X		295	10.7	Walleye/Yellow Perch
Cascade Lake		X		452	5.2	Walleye/Yellow Perch
St. Louis River			X	na	na	Walleye/Yellow Perch
Shagawa			X	2345	14.6	Walleye/Yellow Perch
Lake Superior			X	20,288,000	406	Cisco/Lake Trout
Lake Superior			X	20,288,000	406	Cisco/Lake Trout
Whitewater			X	1212	22.3	Walleye/Yellow Perch
Manganika			X	170	7.3	Walleye/Black Crappie

Table 2. Number of samples collected for PPCP analysis.

Anthropogenic Impact	Waterbodies	Tissue	Water	Sediment
Undeveloped	7	13	7	7
Developed	6	12	6	6
Wastewater Effluent	6	12	6	6
Total	19	37	19	19

Table 3. Fish collection sites, categorized by anthropogenic impact and number of fish collected per species.

Site	Anthropogenic impact	Fish species	# fish
Lake Superior – Grand Portage	Undeveloped	Cisco	20
		Lake Trout	20
Lake Superior – Hovland	Undeveloped	Cisco	18
		Lake Trout	20
Ball Club	Undeveloped	Yellow Perch	20
		Walleye	17
Devilfish	Undeveloped	Yellow Perch	7
		Walleye	12
Homer	Undeveloped	Yellow Perch	20
		Walleye	10
Trout	Undeveloped	Yellow Perch	20
		Walleye	11
Caribou	Developed	Yellow Perch	20
		Walleye	6
Cascade	Developed	Yellow Perch	20
		Walleye	16
Lax	Developed	Yellow Perch	20
		Walleye	20
Poplar	Developed	Yellow Perch	20
		Walleye	20
Vermillion	Developed	Yellow Perch	20
		Walleye	20
Manganika	Discharge related	Crappie	20
		Walleye	16
Shagawa	Discharge related	Yellow Perch	20
		Walleye	20
St. Louis River	Discharge related	Yellow Perch	15
		Walleye	11
Whitewater	Discharge related	Yellow Perch	20
		Walleye	6
Lake Superior – Duluth Harbor	Discharge related	Cisco	20
		Lake Trout	9
Lake Superior – Superior Entry	Discharge related	Cisco	20
		Lake Trout	12

Table 4. Refined Fish Health Index (rFHI) adapted from Adams et al. 1993. Original field designation was used for field records. Refined fish health index values were substituted for statistical analyses.

Variable	Variable Condition	Original field designation	Substituted rFHI value
Eye	Normal; "clear" eye	N	0
	Opaque eye (one or both)	B	30
	Protruding eye (one or both)	E	30
	Hemorrhaging or bleeding in the eye (one or both)	H	30
	Missing one or both eyes	M	30
	Other; phenotype not fitting the above	OT	30
Skin	Normal; no aberrations	N	0
	Mild aberrations	1	10
	Moderate aberrations	2	20
	Severe aberrations	3	30
Pseudobranch	Normal; no aberrations	N	0
	Swollen; convex character	S	30
	Lithic; mineral deposits, white spots	L	30
	Other; condition not covered above	OT	30
Liver	Normal; solid red or light red color	A	0
	"Fatty" liver; "coffee with cream" coloration	C	30
	Nodules in liver; cysts or nodules	D	30
	Focal discoloration	E	30
	General discoloration	F	30
	Other; condition not covered above	OT	30
Fat	No fat apparent in body cavity	0	0
	Mild amount of fat	1	10
	Moderate amount of fat	2	20
	Severe amount of fat	3	30
Spleen	Normal; black, very dark red or red	N	0
	Normal; granular, rough appearance	G	0
	Nodular; containing fistulas or nodules of varying sizes	D	30
	Enlarged; noticeably enlarged	E	30
	Other; gross aberrations not covered above	OT	30
Hindgut	Normal; no inflammation	0	0
	Slight inflammation or reddening	1	10
	Moderate inflammation or reddening	2	20
	Severe inflammation or reddening	3	30
Kidney	Normal; dark red color, lying relatively flat along the length of vertebral column	N	0
	Swollen; enlarged wholly or in part	S	30
	Mottled; gray discoloration	M	30
	Granular; granular appearance and texture	G	30
	Other; aberrations not fitting previous categories	OT	30
Gastrointestinal (GI) parasites	No GI parasites grossly visible	0	0
	One or more GI parasites present	1	30
Peritoneal parasites	No observed parasites	0	0
	Few observed parasites	1	10
	Moderate parasite infestation	2	20
	Numerous parasites	3	30

Reference

Adams, S Marshall, Brown, Allen M, Goede, Ronald W. 1993. A Quantitative Health Assessment Index for Rapid Evaluation of Fish Condition in the Field. Transactions of the American Fisheries Society. 122:1, 63-73, DOI: 10.1577/1548-8659(1993)122<0063:AQHAIF>2.3.CO;2.

Activity 1 Status as of *[July 2018]***:**

Activity 1 Part 2: Analyze sampling data with UMN ecohealth researchers and state and federal toxicologists

Data analysis (Figures are located at the end of this document)

We completed entry of metadata, such as collection dates, collection location latitude and longitude, lake depth and acreage, and fish species tested. We received all the results of Pharmaceuticals and Personal Care Products (PPCP) and hormones found in water, sediment and fish tissue from SGS AXYS Laboratory. The PPCP and hormone data was provided, along with metadata and laboratory verification data, in multiple excel files for each sample type, batch, and location. The raw data was stored in 28 different excel files, and there were also multiple additional PDF and excel files that included shipping documentation, metadata, laboratory verification procedures, and analyte lists. We compiled the raw PPCP and hormone data into one excel file to make the data available for analysis. We organized the additional PDF and excel files to make them available for the team working on this project.

We combined all the data to create a database that included: pharmaceuticals and personal care products and hormones in water, fish, and sediment, fish health index results, metadata, water quality and chemistry, which included Total Organic Carbon, Hardness, Chloride, Dissolved Oxygen, pH, Conductivity, Total Dissolved Solids, Salinity, and Resistance, sediment quality, which included Dissolved Organic Carbon, and heavy metals in water, sediment, and fish livers. All data was audited and verified for accuracy to ensure no errors were made when transferring and manipulating the data. Yvette Ibrahim and a Grand Portage natural resources technician both separately compared the entire raw data to the database to look for errors. Data auditing was completed each time data was manipulated. Additionally, Grand Portage staff participated in multiple meetings internally, with MPCA staff, and with SGS AXYS Laboratory technicians and project leaders to correctly interpret and understand the PPCP and hormone data.

We have received heavy metal data, TOC, water chemistry and contaminants of emerging concern (CEC) data for sediment, water, and fish. All data have been compiled and cleaned. We have begun statistical analysis of fish health data and CEC data. From 2017 data, we screened 141 CECs in all water, sediment, and fish samples. Ninety-three different CECs (66%) were found in water samples, 57 (40%) in sediment samples, and 26 (18%) in fish samples. We identified 1 – 84 detects in water samples per site, 2 – 59 detects in sediment samples per site, and 1 – 6 detects in fish samples per site.

We combined data from 2016 and 2017 for summary analyses of detected contaminants. **Figure 1** displays the percentage of lakes with detections of CECs. With both years combined, 109 contaminants (77%) were detected in water samples. **Figure 2** displays the percentage of sites with detections of CECs in sediment samples. Seventy-one contaminants (50%) were detected in 2016 and 2017. **Figure 3** displays the percentage of sites with detections of CECs in fish samples. In both years combined, 37 contaminants (26%) were detected. Interestingly, three anti-neoplastic drugs (cancer drugs), which prevent replication of DNA, were found in fish samples. Doxorubicin was found in approximately 4% of lakes, Etoposide was found in approximately 12% of lakes, and Melphalan was found in approximately 16% of lakes.

We grouped contaminants into pharmaceutical type and found the highest detection frequencies in hormones and antidepressants, which have considerable effects on fish reproduction and behavior (**Figure 4**). When analyzing CECs in water, sediment, and fish samples combined, wastewater effluent sites have the highest number of CECs (**Figure 5**). When analyzing fish samples alone, developed sites have the highest number of CECs (**Figure 6**). Hormones were screened for in 2016 and 2017; 13 different hormones were identified in water and sediment samples (**Figure 7**).

Teaching
We have incorporated this research as a teaching tool on Ecosystem Health and Pharmaceutical Stewardship in the courses GCC 3020/5020 and CVM 6500, respectively.

Personnel Effort on Project
S. Moore has 15% time on this grant (in-kind) which is roughly equivalent to 24 hr/mo. His activity involves a) overall project management among Grand Portage, UMN, and government agency partners, b) project management and coordination between UMN team leader for UMN-led activities, c) mentoring the graduate student (Deere), d) scientific analysis and communication.
Monthly base activity (~24 hr):
Research team planning/coordination/participation = 10-20 hr/mo
Student mentoring = 2 hr/mo
Analysis and communication = 5-7 hr/mo
Additional project-related activities over past 6 months:
Scientific communication = assisted with project poster presentation and presentations. Oversight of collection, management, and analysis of data, manage project fiscal resources, participate in seasonal project planning, and communicate with Tribal leaders and University investigators.

T. Wolf has 10% time on this grant which is roughly equivalent to 16 hr/mo. Her activity involves a) overall coordination of grant activities between Grand Portage, UMN, and government agency partners, b) project management and coordination between team leaders for UMN-led activities, c) mentoring the graduate student (Deere), d) scientific analysis and communication.
Monthly base activity (~16-18 hr):
Research team planning/coordination/participation = 8-9 hr/mo
Student mentoring = 6 hr/mo
Analysis and communication = 2-3 hr/mo
Additional project-related activities over past 6 months:
Deere project seminar = 10 extra hours of graduate mentoring
Scientific communication = assisted with project poster presentation and discussion by MPCA partner at UMN 3rd International Conference on One Medicine, One Science (iCOMOS) (https://ccaps.umn.edu/documents/CPE-Conferences/iCOMOS/AbstractPosterProceedings.pdf). This is an internationally recognized meeting focusing on environmental and ecosystem health. This included time for planning and delivery (minimum 6 hr [2 hr on poster design and review, 4 hours on conference communication])
Research coordination/relationship building = spent 2 weeks in Grand Portage in May to collect, manage, and analyze data, manage project resources, participate in seasonal project planning, and communicate with Tribal leaders (25% on this grant).

D. Travis has 2% time on this grant which is roughly equivalent to 3.2 hr/mo. Most of his activity is involved with a) mentoring the graduate student (Deere), b) research team planning/participation/coordination and c) scientific analysis and communication.
Monthly base activity (~6 hr)
Graduate student mentoring = 8hr/mo minimum (50% on this grant)
research team planning/coordination/participation = 2-4 hr/mo
Quarterly activity
Deere Project Summary Seminar (this may be last quarter) - 10 extra hours of graduate mentoring
Scientific communication = this quarter coordinated hosting MPCA partner for project poster presentation and discussion at UMN 3rd International Conference on One Medicine, One Science (iCOMOS) (https://ccaps.umn.edu/documents/CPE-Conferences/iCOMOS/AbstractPosterProceedings.pdf). This is an internationally recognized meeting focusing on environmental and ecosystem health. This included time for

planning and delivery (minimum 8 hr [2 hr coordination with MPCA, 2 hr on poster design and review, 4 hours on conference communication])

N. Phelps is contributing 2% effort in-kind towards this project. Most of his activity is involved with project team meetings for planning and coordination of activities and data analysis, and reviewing scientific communication.

