Fish as indicators of ecosystem health: Assessing the impact of contaminants of emerging concern

A DISSERTATION

SUBMITTED TO THE FACULTY OF THE

UNIVERSITY OF MINNESOTA

BY

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IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

Advised by Drs. Tiffany Wolf and Dominic Travis

August 2021

Acknowledgements

I am extremely grateful for my time at the University of Minnesota. I have been challenged and supported in ways I did not know possible, and I could not have asked for a better experience.

I would like to express my deepest gratitude to my advisors, Tiffany Wolf and Dominic Travis. Thank you both for taking me on as a student and challenging me to see the importance of expanding my expertise and seeing our work through a different point of view. Thank you for your advice, patience, and understanding. Tiffany, thank you so much for the time you invested in my development as a scientist. Your guidance in scientific thinking and development was invaluable. Dom, thank you for always being there with support and encouragement. Your insights were integral to my development as an ecosystem health scientist. You always made sure I saw the importance of the path I was on, and for that, I could not be more grateful. I look forward to continuing to work with you both in the next steps of my career. I can never thank you both enough for the opportunities you have offered me that pushed me to grow into the scientist I am today and allow me to follow my dreams.

I would like to thank my committee members, Alex Primus, Seth Moore, and Randy Singer. Alex, thank you so much for welcoming me into the world of fish health. You helped me stay interested and excited about starting research in a field that was at first scary to me. I will be forever grateful for my experience conducting fieldwork in

northeastern Minnesota, and you were and integral part of that. Seth, thank you so much for allowing me to take part in this important research that means so much to you. As I said in my thanks to Alex, my fieldwork in Grand Portage and the Ceded Territory includes some of my favorite experiences in life. Without your support and guidance, this work would not have been possible, so I am extremely appreciative of everything that you do. Randy, I have learned so much from you. Thank you for your support and guidance and for the significant contributions you have made to my training throughout my PhD.

I owe sincere appreciation to my "ecotox team." The biweekly meetings we had throughout my PhD were the driving force behind my progress and success. Mark Ferrey and Mark Jankowski, thank you so much for walking me through the world of toxicology. I told everyone you were my unofficial committee members. I could not have done this PhD without your guidance on chemicals and toxicology. Thank you for always connecting me with the right collaborators, for redirecting me when needed, and for giving me the opportunity to learn from you both. Nick Phelps, thank you for always being there when I asked and for your support and guidance on all things fish health. Thank you to Matteo Convertino for your contributions to my PhD work. I would also like to thank Coreen Hamilton for your support on chemical analyses. Thank you to Summer Streets for working with me on the Aquatic Toxicity Profiles. You both never hesitated to answer the many questions I had, and I really appreciate your help and support. Joanna Borucinska, it was a pleasure to work with and learn from you. I am very glad we were able to host you in Minnesota. It was wonderful getting to know you. Joe

Servadio, I am so happy you joined the group. Thank you for never hesitating to stay late after meetings or responding to my sporadic text messages and emails asking for help or advice. I cannot thank you enough for your support and friendship.

I would like to express sincere thanks to the Grand Portage Band of Lake Superior Chippewa for their trust and partnership. Thank you to the Grand Portage Reservation Tribal Council and Tony Swader for your continued support. I would like to express my appreciation to Yvette Chenaux-Ibrahim, E.J. Isaac, and employees of the Grand Portage Band Trust Lands and 1854 Treaty Authority. Without your help and support, this thesis would not have been possible.

I would like to thank Leila Knoll and Aidan Neher for their support in fieldwork and early discussions of the fish health element of this project in 2017 and Jennifer Hoy-Petersen, Silje Mogstad Finstand, and Jakob Mo for supporting my fieldwork in 2019. Leila and Aidan, you made navigating assessing fish health on the side of lakes in rural, and some not so rural, areas in northeastern Minnesota so much fun. I am so grateful for your help and friendship. Jennifer, Silje, and Jakob, I still cannot believe I was lucky enough to have been provided the opportunity to work with, and learn from, all of you. Jennifer, your friendship means the world to me and I'm so grateful for all that you have done for me.

To my colleagues and those that I have collaborated with on projects during my time as a student at the University of Minnesota, thank you for showing me what it means to be

dedicated scientist and for helping to shape my scientific perspectives. I would like to thank the Wolf Lab members, Tyler Garwood, Marissa Milstein, Luiz Gustavo Rodrigues Oliveira Santos, Katie Marchetto, and Bill Severud. The discussions and conversations I have had with you all, both in and out of lab meetings, have been so impactful throughout my PhD. Through the Travis Lab I have been able to work with, and learn from, George Omondi and Michael Mahero. I have learned so much from you both, thank you. Thank you to the members of the Wildlife Epidemiology Journal Club. It was during these journal clubs that I learned how truly remarkable the people in this program are.

I am forever grateful for the endless support and encouragement from my friends and family. To all of the friends I have made during my time at the College of Veterinary Medicine, the time you have spent listening, offering advice, and teaching me valuable lessons will never be forgotten. Katie Worsley-Tonks, Amy Kinsley, Kim VanderWaal, Cata Picasso, Irene Bueno, and Kaushi Kanankege, your friendship has been truly incredible during my PhD, and I cannot wait for many more years of friendship with you all. To Sahana Kuthyar, sharing this scientific journey with you has been so helpful, thank you so much for your support. Last, but certainly not least, thank you Maggie Baynham for everything you do for me, your love and support means everything.