A. Primus has 5% time on this grant which is roughly equivalent to 8 hr/mo. Most of his activity is involved with a) mentoring the graduate student (Deere), b) research team planning/participation/coordination and c) scientific analysis and communication.
Monthly base activity (~8 hr):
Research team planning/coordination/participation = 4 hr/mo
Student mentoring = 2 hr/mo
Analysis and communication = 2-3 hr/mo
Additional project-related activities over past 6 months:
Deere project seminar assistance = 6 extra hours of graduate mentoring
Scientific communication = Presented the fish health data collected in the summer of 2017 at the 43rd Annual Easter fish health Workshop (Chattanooga, TN, April 9 – 13, 2018). Preparation of abstract and presentation took approximately 16 hours.

M. Convertino has 2% time on this grant that is roughly equivalent to 3.2 hr/mo. Most of his activity is involved with (a) mentoring a PhD student (J. Servadio), (b) research activity, and (c) research participation with other grant investigators.
Monthly base activity (~6 hr)
Graduate student mentoring = 5.5 hr/mo minimum (50% on this grant)
Research team /participation = 0.5 hr/mo
Additional project-related activities over past 6 months
Scientific communication: presented data analytical methods at different research seminars (Hokkaido University and Tokyo Institute of Technology) as well as submission of a conference proceeding about data-based network inference methods for the 2018 Complex Systems Society Annual meeting.

M. Ferrey is contributing 1% effort in-kind towards this project. Most of his activity is involved with project team meetings for planning and data analysis, synthesis of findings, and reviewing scientific communications.

M. Jankowski is contributing 1% effort in-kind towards this project. Most of his activity is involved with project team meetings for planning and data analysis, synthesis of findings, and reviewing scientific communications.

Y. Ibrahim has 25% time on this grant which is roughly equivalent to 42-44 hr/mo. Her activity involves a) coordinating and planning data collection, b) collecting, processing, storing, and shipping data, c) coordinating agencies to complete field collections, and d) compiling data into a database and ensuring data completeness and integrity.
Monthly base activity (~42-44 hr/mo):
Research team planning/coordination/participation = 8 hr/mo
Data entry/analysis/auditing/interpretation = 30 hr/mo
Field collection/planning/coordinating = 4-6 hr/mo

J. Deere has 50% time on this grant which is roughly equivalent to 80 hr/mo. Her activity involves a) coordinating and planning data analysis of chemical and fish health data (see written description of summary analysis and figures), b) evaluating chemical constituents and literature review, and c) compiling database for ordination analysis, d) CEC research and fish health analysis appropriate for doctoral degree.
Monthly base activity (~80 hr/mo):
Research team planning/coordination/participation = 8 hr/mo

Data analysis/auditing/interpretation = 60 hr/mo
Field collection/planning/coordinating = 4-6 hr/mo

Activity 1 Status as of *[January 2019]***:**
Activity 1. Part 1: 18 sites sampled for fish, water, and sediment
Water Collection
Two water sample collections in 2017 were compromised during deployment. The POCIS membranes deployed in Lake Superior at the Duluth and Superior harbor locations were destroyed due to high sediment and water flow. Therefore, we collected water samples at these locations in 2018. We deployed three POCIS disks at each of the Duluth and Superior harbor locations in slightly different areas that would receive less water flow. The POCIS disks were deployed in the same manner as described above for the 2017 collections and were deployed from July 17 to August 30, 2018. The POCIS disks were sent to SGS AXYS Analytical (Sydney, British Columbia, Canada) for analysis.

Activity 1 Part 2: Analyze sampling data with UMN ecohealth researchers and state and federal toxicologists
We received the results of PPCP and hormones found in water collected at the two Lake Superior harbor locations from SGS AXYS Laboratory. The PPCP and hormone data was provided, along with metadata and laboratory verification data, in multiple excel files for each batch. We compiled the raw PPCP and hormone data into one excel file and added it to the database to make the data available for analysis. We organized the additional PDF and excel files to make them available for the team working on this project. All data was audited and verified for accuracy to ensure no errors were made when transferring and manipulating the data. Yvette Ibrahim and a Grand Portage natural resources technician both separately compared the entire raw data to the database to look for errors. Data auditing was completed each time data was manipulated.

Grand Portage staff, UMN Ecohealth researchers, and federal toxicologists participated in bi-weekly meetings to discuss data analysis and interpret findings. All researchers participated in an annual meeting in Grand Portage on August 6, 2018 where members of this workgroup gave presentations on experimental design, methods, summary analysis, analysis of total PPCP detections by media, anthropogenic impact, and pharmaceutical type, fish health analysis, landscape analysis, and ecological concerns based on laboratory research of PPCPs and hormones. The workgroup decided to increase the annual meetings to quarterly. The workgroup met at the University of Minnesota on December 13, 2019 for a quarterly meeting and reviewed project deliverables and timelines, discussed current data analyses, including summary statistics, fish health, and analyzing non-detect data, reviewed current literature, and discussed interpretation of data to ensure understanding among all project partners and that the group is following or exceeding industry standards.

Activity 1 Status as of *[July 2019]***:**
Activity 1 is complete.
Activity 1 Status as of *[January 2020]***:**
Fish were collected from six waterbodies, five of which were previously sampled in this study, to provide additional pathology analyses. Fish were evaluated using the Fish Health Assessment Indices (rFHI) and are also being analyzed for additional histopathological indices and PPCP contaminants. Staff from Grand Portage, 1854 Treaty Authority, and University of Minnesota collected yellow perch from Poplar, Trout, Elbow, Cascade, Whitewater, and Shagawa Lakes (Figure 1) in summer 2019. The lakes were chosen based on anthropogenic impact, with two lakes selected from each category: undeveloped, developed, and discharge related (Table 1). Elbow Lake was a newly chosen site and is considered an undeveloped location.

Fish were processed by Grand Portage staff using the same methods detailed above. Fish tissue has been sent to AXYS Analytical for PPCP analysis. Once we receive data from the lab, data will be processed using the same data validation methods used previously.

Activity 1 Final Report Summary *[August 2020]***:**

ACTIVITY 2: Interpret findings with University of Minnesota ($68,277).

Description:

Statistical analysis will be used to test research hypotheses using conventional means of data analysis. In addition, for spatial hypotheses, contaminant data will be uploaded into GIS software for analyses of spatial distribution. Contaminant distribution maps will be overlaid with data layers containing point sources of environmental contaminants (e.g. mining activities, wastewater effluents), population centers, habitat /development, tribal use (e.g. characterization of water bodies based on level of harvest for subsistence or commercial purposes), and environmental pathways of river basins (channels and hillslope pathways). These new maps will be used to estimate the potential contribution of point and non-point sources to contaminant levels across the environment and identify areas of risk where human consumption of contaminated subsistence species warrants further investigation. Dynamical network models will establish the interdependencies of factors and geographical areas of the system investigated. These data will be used in future eco-hydrological models to describe how contaminants move through the Lake Superior watershed and up into the food web of the ecosystem.

Summary Budget Information for Activity 2:

ENRTF Budget: $ 83,511
Amount Spent: $ 76,851
Balance: $ 6,600

Outcome	Completion Date
1. *Analyze data and develop spatial maps of results*	January 2019
2. *Disseminate findings to MPCA*	March 2019
3. *Evaluate pathways to consumption among GPIR members and plan future strategies*	January 2020

Activity 2 Status as of *[January 2018]***:**

This activity has not yet begun.

Activity 2 Status as of *[July 2018]***:**

Pilot analysis of detection data has begun and has been presented in a few forums identified in the dissemination section of the report.

Activity 2 Status as of *[January 2019]***:**

Data analysis:

Water, sediment, and fish were sampled in 2016 and 2017 for pharmaceuticals, personal care products, alkylphenols, hormones, and other contaminants. In water, a total of 98 contaminants were detected at least once, including several antidepressants and antibiotics, sedatives, drugs to lower cholesterol and high blood pressure, medicine to treat diabetes, anticancer drugs, analgesics, the disinfectants triclosan and triclocarban, and several other pharmaceuticals.

Many of these were also found in corresponding samples of lake sediment and fish. Of the 34 pharmaceuticals detected in fish, DEET, the antidepressant amitriptyline, and the x-ray contrast agent iopamidol were detected the most frequently, in 81%, 61%, and 58% of samples of game fish, respectively. Other pharmaceuticals found in fish included antibiotics, antidepressants, and drugs used in the treatment of diabetes and cancer.

Notably, contaminants were detected at remote locations as well as in lakes with shoreline development. The results of this study are similar to those of previous investigations of surface water conducted on a statewide

basis, suggesting that these contaminants are remarkably widespread in the environment at low concentration. This study, the first in Minnesota to concurrently sample water, sediment, and fish, demonstrates that contaminants such as pharmaceuticals in surface water and sediment are also present in fish.

In collaboration with Minnesota Pollution Control Agency (MPCA), Aquatic Toxicity Profiles (ATPs) were created for the contaminants detected in water samples. Aquatic Toxicity Profiles (Streets & Dobbins, 2017) were developed to characterize the potential for contaminants detected in Minnesota's environment to cause adverse effects on aquatic life. A preliminary ATP report provided by MPCA resulted in six high priority contaminants: Carbamazepine, DEET, Estrone, Gemfibrozil, Mestranol, and Progesterone. We will prioritize these contaminants (and potentially others based on other information) in future analyses. ATPs are currently being created for fish and sediment samples which will provide more information on which contaminants might be of highest concern in our study locations.

A modeling framework has been developed to measure risk factors for elevated levels of CECs in water. Using precipitation, population density of the nearest municipality, and watersheds in Minnesota, the models are designed to identify characteristics of locations with greater chemical levels and generate hypotheses regarding movement across bodies of water. Preliminary results show that some, but not all, CEC levels relate to one or more of these factors. The discharge categories were strong predictors of CEC levels, with discharge related sites having the greatest levels. Having a high number of non-detect data presents a challenge in modeling. Many methods for modeling with non-detect data can lead to inaccurate results due to imposed assumptions. Non-detect data will be handled using Monte Carlo samples for multiple imputations, and this method will be compared to other common methods for handling non-detect data to show the potential benefits of this method.

Personnel Effort on Project

S. Moore has 15% time on this grant (in-kind) which is roughly equivalent to 24 hr/mo. His activity involves a) overall project management among Grand Portage, UMN, and government agency partners, b) project management and coordination between UMN team leader for UMN-led activities, c) mentoring the graduate student (Deere), d) scientific analysis and communication.
Monthly base activity (~24 hr):
Research team planning/coordination/participation = 10-20 hr/mo
Student mentoring = 2 hr/mo
Analysis and communication = 5-7 hr/mo
Additional project-related activities over past 6 months:
Scientific communication: Provided invited presentation for Partners in Action in July and International Association of Great Lakes Research October. Conducted oversight of collection, management, and analysis of data, manage project fiscal resources, participate in seasonal project planning, and communicate with Tribal leaders and University investigators and LCCMR staff and leadership as needed.

T. Wolf has 10% time on this grant which is roughly equivalent to 16 hr/mo. Her activity involves a) overall coordination of grant activities between Grand Portage, UMN, and government agency partners, b) project management and coordination between team leaders for UMN-led activities, c) mentoring the graduate student (Deere), d) scientific analysis and communication.
Monthly base activity (~16-18 hr):
Research team planning/coordination/participation = 8-9 hr/mo
Student mentoring = 6 hr/mo
Analysis and communication = 2-3 hr/mo
Additional project-related activities over past 6 months:
Scientific communication = assisted with project poster presentation development for UMN College of Veterinary Medicine Points of Pride Research Day, October 9, 2018. This included time for abstract and poster editing. (minimum 3-4 hr)

Deere project presentation at International Symposium on Veterinary Epidemiology and Economics, Chiang Mai, Thailand. This is an internationally recognized meeting focusing on epidemiology and the economics of veterinary and ecosystem health. (10 extra hours of graduate mentoring)
Research coordination/relationship building = included 2 trips to Grand Portage in August and September and planning/hosting 1 full-day meeting at UMN in December to manage and analyze data, manage project resources, participate in seasonal project planning, and communicate with Tribal leaders.

D. Travis has 2% time on this grant which is roughly equivalent to 3.2 hr/mo. Most of his activity is involved with a) mentoring the graduate student (Deere), b) research team planning/participation/coordination and c) scientific analysis and communication.
Monthly base activity (~6 hr)

Graduate student mentoring = 8hr/mo minimum (50% on this grant)
research team planning/coordination/participation = 2-4 hr/mo

Quarterly activity

Data analysis meetings (beyond above) = ~10 hour
Deere Project Summary for presentation at international conference (ISVEE) = ~5 hour
Primus/Deere analytical design for further validation of fish health monitoring tool = ~10 hour

A. Primus has 5% time on this grant which is roughly equivalent to 8 hr/mo. Most of his activity is involved with a) mentoring the graduate student (Deere), b) research team planning/participation/coordination and c) scientific analysis and communication.
Monthly base activity (~8 hr):
Research team planning/coordination/participation = 4 hr/mo
Student mentoring = 2 hr/mo
Analysis and communication = 2-3 hr/mo
Additional project-related activities over past 6 months:
Scientific communication = Presented the fish health data collected in the summer of 2017 and some of the basic contaminant data at the 8th International Symposium on Aquatic Animal Health (Charlottetown, PEI, Canada, September 2-6, 2018). Preparation of abstract and presentation took approximately 16 hours. Title: “Initial Findings from an Aquatic Ecosystem Health Study Site”.

N. Phelps is contributing 2% effort in-kind towards this project. Most of his activity is involved with project team meetings for planning and coordination of activities and data analysis, and reviewing scientific communication.

M. Ferrey is contributing 1% effort in-kind towards this project. Most of his activity is involved with project team meetings for planning and data analysis, synthesis of findings, and reviewing scientific communications. Mark has led the summary data analysis for Activity 2.

M. Jankowski is contributing 1% effort in-kind towards this project. Most of his activity is involved with project team meetings for planning and data analysis, synthesis of findings, and reviewing scientific communications. Mark is leading work with EnviroAtlas

Y. Ibrahim has 25% time on this grant which is roughly equivalent to 42-44 hr/mo. Her activity involves a) coordinating and planning data collection, b) collecting, processing, storing, and shipping data, c) coordinating agencies to complete field collections, and d) compiling data into a database and ensuring data completeness and integrity.
Monthly base activity (~42-44 hr/mo):
Research team planning/coordination/participation = 8 hr/mo
Data entry/analysis/auditing/interpretation = 30 hr/mo
Field collection/planning/coordinating = 4-6 hr/mo

J. Deere has 50% time on this grant which is roughly equivalent to 80 hr/mo. Her activity involves a) coordinating and planning data analysis of chemical and fish health data (see written description of summary analysis and figures), b) evaluating chemical constituents and literature review, and c) compiling database for ordination analysis, d) CEC research and fish health analysis appropriate for doctoral degree.
Monthly base activity (~80 hr/mo):
Research team planning/coordination/participation = 8 hr/mo
Data analysis/auditing/interpretation = 60 hr/mo
Field collection/planning/coordinating = 4-6 hr/mo

J. Serviado has 6% time on this grant, which is roughly equivalent to 11 hours per month. He is a post-doctoral associate and his activity involves much of the spatial and hydrological modeling under activity two.

Activity 2 Status as of *[July 2019]***:**
Land use characteristics data analysis
In order to determine if any anthropogenic activity can be linked to contamination in the area, data were collected through the University of Minnesota USpatial office. USpatial provides services to the UMN community relating to spatial data and analyses. The staff at the office were able to collect various data for the state of Minnesota relating to human land use. Data were collected for various factors, and the following were suitable for further analyses.

1. Catchment units
Proximity to bodies of water can impact the abilities of human actions to contaminate bodies of water. While simple proximity is an important factor, the flow of water is also important. To account for this, water catchments were identified in Minnesota at three spatial resolutions. The smallest units were selected, and water catchment units including the bodies of water tested as well as upstream catchment units connected by water were included to create a water catchment area for each site. If more than four catchment units needed to be used to create the catchment area, then a coarser unit was used. This led to the creation of land areas of varying sizes where human activity could potentially lead to contamination in the tested bodies of water.

In addition to the catchment areas, 100 meter buffers were created around each body of water. This was done to represent activities that occur in very close proximity or in direct contact with the bodies of water. Both of these areas were created for analyses.

2. Impervious land
Impervious land measures the amount of land area, as a percentage, that is impenetrable. Water is expected not to be able to travel through pieces of land designated as impervious. Much of the impervious land identified around the study sites represent roadways and other paved surfaces. Data were presented in fine spatial grids, and grid units with more than 50% impervious surface were identified as impervious. The percentages of land within each catchment and within a 100 meter buffer around each body of water were then calculated.

3. Businesses
Precise locations and types of businesses were available. The businesses were categories based on SIC codes to specify the industry. These locations were matched to the catchment areas and buffers to determine the businesses associated with the sample sites. Most of the businesses identified were agricultural, food production, manufacturing, or healthcare related.

4. Wastewater treatment
The locations and output volume of wastewater treatment sites were identified and then matched to the catchment areas and buffers. The volume of flow was also available to measure the relative output of the different treatment facilities.

5. Building footprints
Locations of all buildings were identified and matched to latitude/longitude coordinates. These locations were merged with the catchment areas and lake buffers to determine the numbers of buildings within each. The buildings present include both residential and commercial spaces, and also include those that are not permanently occupied.

6. Population
Population per census block in Minnesota were provided. In order to conform to the catchment areas and 100 meter buffers, the census blocks were modified to estimate the populations. The proportion of land area of each census block within the catchment area was measured, and then the same proportion of the population was assumed to live within the catchment unit or buffer. This allows both the estimated total population as well as the estimated population density to be considered for analyses.

Planned analyses
Variable selection for subsequent statistical analyses will be guided through Redundancy analysis (RDA). The land use data contain variables that are likely to be highly correlated to each other, making the use of all variables inappropriate. Following the RDA, a variable set can be created that will reduce risk of multicollinearity while still providing contextual benefits.

Quality Assurance
We held meetings with a chemist from AXYS Laboratories to determine whether the concentrations of PPCPs and hormones found in water, fish, and sediment samples were valid results. The data was examined by analyzing individual data runs and looked for false positive results in all data collected. We developed quantification limits, as described below, to ensure data accuracy.

Fish samples
Concentrations of chemicals in fish tissue were measured among fish collected at each site. The samples were analyzed by AXYS laboratories, providing detection limits. Quantification limits, being defined as three times the detection limit, are required for values to be accepted as true concentrations.

Statistical models are being developed in order to determine if land use activities relate to differing levels of fish contamination. Additional factors that may be considered in modeling include repeated samples among years at the same site and proximity to other water sources.

Water samples
Water samples were analyzed by AXYS laboratories. The measured POCIS concentration is provided, if detected, as well as associated detection limits. Among detected data, only values above the quantification limit, defined as three times the detection limit, are accepted as true values.

Analyses will be conducted to determine both presence of detection as well as amounts detected. The former will use whether a chemical was detected as a binary outcome, and the latter will use the amounts found. In these analyses, measures will be taken to account for detection and quantification limits such as tobit regression of multiple imputations.

Preliminary summary results
Prior to beginning RDA, correlation matrices were created using Pearson correlation coefficients to identify pairs of variables that would likely be correlated. This was done for both untransformed data and for squared values to investigate nonlinear relationships. For both, as hypothesized, numerous pairs of variables were highly correlated to each other, and therefore would not be suitable to both be present in a statistical model.

Visiting faculty
Joanna Borucinska, a faculty member from the University of Hartford in Connecticut, was brought to the University of Minnesota through an Institute on the Environment grant based on this project. Dr. Borucinska visited between July 15 and July 19 to provide expertise in fish pathology and laboratory methods. During her visit, she was updated on the scope and aims of the project and described her experiences and expertise. She also assisted in the laboratory analysis of liver samples for contamination. Additionally, Dr. Borucinska presented on her work in pathology to the public through the University of Minnesota College of Veterinary Medicine on July 18, 2019.

Activity 2 Status as of *[January 2020]***:**
Our first manuscript was submitted and is under review at Science of the Total Environment. The editor sent comments from six reviewers so we are currently revising the manuscript and will resubmit no later than March 5, 2020. Our manuscript (*Characterization of contaminants of emerging concern in aquatic ecosystems utilized by Minnesota tribal communities)* reports the chemicals we detected from 28 lakes over three years (2016-2018). 116 of the 158 chemicals were detected at least once across all media. Up to 83 chemicals were detected at locations impacted by wastewater, while 17 chemicals were detected in remote lake water that lacked any source of contamination **(Figure 8)**. No significant difference was found in the number of contaminants detected in lakes with developed land use and lakes without direct anthropogenic impact. Twenty-two chemicals were detected from developed and undeveloped sites that were not detected in surface water from wastewater impacted sites. A total of 101 CECs were detected in water (POCIS), 67 in sediment, and 35 in fish tissue **(Figure 9)**. The following CECs were detected in all media: 1) 10-hydroxy-amitriptyline, 2) Amitriptyline, 3) Azithromycin, 4) Caffeine, 5) Citalopram, 6) Cocaine, 7) DEET, 8) Diphenhydramine, 9) Enrofloxacin, 10) Fluoxetine, 11) Metformin, 12) Miconazole, 13) Sertraline, 14) Venlafaxine, and 15) Verapamil. We grouped all CECs into 23 different primary use categories. Contaminant classes were updated from the previous categorization **(Figure 4)** to primary use categories **(Figure 10)** based on further literature review and guidance from toxicologists on the team. We plan to follow this submission with a manuscript more thoroughly discussing the implications of our findings and how we are prioritizing contaminants based on Aquatic Toxicity Profiles (ATPs).

ATPs have been completed based on the methods created by Minnesota Pollution Control Agency and manuscript preparation has begun. Based on partial ATPs on water, sediment, and fish, the highest priority contaminants include the following: DEET, clotrimazole, estrone, progesterone, carbamazepine, gemfibrozil, and mestranol. These priority contaminants are the primary response variables being used in regression analyses. So far, the maximum number of predictors we have investigated that do not have multicollinearity (correlation) issues are human population size, number of wastewater facilities, and number of residential nursing homes in the catchment unit. There is preliminary evidence that human activity is associated with water contamination. For example, detection of gemfibrozil was more likely in bodies of water with higher numbers of wastewater treatment facilities and residential nursing homes in the catchment area. However, models will be expanded to account for repeated measures at selected sites. More predictor variables are also being explored using the gradient analysis method appropriate for our data, redundancy analysis (RDA). For RDA, contaminant concentration are the response variables and various spatial data are the explanatory variables. To date, no clear patterns have been discerned through RDA, but this investigation is ongoing. In addition to exploring RDA with individual contaminants, we will group contaminants by primary use categories to explore whether observed patterns in chemicals (stratified by primary use category) are related to environmental variables.