To my family, I am not sure I can put into words my appreciation for you all. The pathways and adventures that have led me to Minnesota to pursue a PhD would not have been possible without my parents, Lisa and Steve, and my brothers, Bradley, Justin, and Evan. Mom, thank you for your endless support and encouraging me to chase my dreams.

Your “never say no” philosophy led me down this path and I could not be more grateful. Brad, you inspired me to pursue a career I never knew possible - thank you for setting the best example and supporting me in following in your footsteps. Justin, I would not be the person I am today without you - thank you for your endless love and support. Evan, your drive and ambition inspires me - thank you for being the comedic encouragement I need to get through this life and for helping me through coding issues for hours after I say, “It’ll only take 30 minutes.” To my grandparents, Gma and Gpa Lem, thank you for your love and support throughout my educational career. To Lekha, Carson, and Carrie, I believe I have the best brothers in the world, so I am not surprised they chose amazing women to share their lives with - thank you for your love and support. Thank you to my niece, Mira, your cuteness and excitement for life are always the pick-me-up I need. I cannot end without thanking my dog, Chloe. She has been with me throughout all my graduate studies, and she provides me daily joy that has been the best support I could have asked for during this journey.

Dedication

I dedicate this work to my mom, Lisa, who never lets me forget that I can do anything I set my mind to.

Abstract

Water is arguably the most essential natural resource in the world, yet the use of industrial, healthcare, and household products threaten freshwater ecosystems. Contaminants of emerging concern (CECs) are a diverse group of chemicals - often defined as chemicals that were previously unknown, unrecognized, or unregulated - that comprise pharmaceuticals, personal care products, and hormones. CECs now have a ubiquitous distribution worldwide and their presence is only increasing as quantitative detection limits continue to be lowered and new chemicals make their way onto the global market. Concern over their biological effects at the molecular, organism, and population level in aquatic ecosystems is also increasing. CECs are identified throughout the Great Lakes Basin and may have a variety of adverse effects on aquatic life. However, data describing the specific risks these contaminants pose to human, wildlife, and environmental health are scarce.

The goal of this thesis was to characterize CECs in freshwater ecosystems of northeastern Minnesota and evaluate their potential impact on the health of subsistence fish species. We investigated CECs and fish health within the Grand Portage Indian Reservation (GPIR) and 1854 Ceded Territory, where the Grand Portage Band of Lake Superior Chippewa rely on subsistence hunting, fishing and gather as the foundation for their culture and way of life. Thus, to establish a baseline understanding of CECs on these Tribal lands and their potential impact on fish health, we assessed important subsistence fish species in waterbodies that have value as fish harvesting locations for Band

members. Further, due to a gap in knowledge regarding the distribution of CECs in rural and Tribal areas, we targeted waterbodies along a spectrum of anthropogenic pressures: waterbodies with no human development along their shorelines, those with development, and those directly impacted by wastewater effluent.

Chapter 1 provides background for why it is essential that we better understand the potential impact CECs might be having on aquatic ecosystems, and thus Ojibwe culture. **Chapter 2** characterizes the occurrence of CECs in water, sediment, and subsistence fish species in 28 locations. We detected 117 different chemicals in water, sediment, and/or fish in wastewater effluent-impacted, developed, and undeveloped sites. **Chapter 3** prioritizes the chemical hazards of the detected chemicals through a rapid assessment of chemical-specific information - including detection frequency, persistence, endocrine disruption, toxicity, and bioaccumulation - to evaluate the potential for these contaminants to cause adverse effects on aquatic life. We identified 50 contaminants in water, 21 in sediment, seven in fish as high priority, including antimicrobials, antihistamines, antidepressants, cardiovascular modulating agents, and insect repellant. **Chapter 4** evaluates the health of wild fish exposed to CECs across varying anthropogenic pressures. We compared the utility of three different approaches that could be used to evaluate the health of fish exposed to CECs: a refined fish health assessment index (rFHI), a histopathological index, and high-throughput (ToxCast) *in vitro* assays. We mapped adverse outcome pathways (AOPs) associated with identified ToxCast assays to determine potential impacts across levels of biological organization within the aquatic system. The health of fish in undeveloped sites was as poor, or sometimes poorer,

than fish in developed and wastewater effluent-impacted sites. **Chapter 5** is a general discussion to conclude the relevance of this work and explore important future directions. Collectively, this thesis provides evidence of the potential hazards of CECs and their impact on fish health in a region that is important for sustaining Indigenous culture through subsistence fishing.