Dr. Borucinska has created histopathological indices on fish tissues collected from 6 of the study locations. Dr. Borucinska will stain lesions of interest to further explore etiology. The histopathological indices will be compared to the refined Fish Health Assessment Indices (rFHI) that were conducted during the same sampling period. We will assess the rFHI using common measures of validity, sensitivity and specificity, using the histopathological indices as the gold standard. We are also awaiting CEC data from AXYS from fish collected during this same sampling period so we can statistically analyze fish health and contamination.

Grand Portage staff, UMN Ecohealth researchers, and federal toxicologists continued to participate in bi-weekly meetings to discuss data analysis and interpret findings. Additionally, these meetings are used to discuss manuscript preparation.

Activity 2 Final Report Summary *[August 2020]***:**

V. DISSEMINATION:

Description:

The core intent of this project is to provide science-based information for assessing and managing risks associated with sustainable cultural use of natural resources and interaction with the environment in and around the grand Portage Tribal area. Our goal is also to communicate our findings in both the policy and scientific arenas. In order to reach this broad range of stakeholders, we anticipate the following public and scientific communications.

1) Tribal and regional audience:
- local public radio program will provide updates on study results
- results and recommendations will be made to Tribal Council, including potential warnings and advisories for risk management and food safety

2) Minnesota Pollution Control Agency:
- this project fills a data gap in MPCA maps and will allow for a more complete picture of MN. Thus, inclusion of this area in MPCA communication pieces will be carried forward to the general public and interested stakeholders.

3) LCCMR policy-based audience
- the final report should be useful in several ways. First, maps with baseline threat data will add science to general policy discussions. Second, this project will help prioritize long terms monitoring strategies that could be useful to several State Agencies (DNR, DPH, MPCA specifically). We believe findings will also inform LCCMR review community regarding the potential priority for this issue in the future. That is, GPIR could be considered one of the least "at risk" places from a land use standpoint in the State. If we verify considerable contamination here, as we believe we will, this project may provide stimulus for further investigation across the State AND help validate new scientific tools that can assist in this endeavor.

4) Scientific Audience
- a minimum of two peer-reviewed papers, one for each objective, will be submitted to peer reviewed journals. These publications will communicate scientific results, as well as sampling methods, diagnostic methodologies and system-based analyses.

5) UMN Education/Extension programs
-Dr. Phelps is the director of the UMN aquatic invasive spp. Center, and Dr. Primus the aquatic animal health lead for the UMN CVM. As such, both play a large extension role in the State of MN. They will incorporate important parts of these findings in extension products for the State.
-Drs. Convertino, Wolf and Travis have overlapping appointments in the UMN School of Public Health and Veterinary Medicine (ecosystem health). They teach courses to undergraduate, graduate and professional students, as well as in the Grand Challenge Curriculum. These findings will be incorporated into already developed case studies in all of the above.

This project is unique in that it will focus on system-wide exposures across a variety of aquatic systems and subsistence fish species in the Lake Superior watershed. Data collected will be analyzed for presence/absence of

both traditional/known hazards, as well as CECs across a large landscape, allowing for prioritization of in depth studies on food safety and security. Specific outputs in the final report will include:

Spatial distribution of CECs and metal occurrence data from 27 locations within Northeastern MN and the Lake Superior watershed. This data will be derived from surface water samples, pooled fish samples of species-specific samples from important subsistence species for local populations: walleye/yellow perch and lake trout/cisco (the number of species-specific samples may be limited by availability at each site). We will also relate measures of fish health and parasite loading to CEC's and landuse. These data may later be linked to a water flow model for this watershed system.

Quantitative data of contaminants present in fish and water samples from each location. Depending upon the results gathered, these data will potentially allow us to identify current chemical risks to biological systems and/or public health. Regardless of our findings, however, these data will allow us to start developing hypotheses about pathway-based biological effects (e.g. neuroendocrine, cell-cycle specific, etc.) of contaminants found at each location, as well as potential adverse outcomes.

Detection of potential contaminant pathways (sources and transport networks) and attribution of their variability to human and environmental dynamics

By evaluating contaminants across a gradient of human development, ranging from known point sources such as wastewater treatment plant effluent streams to remote wilderness, we will be able to detect how emerging pollutants may be transferred across the landscape and through biota. Spatial mapping of pollutants and biota will be used to determine likely pathways through which CEC's and metals move through the environment. Once known, specific management actions can be developed to reduce the spread of contaminants in the environment through these pathways.

Identification of at-risk areas and/or populations

Results from our analyses will identify sites or water bodies that are at risk for biological impacts on aquatic species or contaminant exposure to humans via consumption of subsistence species based on chemical detections. Such sites will form the basis of future monitoring research and be the focus of future management plans.

Dissemination Status as of *[January 2018]***:**

Video on Pharmaceuticals in the environment was developed and posted on YouTube
https://www.youtube.com/watch?v=FIxVQU2gSBA&list=PLnC4yTjRBxQeDnfIW1Oxd2BlHnBKc4_bY&index=5

Deere, J. The use of a refined Fish Health Assessment Index to evaluate fish health in Northeastern Minnesota. Presentation at the College of Veterinary Medicine Graduate Student Seminar. Minneapolis, MN. January 25, 2018.

Abstracts

Session: One Health - science communication, ethics, policy, evaluation and education, International Conference on One Medicine One Science. Minneapolis, MN. April 29 – May 2, 2018.

Title: Mitigating the Risk of Emerging Contaminants through Surveillance and Education

M Ferrey, Minnesota Pollution Control Agency, USA; T Wolf, University of Minnesota, USA; S Moore, Grand Portage Band of Lake Superior Chippewa, USA; D Travis, University of Minnesota, USA; A Primus, University of Minnesota, USA; N Phelps, University of Minnesota, USA; M Jankowski, U.S. EPA, USA.

The presence of pharmaceuticals and other commercial and industrial chemicals in the aquatic environment is well documented by hundreds of reconnaissance studies over the past two decades. These contaminants are surprisingly widespread in surface water, often appearing in lake water without an apparent source. Many of these contaminants are toxic or endocrine active, adversely affecting fish and wildlife at part per trillion concentrations. In addition, trace levels of antibiotics in surface water are suspected of promoting bacterial antibiotic resistance. In the absence of environmental regulatory standards for these chemicals, the challenge is

to combine the measurements of contaminants in the environment with an understanding of their effects on ecosystems and with efforts aimed at preventing their release to the environment. Predicated on the earlier monitoring results of chemicals of emerging concern in surface water by government agencies, we are analyzing these chemicals in surface water, sediment, and fish tissue on the Grand Portage Indian Reservation and surrounding region, with the goal of understanding whether these chemicals are exerting adverse effects on fish species and, subsequently, on the traditional subsistence hunting and fishing that is of cultural importance to the Grand Portage Band of Chippewa. Initial study reveals that several of these contaminants are present in samples of water, sediment, and fish tissue, including the x-ray contrast agent iopamidol, the antidepressants sertraline and amitriptyline, triclocarban, and naproxen. Recognizing the importance of preventing the release of pharmaceuticals to surface water, the Univ. of Minnesota College of Veterinary Medicine and the Minnesota Pollution Control Agency have formed a unique partnership to promote research, education, and communication on the ecological risks posed by pharmaceuticals and the importance of pharmaceutical stewardship to prevent their unintended release to the environment.

SETAC North America 38th Annual Meeting
Session: One Health Nov. 14, 2017
Title: Evaluation of Anthropogenic Micropollutants in Waters and Subsistence Species Used by the Minnesota Chippewa
S. Moore, Grand Portage Band of Lake Superior Chippewa / Biology and Environment; T. Wolf, University of Minnesota / College of Veterinary Medicine; M.L. Ferrey, Minnesota Pollution Control Agency / Environmental Outcomes; M.D. Jankowski, USEPA / Office of Environmental Review and Assessment, Region 10; M. Convertino, University of Minnesota / School of Public Health; N. Phelps, University of Minnesota / Minnesota Aquatic Invasive Species Research Center; A. Primus, D. Travis, University of Minnesota / College of Veterinary Medicine.
Although the One Health Paradigm - a holistic, collaborative, systemsbased approach to health research and policy - currently enjoys a wave of energy and funding, most of the emphasis to date has been on zoonotic and emerging infectious diseases. Models that emphasize chronic diseases and ecotoxicology are needed. Through a unique collaboration between the Minnesota Chippewa Tribe, Grand Portage Indian Reservation, University of Minnesota's College of Veterinary Medicine's Ecosystem Health Division and School of Public Health, Minnesota Pollution Control Agency, and the US Environmental Protection Agency, we are assessing threats by chemical pollutants to the sustainable use of tribal natural resources within key lacustrine habitats and fish species used by the Minnesota Chippewa, possibly threatening the very culture of the Minnesota tribes using these resources. This project is unique in that it is focused on system-wide exposures and responses across a variety of aquatic systems and subsistence fish species in the Lake Superior watershed, an understudied location. Initial efforts have focused on monitoring the presence of over 150 heavy metals, endocrine active chemicals, pharmaceuticals, and other commercially available chemicals within the tissues of subsistence fish species, waters, and sediment. In 2016, at least one chemical was detected in all water, sediment, and fish samples from fourteen locations, with a range of 3-10 chemicals found in fish, 1-30 chemicals found in sediment, and 3-64 chemicals found in water. Chemicals were detected at ng/l levels. one site, 64 chemicals were detected in water, 30 in sediment, and 7 in fish. Some chemicals detected in fish were detected across a large percentage of sites; betamethasone, fluticasone propionate, amitriptyline, and DEET were detected in fish from more than 60% of the sites. The results of this work will assist in guiding land and chemical management practices and promote a sustainable food and ecosystem within the Grand Portage Indian Reservation.

Dissemination Status as of *[July 2018]***:**
Presentations:
2018. Moore S.A., Wolf T.M., Ferrey, M., Jankowski, M. Convertino, M., Phelps, N., Primus, A.,Travis, D. Chemicals of emerging concern in waters, sediments, and subsistence fish used by the Grand Portage Band of Chippewa. Bureau of Indian Affairs Partners in Action. Milwaukee, WI. July.

Moore, S., Wolf, T., Ferrey, M., Jankowski, M., Deere, J., Ibrahim, Y., Convertino, M., Phelps, N., Primus, A., Travis, D. Chemicals of emerging concern in waters, sediments, and subsistence fish used by the Grand Portage Band of Chippewa. Invited presentation. Lake Superior Workgroup, Beaver Bay, MN. June 14, 2018.

Ferrey, M., Wolf, T., Moore, S., Travis, D., Primus, A., Phelps, N., Jankowski, M., Convertino. Mitigating the Risk of Emerging Contaminants through Surveillance and Education. Poster presentation at the International Conference on One Medicine One Science. Minneapolis, MN. April 29 – May 2, 2018.