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List of Abbreviations

ACC	Activity Concentration at Cutoff
AFB1	Aflatoxin B_1
AO	Adverse Outcome
AOP	Adverse Outcome Pathway
ATPs	Aquatic Toxicity Profiles
BAF	Bioaccumulation Factor
BCF	Bioconcentration Factor
CECs	Contaminants of Emerging Concern
ChV	Chronic Values
DEET	*N,N*-Diethyl-*meta*-toluamide
E2	Estradiol
EARs	Exposure-Activity Ratios
ECOSAR	Ecological Structure Activity Relationships
EDSP	Endocrine Disruptor Screening Program
EE2	17α-ethynylestradiol
EPA	Environmental Protection Agency
EPISuite	Estimation Programs Interface Suite
ESH	Ecosystem Health
ESI	Electrospray Ionization
GPIR	Grand Portage Indian Reservation
H&E	Hematoxylin and Eosin
HAI	Health Assessment Index
HDPE	High-Density Polyethylene
HLB	Hydrophilic-Lipophilic Balance
HPLC	High Performance Liquid Chromatograph
KE	Key Events
KM	Kaplan-Meier

K_{OC}	Organic Carbon-Water Partition Coefficient
K_{OW}	Octanol-Water Partition Coefficient
LC50	Lethal Concentration 50%
LC-MS/MS	Liquid Chromatography with Tandem Mass Spectrometry
logP	Octanol-Water Partition Coefficient
MAVT	Multi Attribute Value Theory
MCDA	Multi-Criteria Decision Analysis
MIE	Molecular Initiating Events
MMCs	Melanomacrophage Centers
MRM	Multiple Reaction Monitoring
NA	Not Applicable
PCNA	Proliferating Cell Nuclear Antigen
pKa	Acid Dissociation Constant
$PM_{2.5}$	Particulate Matter 2.5
POCIS	Polar Organic Chemical Integrative Sampler
PPCPs	Pharmaceuticals and Personal Care Products
QSAR	Quantitative Structure-Activity Relationship
rFHI	Refined Fish Health Assessment Index
SD	Standard Deviation
SeqAPASS	Sequence Alignment to Predict Across Species Susceptibility
SMA	Smooth Muscle Actin
SPE	Solid-Phase Extraction
$t_{1/2}$	Half-life
TEST	Toxicity Estimation Software Tool
ToxCast	Toxicity Forecaster
TSC	Tissue Screening Concentrations
WHO	World Health Organization
WWTP	Wastewater Treatment Plant

Chapter 1

Introduction

Despite the undeniable importance of water to human, animal, and environmental health, freshwater ecosystems are threatened by everyday human activities that use chemicals in consumer, healthcare, and personal care products. Chemicals that are previously unknown, unrecognized, or unregulated - referred to as contaminants of emerging concern (CECs) and include pharmaceuticals, fragrances, parabens, plasticizers, flame retardants, nanoparticles, among others - are ubiquitous in surface waters worldwide (Nilsen et al., 2019; Valbonesi et al., 2021). Due to the advancement of analytical instrumentation and methodology, the ability to detect extremely low concentrations of CECs is increasing (Poynton & Robinson, 2018). Thus, CECs are discovered in aquatic environments where they were previously unrecognized, and they can have adverse effects on fish and aquatic ecosystems. For example, antidepressants can alter reproductive and antipredator behaviors and contraceptive hormones can cause entire populations to collapse, even at part per trillion concentrations (Dzieweczynski et al., 2016; Fent et al., 2006; Fursdon et al., 2019; Grabicova et al., 2015; Jorgenson et al., 2018; Kidd et al., 2007; Martin et al., 2019). The potential risks to human and environmental health, frequency of occurrence, and unknown source and fate of CECs elevates the importance of understanding the presence of these chemicals in the environment and their effects on fish, aquatic ecosystems, and ultimately all forms of life.

The distribution and fate of CECs in the aquatic environment is largely unknown (Wilkinson et al., 2017) and there is increasing evidence that nonpoint sources of pollution contribute to CEC presence in rural environments once thought pristine (Elliottand VanderMeulen 2017; Ferrey et al. 2015, 2018, 2020). While the original source of most CECs would be traced back to manufacturing plants, their routes of environmental transport after leaving the plants become more complex and are due to their use and disposal by people, rather than simply a by-product of manufacturing. These chemicals can enter aquatic systems through effluent from wastewater treatment plants (Blair et al., 2013; Kathy E. Lee et al., 2011), onsite wastewater treatment systems (Baker et al., 2014; Schaider et al., 2017), stormwater systems (Fairbairn et al., 2018), and even precipitation (Ferrey et al., 2018). CECs enter the water cycle as parent compounds, metabolites, or transformation products, and they can sorb to microplastics, sediment or bioaccumulate (Wilkinson et al., 2017).

Pharmaceuticals are undeniably integral in maintaining a healthy population of both humans and livestock; however, they are designed to have a specific mode of action even at low concentrations. Their presence in the environment impacts non-target animals, especially aquatic organisms that are continuously exposed through their habitats across their entire lives. Antidepressants, such as fluoxetine and citalopram, can alter reproductive and antipredator behaviors in freshwater fish and crayfish (Dzieweczynski et al., 2016; Fursdon et al., 2019; Martin et al., 2017, 2019; Pelli and Connaughton, 2015; Reisinger et al., 2021). Illicit drugs, such as methamphetamine, can elicit addiction and alter behavior in wild fish (Horký et al. 2021). Endocrine

disrupting pharmaceuticals, such as the synthetic estrogen 17α-ethynylestradiol (EE2), lead to the production of the vitellogenin, which causes feminization of male fathead minnows (Kidd et al., 2007) and is correlated with increased human population size Desforges et al., 2010). Additionally, many CECs affect fish behavior (Brodin et al., 2017; Painter et al., 2009; Reisinger et al., 2021), physiology (Capaldo et al., 2018), reproductive biology (Schoenfuss et al., 2008; Tetreault et al., 2012; Writer et al., 2010), and genetic expression (Martinović-Weigelt et al., 2014; Pomati et al., 2007).

In addition to the organism level, CECs can impact entire food webs and cause population-level effects. CECs can biomagnify up the food chain, causing species that consume other contaminated organisms to be exposed to CECs. Brown trout feeding on aquatic invertebrates consumed antidepressants at as much as one-half of a human's therapeutic dose (Richmond et al., 2018). CECs can even jeopardize the structure and function of entire ecosystems. For example, exposure to low concentrations of EE2 led to a near extinction of fathead minnows (Kidd et al., 2007) and the nonsteroidal anti-inflammatory drug, diclofenac, caused the vulture population in Pakistan to significantly decline (Oaks et al., 2004).

Fish are widely used as indicators of aquatic ecosystem health (Łuczyńska et al., 2018; Van Der Schalie et al., 1999; Whitfield & Elliott, 2002), as they provide a biological endpoint of exposure (Stentiford et al., 2003). Due to their high trophic position in food webs, they may accumulate contaminants at toxicologically relevant concentrations (Ali & Khan, 2018). The trophic transfer of potentially toxic contaminants in food chains has

important implications for human health; therefore, understanding how fish are affected by CEC exposure can inform the potential risk of CECs to human health (i.e., food safety). Additionally, CECs may represent a food security issue. Therefore, before understanding the potential threats to human health, we must recognize the role CECs are playing in fish health.

While lethality is often thought of as the endpoint of toxicity testing, lethality is only one endpoint of toxicant exposure, and it is directly encountered in nature less often. The sublethal effects that adversely affect growth and reproduction are more likely to be seen in natural aquatic systems (Nikinmaa, 2014). These sublethal effects can be harder to detect in commonly used fish population health surveys (Pope et al., 2010). Further, there are multiple levels of biological organization that can be measured as indicators of toxicant exposure, from the molecular level to the entire ecosystem (Nikinmaa, 2014), and understanding how environmental contaminants might impact each level requires different tools. The complexity of the toxicology of CECs further complicates this matter. Within an organism, there can be different target site interactions and effects at the molecular level, which can lead to damage at the cellular level and tissue level. These interactions can then lead to impacts at the individual level, which can ultimately lead to population-level effects (Poynton & Robinson, 2018).

Condition indices (e.g., condition factor, hepatosomatic index, and gonadosomatic index) have long been used to document fish stressors (Adams & Ryon, 1994; Blazer et

al., 2014; Bolger & Connolly, 1989) and necropsy-based assessments have been developed and implemented in natural environments in the context of broad environmental stressors (Adams et al., 1993; Blazer et al., 2018; Lang et al., 2017). However, these tools can be relatively subjective and lack sensitivity and specificity, particularly in situations in which differences in environmental conditions may be subtle. For example, matings of fathead minnows exposed to estrogenic compounds produced a lower level of viable eggs (Brian et al., 2007). An increasingly powerful tool used to elucidate the impacts of chemicals at various levels of biological organization is the adverse outcome pathway (AOP) framework, which organizes information across different levels and reveals key events that lead from one level to the next (Ankley et al., 2010; Poynton & Robinson, 2018). The combined implementation of these tools can help reveal the potential impacts CECs have on fish health, and ultimately ecosystem health.

In addition to being important as indicators of ecosystem health, fish are an important species to Indigenous populations, both through cultural and subsistence lenses. Further, many Indigenous peoples have value systems that are intrinsically linked to the freshwater in which these fish reside (Noble et al., 2016). Fish consumption advisories are often put into place in response to dangerous levels of contaminants in the environment; however, these advisories often disregard the significant value that the practices of catching, harvesting, preparing, and eating fish have on the lives of Indigenous people. Recently, important attention has been brought to the cultural and health implications of fish advisories on Native American communities (Gagnon,

2016; Hoover, 2013). In the United States, there is a long history of Indigenous lives being adversely affected by water pollution and contamination that should not be ignored. Therefore, understanding the impact of CECs on fish health is beyond a food safety and security matter, but also has special importance for addressing issues of environmental justice and food sovereignty.

As the number and concentration of environmental contaminants continues to rise in natural bodies of water around the world, our ability to evaluate the effects of these contaminants and their synergistic effects on fish, aquatic ecosystems, and ultimately all forms of life will continue to escalate in importance. **The goal of this thesis was to characterize CECs in freshwater ecosystems of northeastern Minnesota and evaluate their potential impact on the health of subsistence fish species.** Three main objectives contributed to this goal: 1) survey water, sediment, and subsistence fish species within Tribal lands and adjacent territory and assess the presence of CECs across varying anthropogenic pressures: waterbodies with no human development along their shorelines, those with development, and those directly impacted by wastewater effluent; 2) perform a rapid-screening assessment and prioritization of detected CECs based on their potential environmental hazard, identify waterbodies in the study region that contain high priority CECs, and inform future monitoring, assessment, and potential remediation in the study region; and 3) evaluate the health of wild fish exposed to CECs in waterbodies along a spectrum of anthropogenic pressures across northeastern Minnesota by using three fish health metrics: a refined fish health assessment index (rFHI), a histopathological index, and high-throughput

(ToxCast) *in vitro* assays.