Primus, A. A Case Study in the Use of Select Fish Health Indices as a Means of Evaluating Ecosystem Health. Presentation at the 43rd Annual Easter fish health Workshop. Chattanooga, TN. April 9 – 13, 2018.

Dissemination Status as of *[January 2019]***:**

Presentations:

Deere, J., Primus, A., Moore, S., Neher, A., Knoll, L., Convertino, M., Phelps, N., Jankowski, M., Ferrey, M., Travis, D., Wolf, T. The use of a refined Fish Health Assessment Index to evaluate contaminants of emerging concern on fish health in northeastern Minnesota, USA. Oral presentation at the 15th International Symposium of Veterinary Epidemiology and Economics. Chiang Mai, Thailand. November 12 – 16, 2018.

Abstract

Water is arguably the most essential natural resource in the world; yet, freshwater ecosystems are threatened by many human activities. Contaminants of emerging concern (CECs), such as pharmaceuticals and personal care products, are increasingly detected in the environment, but the distribution of such contaminants in northeastern Minnesota is currently unknown. Consequently, we aimed to evaluate the health of subsistence fish species harvested by an indigenous community, at sites with detected CECs and different anthropogenic impact, using a refined Fish Health Assessment Index (rFHI) and ectoparasite prevalence.

Materials and methods: We collected 545 fish from 17 sites – categorized as developed, undeveloped, and wastewater effluent according to a multi-criteria selection process – in the Lake Superior watershed. The rFHI contains numerical scores from 10 fish health variables that were summed to provide a total score for each fish examined. A higher score may be associated with lower health. We identified ectoparasites by examining gill and skin wet-mount preparations via microscopy.

Results: For Lake Superior, fish captured at wastewater effluent sites (rFHI mean = 91.19) had higher scores than fish at undeveloped sites (rFHI mean = 59.04), whereas fish at inland sites did not follow a consistent pattern. Overall, Lake Superior fish (rFHI mean = 72.57) had higher rFHI scores than fish at inland sites (rFHI mean = 42.78). Liver, fat, and internal parasitism scores contributed most to the total rFHI score. There were fewer ectoparasites in fish from Lake Superior sites than inland sites, which could be related to capture method or to the conditions in Lake Superior.

Conclusions: Given the observed differences in rFHI scores and parasite prevalence between inland and Lake Superior sites, a unique set of rFHI and parasite metrics need to be employed for different sites. Metrics from this study will be further evaluated in association with CEC levels across these human-impacted sites.

2018. Moore S.A., Wolf T.M., Ferrey, M., Jankowski, M. Convertino, M., Phelps, N., Primus, A., Travis, D. Chemicals of emerging concern in waters, sediments, and subsistence fish used by the Grand Portage Band of Chippewa. International Association of Great Lakes Research, State of Lake Superior Conference. Houghton, MI. October.

Abstract

The Grand Portage Indian Reservation and partners are assessing threats by chemical pollutants to the sustainable use of tribal natural resources within lacustrine habitats and fish species used by the Minnesota Chippewa, threatening the culture of the Minnesota tribes. Many pharmaceutical chemicals are toxic or

endocrine active, adversely affecting fish and wildlife at part per trillion concentrations. We surveyed for presence of heavy metals, endocrine active chemicals, pharmaceuticals, and synthetic hormones within subsistence fish species, waters, and sediment.
We sampled 28 sites for water, sediment and fish tissue and screened for 141 CECs and 17 hormones. At least one chemical was detected in all water, sediment, and fish samples from all locations, with a range of 1-10 chemicals found in fish tissue samples per site, 1-59 chemicals found in sediment samples per site, and 1-84 chemicals found in water samples per site. In water samples, 109 CECs and hormones were found. In sediments, 71 CECs and hormones were found. In fish, 37 CECs were found. Some chemicals were detected across a large percentage of sites and sample types. Our results will guide land and chemical management practices and promote a sustainable food and ecosystem within the Grand Portage Indian Reservation.

Dissemination Status as of *[July 2019]***:**
Presentations:
2019. Moore S.A., Wolf T.M., Ferrey, M., Jankowski, M. Convertino, M., Phelps, N., Primus, A.,Travis, D. Chemicals of emerging concern in waters, sediments, and subsistence fish used by the Grand Portage Band of Chippewa. Minnesota Contaminants Roundtable. June 25, 2019, Mounds View,MN.

https://www.youtube.com/watch?v=mHgCitsaPCA&list=PLnC4yTjRBxQeDnfIW1Oxd2BlHnBKc4_bY&index=2&t=13s

Dissemination Status as of *[January 2020]***:**
Presentations
Alex Primus and Jessica Deere presented a talk titled “Fish health and environmental contaminants: A case study from Minnesota lakes” to the University of Oslo Biology Department on August 28, 2019.

Jessica Deere presented a talk titled “Characterization of contaminants of emerging concern in aquatic ecosystems utilized by Minnesota tribal communities” to the UMN Veterinary Public Health Residents during their rounds on December 6, 2019.

Yvette Ibrahim presented a talk titled “Chemicals of emerging concern in waters, sediments, and subsistence fish used by the Grand Portage Band of Chippewa” to the Minnesota Sate Park’s Statewide Naturalist Meeting in Grand Portage, MN on October 23, 2019.

Publications
Deere, J.R., Moore, S., Ferrey, M., Jankowski, M.D., Primus, A., Convertino, M., Servadio, J.L., Phelps, N.B.D., Hamilton, M.C., Chenaux-Ibrahim, Y., Travis, D.A., Wolf, T.M. Characterization of contaminants of emerging concern in aquatic ecosystems utilized by Minnesota tribal communities. *Science of the Total Environment. Under Review.*

Awards
Jessica Deere was awarded a UMII MnDRIVE Graduate Assistantship to the amount of $35,380 for a project titled “Assessing the impact of contaminants of emerging concern on subsistence fish species” to continue her work on this project for her dissertation.

Dissemination Final Report Summary *[August 2020]***:**

VI. PROJECT BUDGET SUMMARY:
A. Preliminary ENRTF Budget Overview:
***This section represents an overview of the preliminary budget at the start of the project. It will be reconciled with actual expenditures at the time of the final report.**

Budget Category	$ Amount	Overview Explanation
Personnel:	$ 48,980	Tribal personnel for project field effort
Professional/Technical/Service Contracts:	$331,555	University of Minnesota (analysis and interpretation) and Sample detection laboratory (chemical analysis)
Travel Expenses in MN:	$ 19,465	Field trips for university and tribal staff visits
TOTAL ENRTF BUDGET:	**$400,000**	

Explanation of Use of Classified Staff:

Explanation of Capital Expenditures Greater Than $5,000:

Total Number of Full-time Equivalents (FTE) Directly Funded with this ENRTF Appropriation:
0.67
Total Number of Full-time Equivalents (FTE) Estimated to Be Funded through Contracts with this ENRTF Appropriation:
3

B. Other Funds:

Source of Funds	$ Amount Proposed	$ Amount Spent	Use of Other Funds
Non-state			
	$	$	
State			
	$	$	
TOTAL OTHER FUNDS:	**$**	**$**	

In-kind Services To Be Applied To Project During Project Period:			
	Principle Investigator: Seth Moore 15% FTE x 2.5 yrs (11,850 + 38% fringe)	$40,882	Secured
	EPA Research Ecotoxicologist - Mark Jankowski 1% FTE salary and 34% fringe	$2,381	Secured
	UMN Fish Health Specialist: Nicholas Phelps - 2% FTE x 2.5 yrs (12,592 + 34% fringe)	$8,417	Secured
	MPCA Environmental Scientist - Mark Ferrey salary and 34% fringe	$9,527	Secured
	Field logistics and personell for sample collection (crew of 3, vessel, fuel $888/day X 40 field days)**	$35,520	Secured
	Preliminary sample collection and analysis by Grand Portage ***	$140,000	Secured
	Laboratory resources at UMN College of Veterinary Medicine:		
	Lab facilities and equipment	$25,000	Secured
TOTAL OTHER FUNDS			**$281,727**

VII. PROJECT STRATEGY:

A. Project Partners:

Partners receiving ENRTF funding

- *Tiffany Wolf, DVM/PhD, Research Associate, University of Minnesota, $31,009, UMN project manager*
- *Dominic Travis, DVM, Associate Professor,University of Minnesota, $11,093, UMN Ecohealth Lead*
- *Mateo Convertino, PhD, Assistant Professor, University of Minnesota, $8,452, Spatial Modeler*
- *Alex Primus, DVM/PhD, Research Associate, University of Minnesota, $11,725, Fish health specialist*

Partners NOT receiving ENRTF funding

- *Seth Moore, PhD, Director of Biology and Environment, Grand Portage Band of CHippewa, Overal project manager*
- *Nick Phelps, PhD, Assistant Professor, University of Minnesota, Fish health specialist*
- *Mark Ferrey, Ecotoxicologist, Minnesota Pollution Control, Toxicology expert*
- *Mark Jankowski, PhD, Ecotoxicologist, Evironmental Protection Agency, Toxicology expert*

B. Project Impact and Long-term Strategy:
This project is unique in that it will focus on system-wide exposures across a variety of aquatic systems and subsistence fish species in the Lake Superior watershed. Data collected will be analyzed for presence/absence of both traditional/known hazards, as well as CECs across a large landscape, allowing for prioritization of in depth studies on food safety and security. Tools used to characterize predicted biological effects and spatial mapping will be employed to interact with managers and other decision/policy makers to determine long term chemical and biodiversity monitoring locations key for management and cultural sustainability.

The core intent of this project is to provide science-based information for assessing and managing risks associated with sustainable cultural use of natural resources and interaction with the environment in and around the grand Portage Tribal area. In this case, we will continue ongoing research known contaminants, while increasing baseline knowledge regarding micro pollutants (emerging contaminants) as it interacts traditional fishing practices by the people of the Grand Portage Tribe. Secondarily, this work fills a data gap in ongoing monitoring projects conducted by the MPCA. The emerging nature of "emerging contaminants" also implies that findings will be new, and thus interesting to the general public, specifically outdoor/sportsmen and tourism industries in Northern MN. Our goal is also to communicate our findings in both the policy and scientific arenas.

C. Funding History:

Funding Source and Use of Funds	Funding Timeframe	$ Amount
University of Minnesota	2015	$20,000
U.S. EPA Great Lakes Restoration Initiative	FY2016	$45,000
U.S. EPA Great Lakes Tribal Initiative	FY2016	$75,500

VIII. REPORTING REQUIREMENTS:

- **The project is for 2.5 years, will begin on 07/01/2017, and end on 01/31/2020.**
- **Periodic project status update reports will be submitted** *[July 30]* **and** *[January 31]* **of each year.**
- **A final report and associated products will be submitted between June 30 and August 15, 2020**.