We investigated CECs and fish health within the Grand Portage Indian Reservation (GPIR) and 1854 Ceded Territory in Minnesota, USA. In 1854, the Grand Portage Band of Lake Superior Chippewa entered a treaty whereby they ceded ownership of their lands to the United States while retaining their right to hunt, fish, and gather on the lands that are now the northeastern portion of Minnesota. The Grand Portage Chippewa are part of a larger Native American group known as the Anishinaabe. Subsistence hunting, fishing, and gathering form the foundation of Chippewa culture. For the Ojibwe, natural resources are cultural resources, they cannot be separated, and one is dependent on the other (Stults et al., 2016). Therefore, the continued longevity of Ojibwe culture and way of life (including both physical and mental health) depends on the protection and preservation of regional resources on which they subsist.

Understanding the potential hazards that CECs pose to subsistence fish species and the aquatic systems in which they live is necessary to protecting Ojibwe culture. Thus, to establish a baseline understanding of the distribution of CECs on these Tribal lands and their potential impact on fish health, we targeted important subsistence fish species in waterbodies that have value as a fish harvesting location for Band members. The focus fish species were walleye (ogaa in Ojibwe; *Sandervitreus*) and yellow perch (asaawens; *Perca flavescens*) collected from inland lakes and cisco (odoonibiins; *Coregonus artedi*) and lake trout (namegos; *Salvelinus namaycush*) collected from Lake Superior sites. We targeted two key subsistence and recreational species of different trophic levels from

each location (i.e., inland lakes and Lake Superiorsites) due to their importance to the Grand Portage Band as well as the role they play in the aquatic food web, acting as a bridge to human consumers and potential risk.

In collaboration with the Grand Portage Band of Lake Superior Chippewa, this thesis was undertaken through the development of a partnership in ecosystem health. The nascent discipline of ecosystem health, grounded in transdisciplinary science and diverse ways of knowing, seeks to optimize the priorities of human, animal, and environmental health. Ecosystem health merges the theories and methods of ecological and health sciences, thus balancing sustainable human and animal health with management of ecosystems (Wilcox et al. 2004). The common, overarching purpose of using the ecosystem health method is to better understand the connections between nature, society, and health and how drivers of social and ecosystem change ultimately influence human health and well-being (Wilcox and Kueffer 2008). Through this partnership, we have an ecosystem health monitoring network aimed at protecting natural resources for the sustainable provision of wildlife health, ecosystem services, and Indigenous cultural practices.

For objective 1 **(Chapter 2)**, we first characterized the occurrence of CECs in freshwater ecosystems utilized by a Minnesota Tribal community. Due to a gap in knowledge regarding the distribution of CECs in rural and Tribal areas, we surveyed water, sediment, and subsistence fish species across varying anthropogenic pressures within the Grand Portage Indian Reservation (GPIR) and 1854 Ceded Territory:

waterbodies with no human development along their shorelines, those with development, and those directlyimpacted by wastewater effluent. We detected 117 different contaminants in water, sediment, and/or fish from 28 locations across northeastern Minnesota. We detected CECs most frequently at wastewater effluent-impacted sites, but contamination also occurred in remote, undeveloped locations with no obvious point source of pollution. The high detection frequencies of contaminants including pharmaceuticals - such as hormones, antidepressants, and antimicrobials and the insect repellant, DEET - raises questions about the safety and security of subsistence foods for Indigenous communities.

The wide variety of CECs present in freshwater ecosystems utilized by the Grand Portage Band for subsistence raised additional questions about the sources of these chemicals and their potential hazards on the biological systems of fish. For objective 2 **(Chapter 3)**, we prioritized the chemical hazards of the 117 CECs detected in water, sediment, and fish through a rapid assessment of chemical-specific information - including detection frequency, persistence, endocrine disruption, toxicity, and bioaccumulation - to evaluate the potential for environmental contaminants to cause adverse effects on aquatic life. We identified 50 contaminants in water, 21 in sediment, and seven in fish as high priority. Among high priority contaminants were antimicrobials, antihistamines, antidepressants, cardiovascular modulating agents, and insect repellant.

Given the breadth of high priority contaminants in fish and the ecosystems in which

they reside, for objective 3 **(Chapter 4)**, we evaluated the health of wild fish exposed to CECs across a spectrum of human pressures. Then, we compared the utility of three different approaches that could be used to evaluate the health of fish exposed to CECs: a refined fish health assessment index (rFHI), a histopathological index, and high-throughput (ToxCast) *in vitro* assays. We mapped AOPs associated with identified ToxCast assays to determine potential impacts across levels of biological organization within the aquatic system. The health of fish in undeveloped sites was as poor, or sometimes poorer, than fish in developed and wastewater effluent-impacted sites.

This thesis describes the patterns of CECs in Lake Superior and surrounding waterbodies and determines the potential impact these chemicals have on important subsistence fish species. A better understanding of the relationship between CECs and fish health helps prioritize risk management research efforts while also supporting the sustainability of Ojibwe culture and way of life.

Chapter 2

Occurrence of contaminants of emerging concern in aquaticecosystems utilized by Minnesota tribal communities*

*Chapter published as: **Deere JR**, Moore S, Ferrey M, Jankowski MD, Primus A, Convertino M, Servadio JL, Phelps NBD, Hamilton MC, Chenaux-Ibrahim Y, Travis DA, Wolf TM. (2020). Occurrence of contaminants of emerging concern in aquatic ecosystems utilized by Minnesota tribal communities. *Science of The Total Environment*,*724*(138057), 1–14. https://doi.org/10.1016/j.scitotenv.2020.138057

Overview

Pharmaceuticals, personal care products, hormones, and other chemicals lacking water quality standards are frequently found in surface water. While evidence is growing that these contaminants of emerging concern (CECs) – those previously unknown, unrecognized, or unregulated – can affect the behavior and reproduction of fish and wildlife, little is known about the distribution of these chemicals in rural, tribal areas. Therefore, we surveyed the presence of CECs in water, sediment, and subsistence fish species across various waterbodies, categorized as undeveloped (i.e., no human development along shorelines), developed (i.e., human development along shorelines), and wastewater effluent-impacted (i.e., contain effluence from wastewater treatment plants), within the Grand Portage Indian Reservation and 1854 Ceded Territory in northeastern Minnesota, U.S.A. Overall, in 28 sites across three years (2016-2018), 116 of the 158 compounds tested were detected in at least one form of medium (i.e., water, sediment, or fish). CECs were detected most frequently at wastewater effluent-impacted

sites, with up to 83 chemicals detected in one such lake, while as many as 17 were detected in an undeveloped lake. Although there was no statistically significant difference between the number of CECs present in developed versus undeveloped lakes, a range of 3 – 17 CECs were detected across these locations. Twenty-two CECs were detected in developed and undeveloped sites that were not detected in wastewater effluent-impacted sites. The detection of CECs in remote, undeveloped locations where subsistence fish areharvested, raises scientific questions about the safety and security of subsistence foods toindigenous communities. Further investigation is warranted so that science-based solutions to reduce chemical risks to aquatic life and people can be developed locally andperhaps be informative for indigenous communities elsewhere.

Introduction

An estimated 80% of global wastewater returns to ecosystems untreated (WWAP, 2017), placing a contaminant burden on surface waters that poses risks to ecosystem health (Vorosmarty et al., 2010). Contaminants of emerging concern (CECs) – chemicals that are previously unknown, unrecognized, or unregulated (Nilsen et al., 2019) – are widespread in surface water (Daughton & Ternes, 1999; Richardson & Kimura, 2019) and comprise a wide variety of chemicals, such as human and veterinary pharmaceuticals,personal care products, and hormones (Ekman et al., 2013). Most existing research has focused on established point sources of these contaminants to rivers and streams (Barber et al., 2000; Barber et al., 2007; Fairbairn et al., 2018; Kiesling et al., 2019; Kolpin et al., 2002; Lee et al., 2010; Lee et al., 2011), with fewer monitoring studies focused on inland freshwater lakes (Blair et al., 2013; Writer et al.,

2010). Investigations of lakes has revealed CECs in remote areas unaffected by wastewater effluent (Elliott & VanderMeulen, 2017; Writer et al., 2010), and a study of randomly selected lakes in the state of Minnesota, USA, showed that numerous CECs, including antibiotics, antidepressants, insect repellant, illicit drugs, and other chemicals were detected in lakeslacking any obvious source of contamination (Ferrey et al., 2015).

CEC occurrence may be associated with the level of watershed disturbance (Baldwin et al., 2016; Ferrey et al., 2015; Kiesling et al., 2019; Sengupta et al., 2014) or atmospheric deposition (Ferrey et al., 2018; Hageman et al., 2006; Lyons & Benvenuti, 2016; Newtonet al., 2014), the latter particularly important where human development is minimal. Evenat low concentrations, CECs can affect the physiology and behavior of fish (Brodin et al., 2017; Capaldo et al., 2018; Painter et al., 2009) and molluscs (Fong, 1998; Fong & Ford, 2014), and may jeopardize the structure and function of entire ecosystems(Kidd et al., 2007; Oaks et al., 2004). However, despite increasing evidence that some of these chemicals may cause adverse effects in aquatic systems, pollution reduction strategies for these chemicals are lacking due to an absence of water quality standards and other toxicity benchmarks. Although evidence on the occurrence of CECs in urban areas of the Great Lakes Basin (Choy et al., 2017; Elliott et al., 2017; Great Lakes Chemicals of Emerging Concern Advisory Work Group, 2009; Hull et al., 2015; Klecka et al., 2010) and global aquatic systems is rapidly accumulating (Arukwe et al., 2012; Česen et al., 2019; Glassmeyer et al., 2017; Kolpin et al., 2002), less is known about the distribution of these CECs in rural and Native American tribal areas near the Great Lakes.

Indigenous communities and traditional cultures are particularly vulnerable to contaminated water, as they are culturally dependent upon aquatic ecosystems for food and economic security (Kuhnlein & Chan, 2000). In Minnesota, the Anishinaabeg Indigenous peoples of the Grand Portage Band of Lake Superior Chippewa harvest subsistence species – such as moose (mooz in Ojibwe), walleye (ogaa), cisco (odoonibiins), and lake trout (namegos) – and place cultural value on many native fish, wildlife, and plant species (Stults et al., 2016). For this study, our partnership with the Grand Portage Band was developed in accordance with Ecosystem Health (ESH) principles. ESH merges the theories and methods of ecology, health sciences, and policy,balancing sustainable human and animal health with management of ecosystems (Wilcox et al., 2004). Partnership and team development were undertaken through a formal stakeholder engagement process under the principles outlined by Charron (2012) with the goal of creating an ESH monitoring network aimed at protecting natural resources for the sustainable provision of wildlife health, ecosystem services, and indigenous cultural practices in Grand Portage, Minnesota.