IX. VISUAL COMPONENT or MAP(S):
Figures

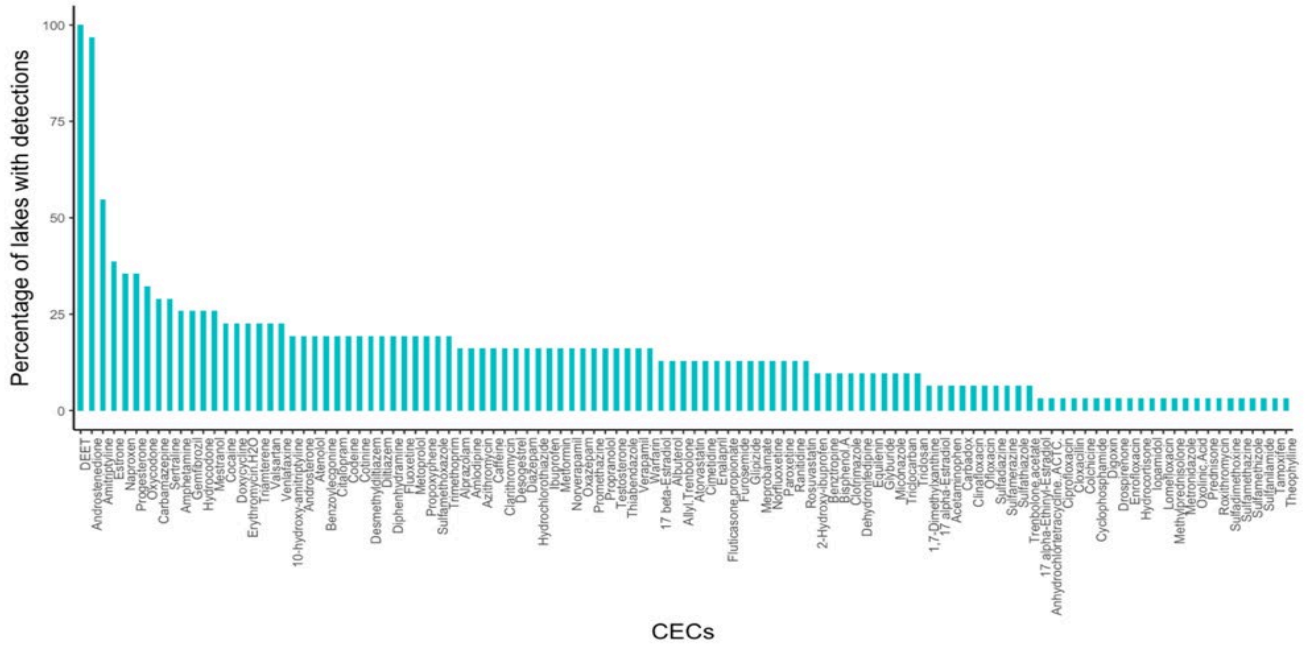

Figure 1. Contaminants of emerging concern (CECs) across lakes in NE Minnesota in 2016 and 2017.

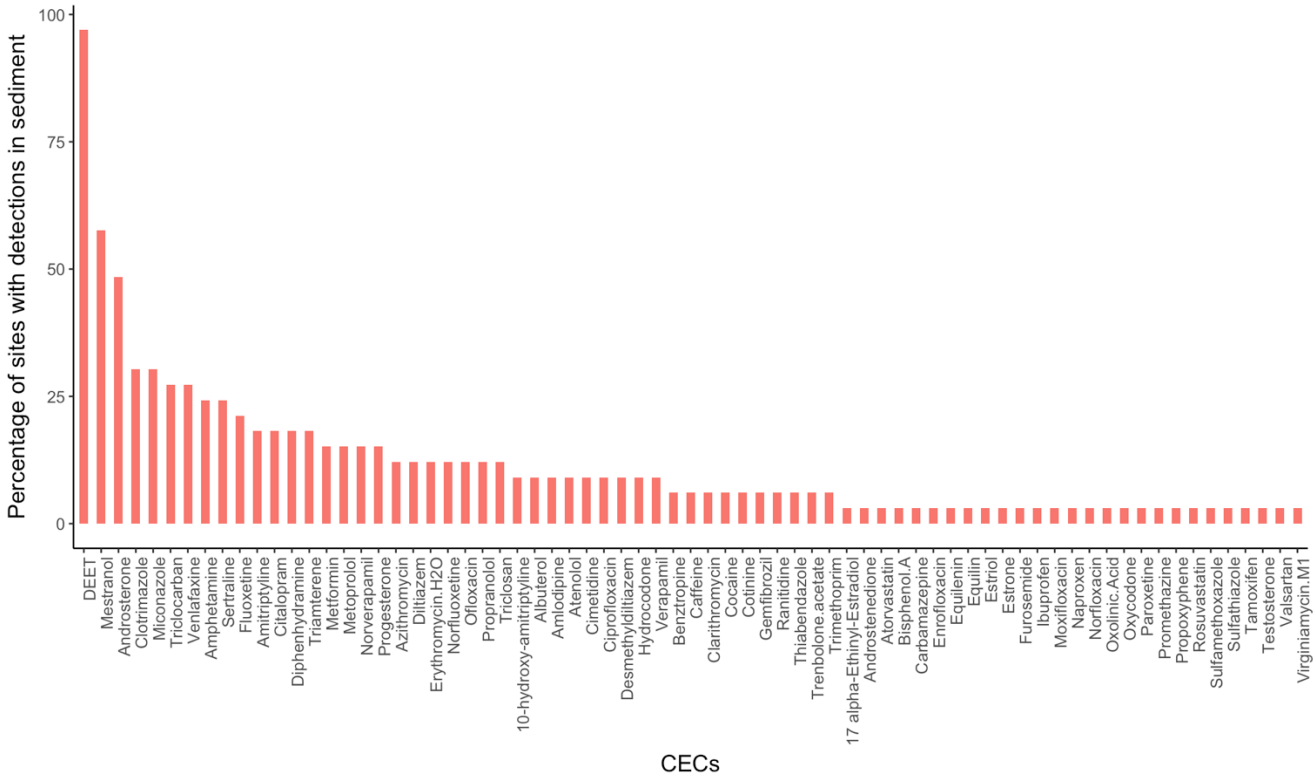

Figure 2. Contaminants of emerging concern (CECs) in sediment across sites in NE Minnesota in 2016 and 2017.

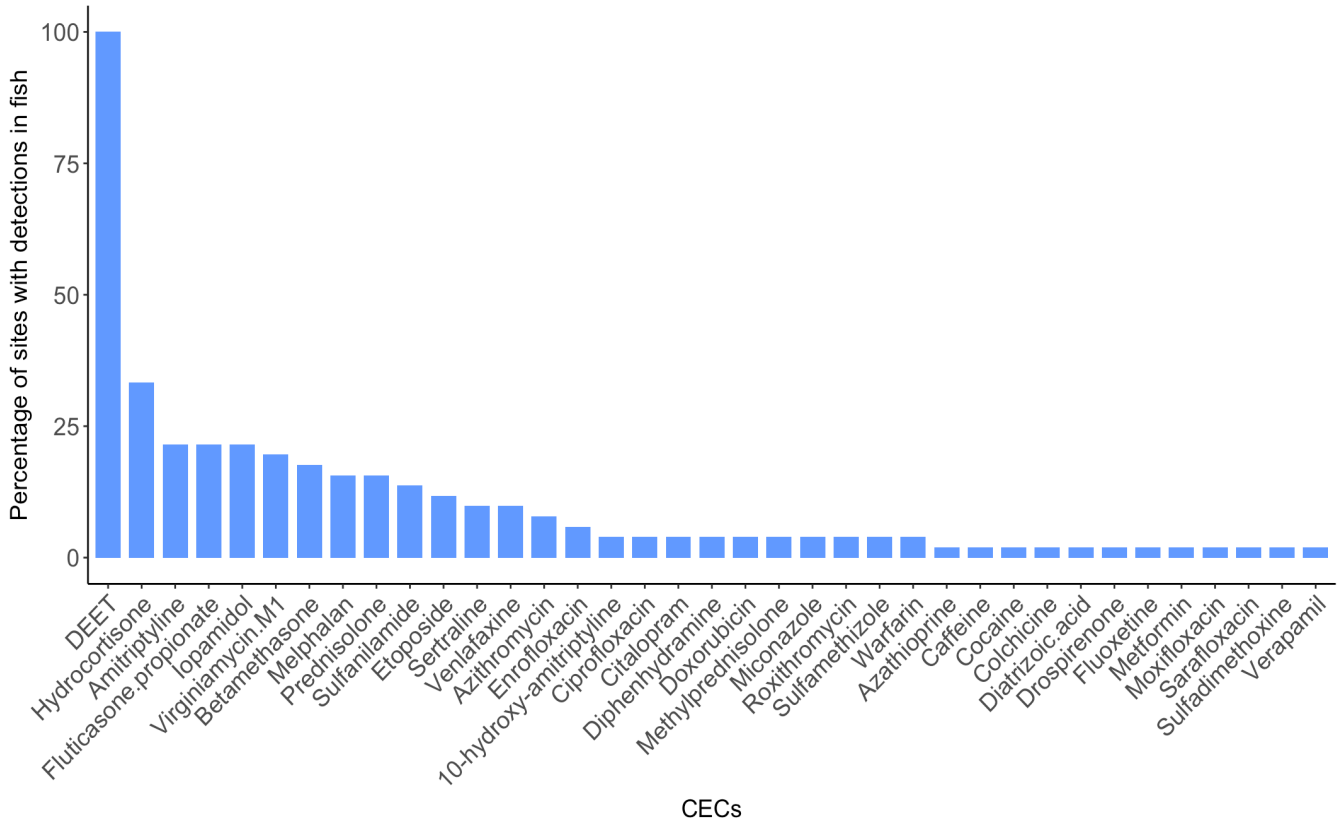

Figure 3. Contaminants of emerging concern (CECs) in sediment across sites in NE Minnesota in 2016 and 2017.

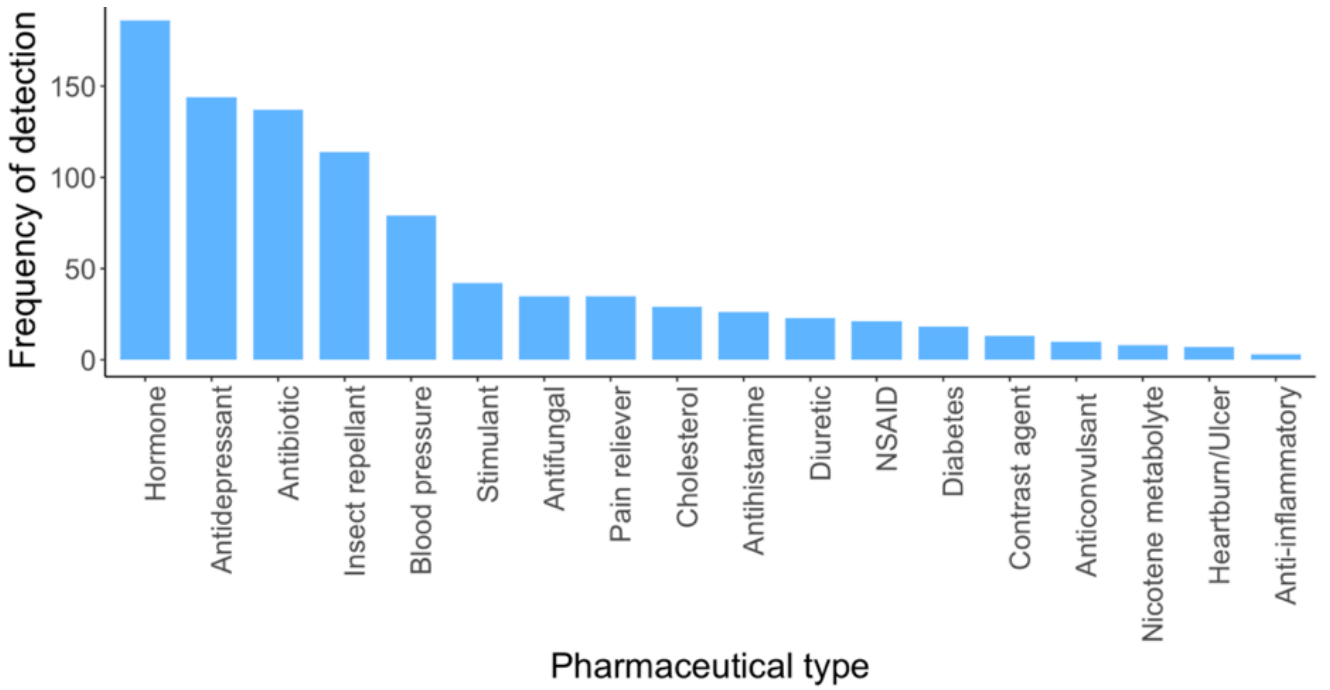

Figure 4. Pharmaceuticals found in water, sediment and fish across NE Minnesota in 2016 and 2017.