To address the gap in knowledge regarding the distribution of CECs in rural and tribal areas, we surveyed water, sediment, and subsistence fish species within tribal lands and adjacent territory, much of which is within the watershed of the largest freshwater lake inNorth America, and assessed the presence of contaminants across varying anthropogenicpressures: waterbodies with no human development along their shorelines, those with development, and those directly impacted by wastewater effluent. This initial study characterizes the distribution of CECs across key waterbodies for

subsistence fishing ontribal lands in and near the Lake Superior watershed.

Materials and methods

Study site and site selection

This study was conducted in two regions in northeastern Minnesota upon which the Anishinaabeg depend for subsistence, the Grand Portage Indian Reservation (GPIR) and the 1854 Ceded Territory **(Figure 1)**. The reservation land base encompasses approximately 192 km^2, bordered by Ontario, Canada to the north, Lake Superior to the east and south, and a mixture of federal, state, and private land to the west. In addition, the Grand Portage Band of Chippewa retains treaty-reserved rights to hunt, fish, and gather in the 1854 Ceded Territory, an area of 20,234 km^2 covering much of northeastern Minnesota. The Ceded Territory comprises nearly 2,540 lakes, each larger than 40,000 m^2 and encompassing a total of nearly 2,025 km^2 of water, 300 km^2 of wetlands, approximately 9,000 km of rivers and streams, and nearly 6000 km^2 of Lake Superior itself.

A cross-sectional survey design was used across 14 waterbodies in 2016, 19 waterbodiesin 2017, and two waterbodies in 2018, leading to a total of 28 unique waterbodies acrossall years **(Supplementary Table S1)**. Five sites were sampled in both 2016 and 2017. Polar Organic Chemical Integrative Sampler (POCIS) deployment canisters, used to sample water, were destroyed at two sites in 2017; therefore, we resampled those sites in 2018. Sites included inland lakes as well as locations along the Lake Superior coast. Candidate sites were selected by land use,