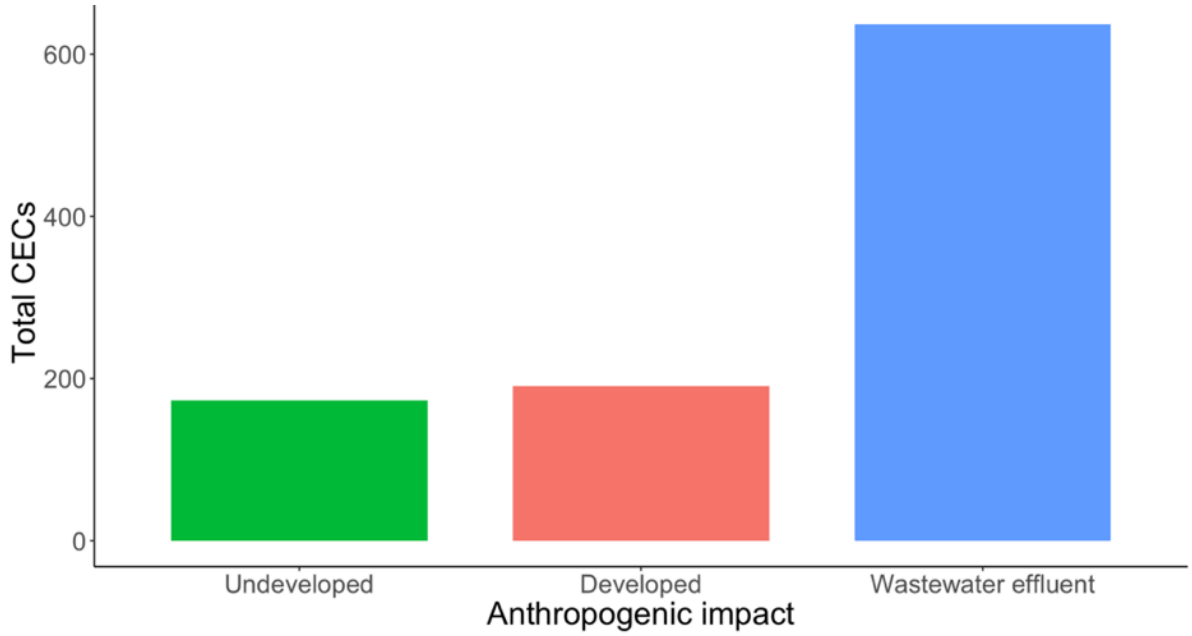

Figure 5. Contaminants of emerging concern (CECs) in water, sediment and fish across NE Minnesota in 2016 and 2017.

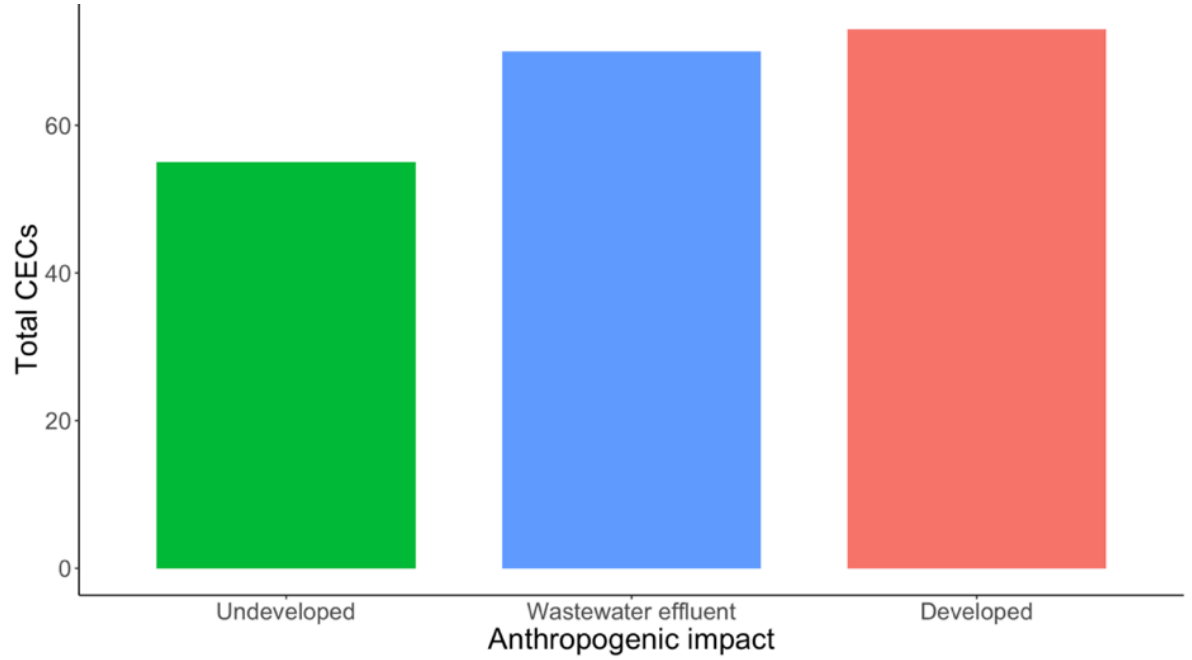

Figure 6. Contaminants of emerging concern (CECs) in water, sediment and fish across NE Minnesota in 2016 and 2017.

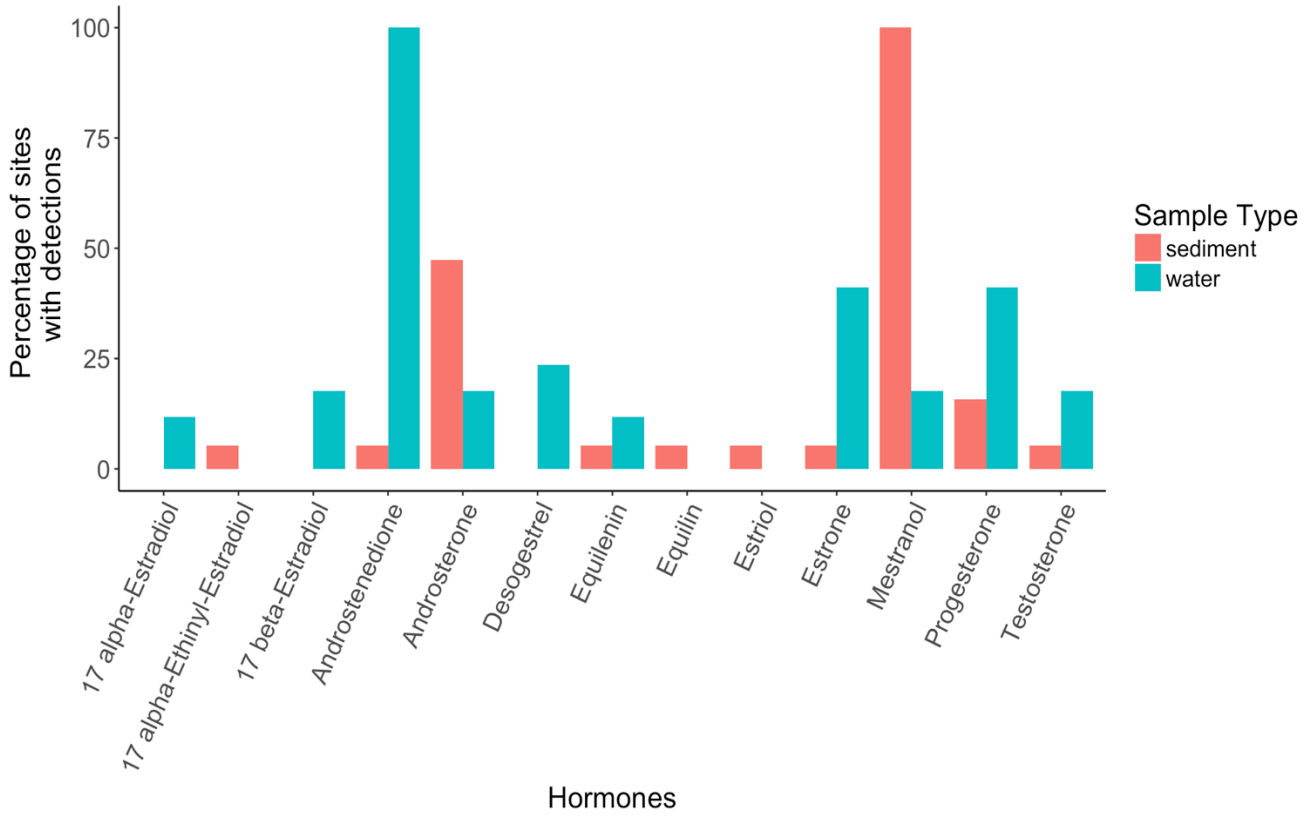

Figure 7. Detections of hormones in water and sediment across sites in NE Minnesota in 2017.

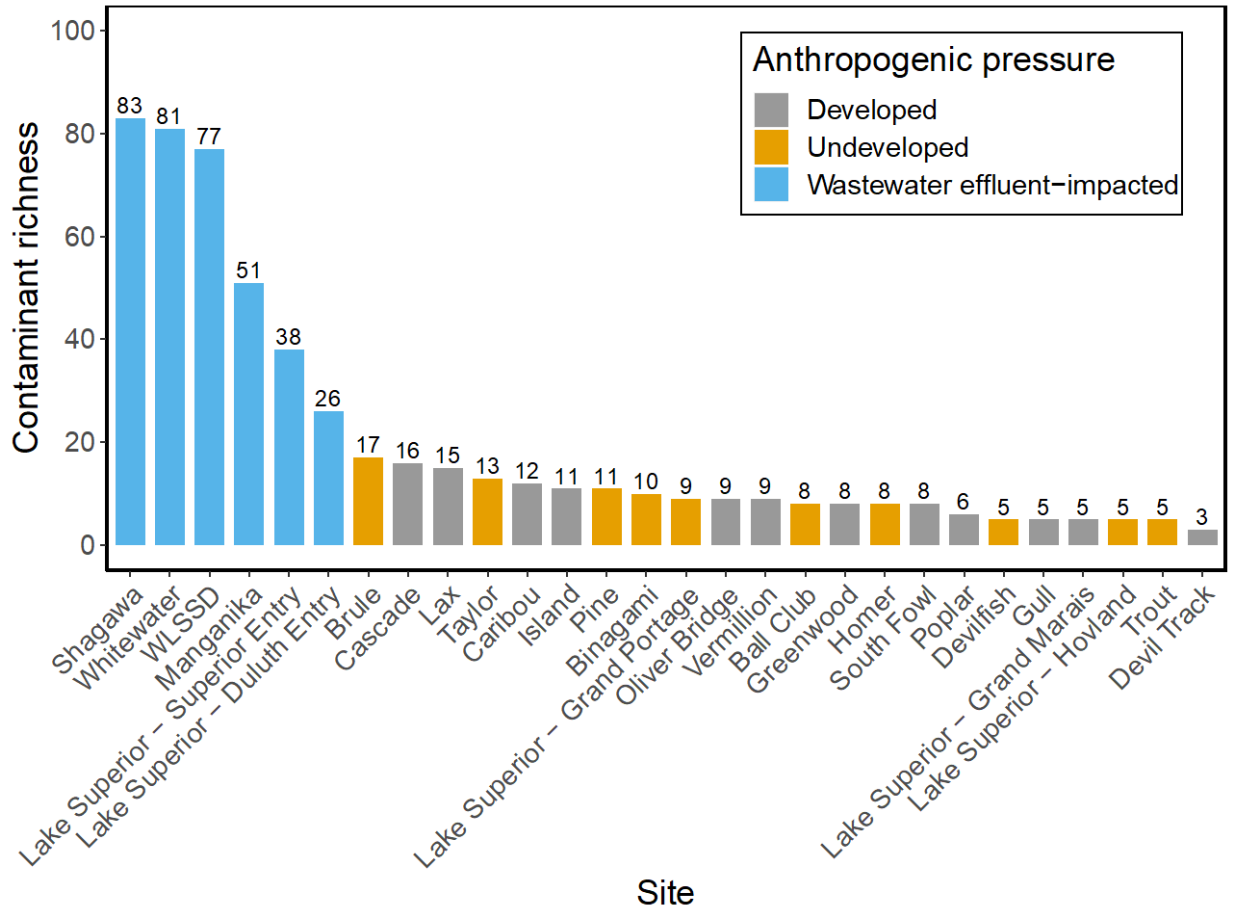

Figure 8. Number of unique contaminants detected across sites in northeastern Minnesota in 2016-2018.

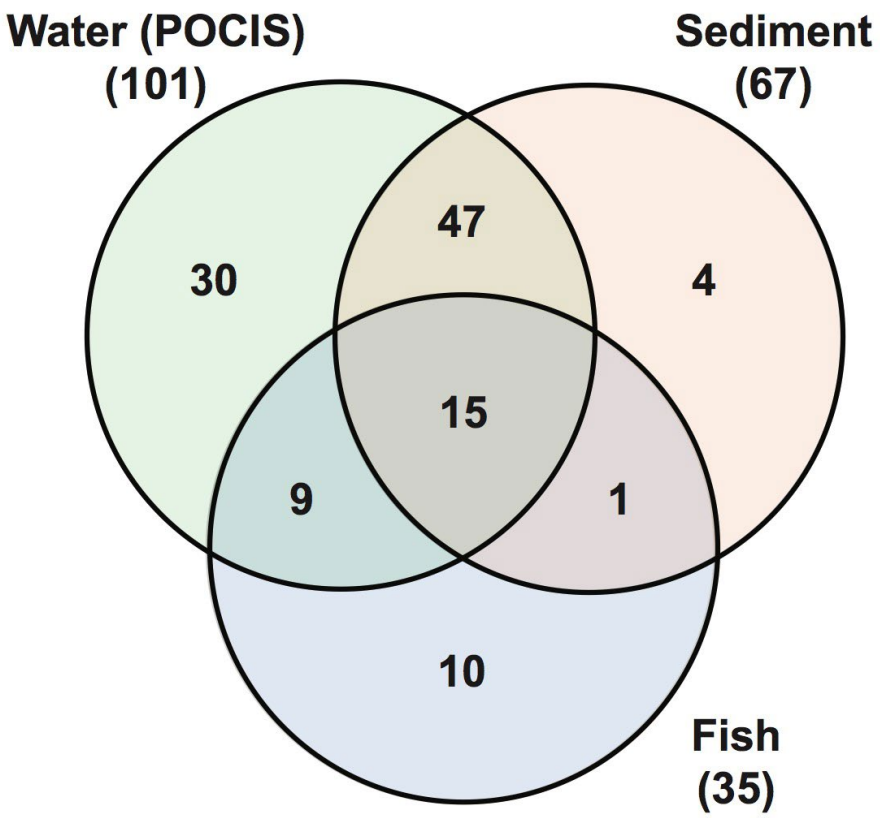

Figure 9. A Venn diagram representing the number of unique and shared contaminants in all media. The number in parentheses represents the total number of contaminants detected in each media. The following 15 CECs were detected in all media: 1) 10-hydroxy-amitriptyline, 2) Amitriptyline, 3) Azithromycin, 4) Caffeine, 5) Citalopram, 6) Cocaine, 7) DEET, 8) Diphenhydramine, 9) Enrofloxacin, 10) Fluoxetine, 11) Metformin, 12) Miconazole, 13) Sertraline, 14) Venlafaxine, and 15) Verapamil.

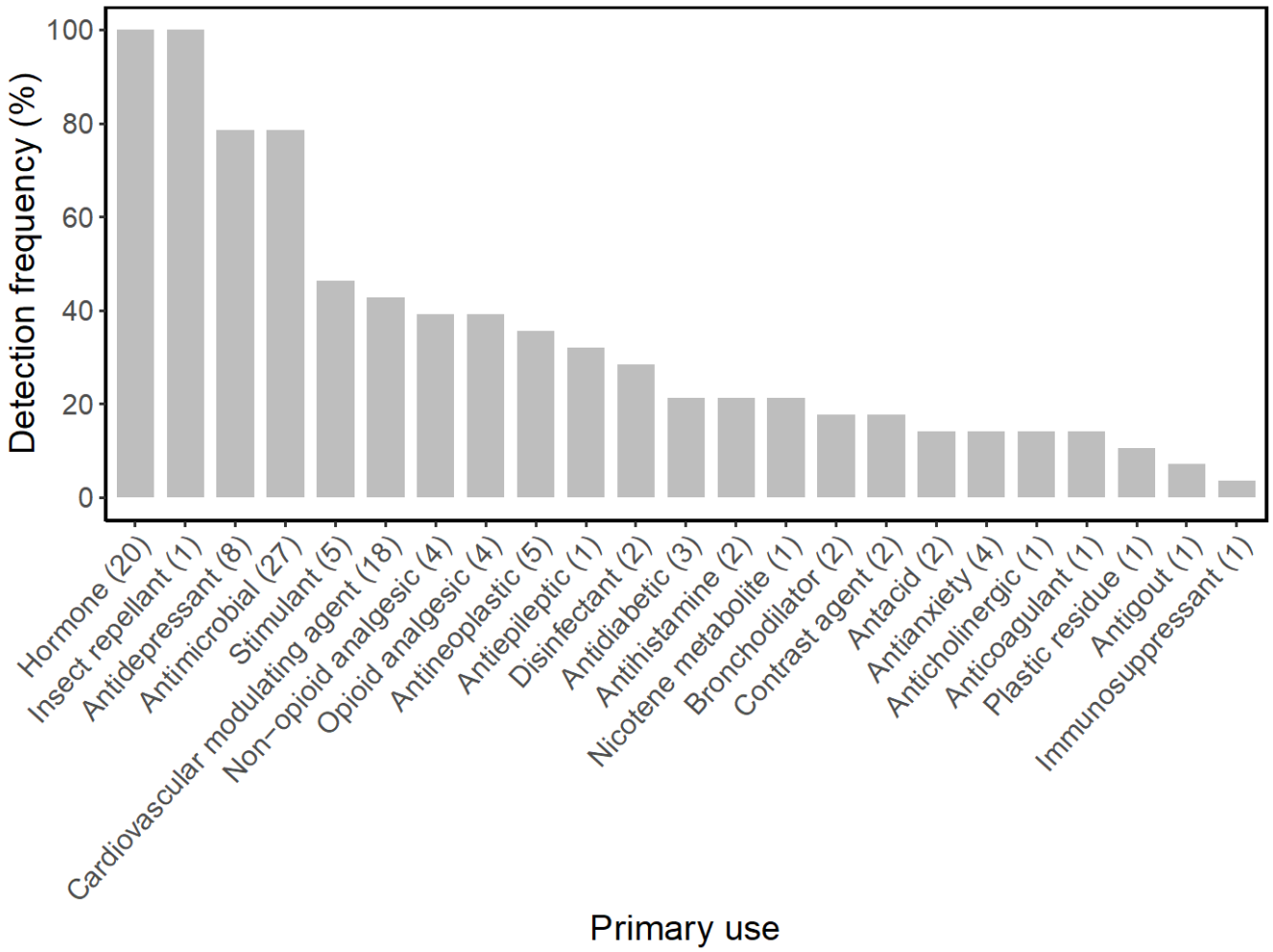

Figure 10. Frequency of contaminants detected in northeastern Minnesota in 2016-2018. Contaminants are grouped by primary use category. The number in parentheses represents the number of possible compounds detected in each category. If the contaminant was detected in any media at each site, it was considered a detection.

Environment and Natural Resources Trust Fund
FINAL M.L. 2017 Project Budget

Project Title: *Evaluation of anthropogenic micro pollutants in subsistence species used by the Grand Portage Band of Lake Superior Chippewa*
Legal Citation: *M.L. 2017, Chp. 96, Sec. 2, Subd. 04g*
Project Manager: Seth Moore
Organization: *Grand Portage Band of Lake Superior Chippewa*
M.L. 2017 ENRTF Appropriation: $ *400,000*
Project Length and Completion Date: June 30, 2020
Date of Report: 08/15/2020

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	Activity 1 revised Budget	Amount Spent	Activity 1 Balance	Activity 2 Budget	Amount Spent	Activity 2 Balance	TOTAL BUDGET	TOTAL BALANCE
BUDGET ITEM	***Collect data on chemicals of emerging concern from 18 waterbodies by sampling fish, water, and sediments .***			***Interpret findings with University of Minnesota***				
Personnel (Wages and Benefits) overall								
Grand Portage Biologist: Yvette Chenaux- 25% FTE x 2.5 yrs (21,000 + 38% fringe)	$13,746.00	$13,746	$0	$15,234	$15,234	$0	$28,980	$0
Professional/Technical/Service Contracts								
UMN contract: T. Wolf 10% FTE, A. Primus 5%FTE, D. Travis 2% FTE, M. Convertino 2% FTE, Post Doc 6% FTE, Grad Student 50% FTE	$84,743	$84,743	$0	$68,277	$61,617	$6,660	$153,020	$6,660
Sample Procesing: AXYS LABS: Analysis of contamination with micropullutants (78 samples @ $2,500 sample)	$195,000	$192,688	$2,312				$195,000	$2,312
1854 Treaty Authority: Field Work: Logistics and sample collection and transport ($10,000 per field season)	$20,000	$20,000	$0				$20,000	$0
Travel expenses in Minnesota								
Grand Portage Staff: 5 two-day trips for 2 staff	$3,000	$396	$2,604				$3,000	$2,604
COLUMN TOTAL	**$316,489**	**$311,573**	**$4,916**	**$83,511**	**$76,851**	**$6,660**	**$400,000**	**$11,576**