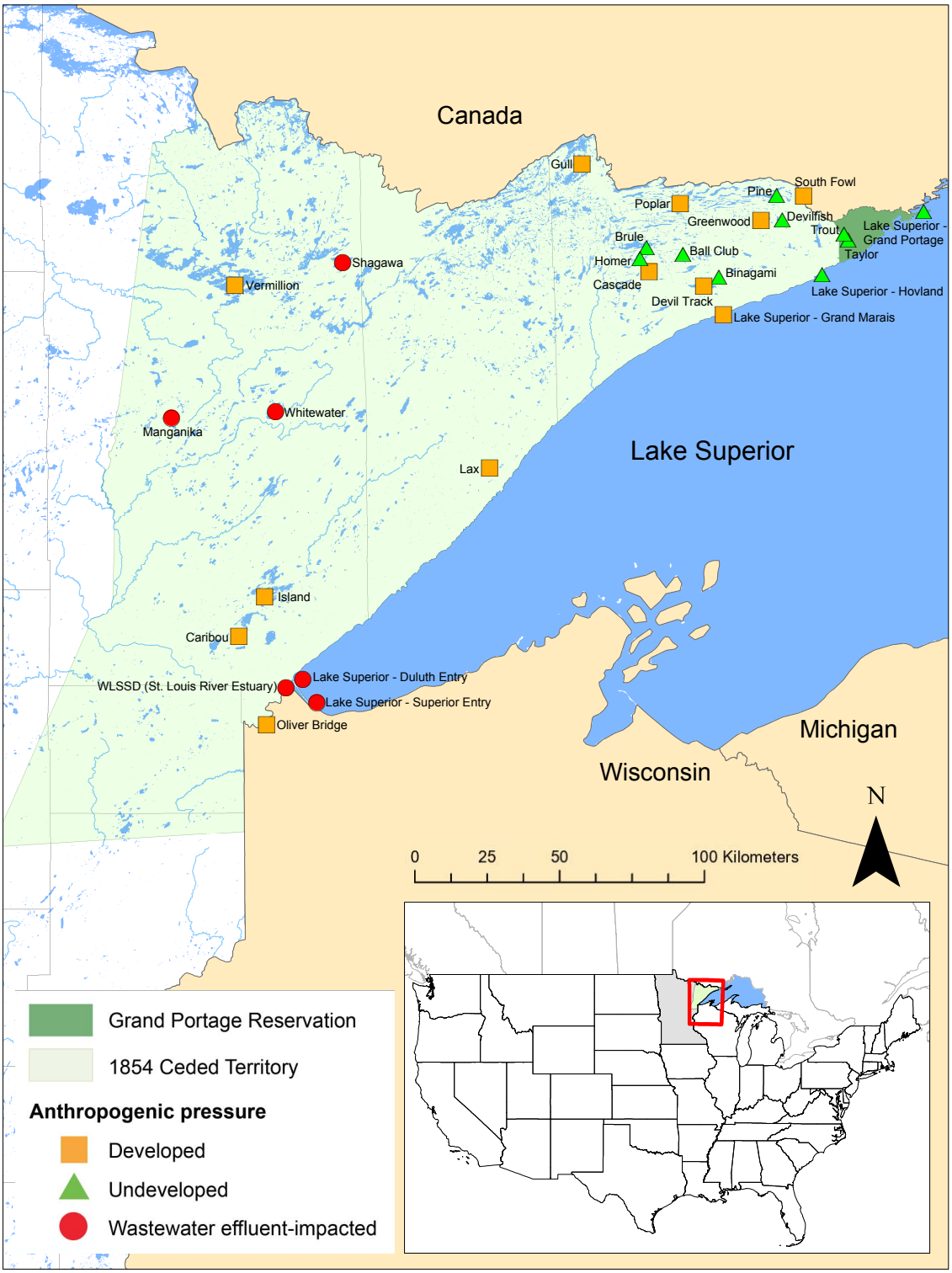

Figure 1. Sites sampled from 2016 – 2018 in northeastern Minnesota.

proximity to potential point sources of contaminant release (e.g., downstream of wastewater effluent or mining activities and several point-source free sites), presence of important subsistence fish species, and value as a fish harvesting location for tribal members.

Candidate sites were categorized by anthropogenic impact: 1) wastewater effluent-impacted water, 2) various human developments, and 3) undeveloped areas. The wastewater effluent-impacted category includes sites that receive discharge from a wastewater treatment plant. The developed category includes sites with any level of shoreline development, including human residences and businesses. The undeveloped category includes sites with no shoreline development, with the exception of one lake with one seasonal residence.

We made final selection of sampling sites by using a multi-criteria decision analysis (MCDA), where multiple variables were considered in ranking the candidate sites (Convertino & Valverde, 2013). Site selection was driven by two contrasting criteria: 1) to select the maximum number of sites with varying drainage density, defined as the number of streams for the drainage area at that site, belonging to independent drainage basins and 2) to select sites that were hydrologically connected with varying drainage path length, defined as the length along waterbodies between hydrologically connected sites. Drainage density and path length were calculated based on elevation. We identified seven clusters of sites that were hydrologically independent, meaning that any water flow in the basins to which the sites belong is not expected to move into other basins. To avoid

any bias in the final site selection, the equally weighted criteria were used in a Multi Attribute Value Theory (MAVT) model (Convertino & Valverde, 2013). The value function, incorporating the two criteria, was optimized via a Pareto optimization algorithm (Convertino & Valverde, 2013), constrained by the limiting requirements of including four sites from each category (i.e., wastewater-effluent impacted, developed, undeveloped) and two sites in Lake Superior.

Sample collection and storage

We collected samples of water, sediment, and fish from each waterbody in 2016 (June – November) and 2017 (July – October), and water only in 2018 (July – August). Water was sampled using POCIS (Environmental Sampling Technologies, St. Louis, Missouri),which are passive samplers used to collect a time integrated sample for potentially bioavailable hydrophilic organic chemicals that is more representative (than grab sampling) of what aquatic organisms are exposed to over a given time period and covering several precipitation events. POCIS sampling represents respiratory exposure ofaquatic organisms to dissolved chemicals and can be used to determine a time-weighted average concentration of water-soluble organic contaminants (Alvarez et al., 2004). We deployed three POCIS disks, combined into a single sample for analysis, per waterbody to improve analyte detectability. The POCIS disks were placed in the appropriate canisterand carrier (Environmental Sampling Technologies) and submerged under twelve inches of water for a minimum of 30 days during July – August each year. Before and after deployment, POCIS disks were stored in airtight metal containers. They were immediately frozen after collection and subsequently sent to SGS AXYS

Analytical (Sidney, British Columbia, Canada) for testing of 158 chemicals, including hormones, antibiotics, antidepressants, and more **(Supplementary Table S2)**.

We collected grab samples of sediment in 250 mL high-density polyethylene (HDPE) containers from the top 10 cm of sediment from each waterbody using either a Ponar dredge or sediment siphon (hand corer), whichever was most appropriate for the sedimenttype and water depth (U.S. EPA, 2001). Samples were taken in close proximity to where the POCIS were deployed. All materials used were washed with detergent and tap water and subsequently rinsed with distilled water. Samples were stored on ice immediately after collection and frozen at -18°C until overnight shipment to SGS AXYS for testing ofthe same 158 compounds as water.

We targeted important subsistence and recreational fish species of different trophic levelsfor sampling. In 2016, we sampled fish species opportunistically and in 2017, walleye (*Sander vitreus*) and yellow perch (*Perca flavescens*) were targeted from inland sites and lake trout (*Salvelinus namaycush*) and cisco (*Coregonus artedi*) were sampled from Lake Superior sites. Two lakes did not contain yellow perch, so we collected only walleye at Binagami Lake and walleye and black crappie (*Pomoxis nigromaculatus*) at Manganika Lake. We utilized fish collection gear and protocols appropriate for the water body and target species. In inland lakes, we used multiple methods, including boat-operated electrofishing, 24-hour set experimental gill nets of multiple mesh sizes ranging from 1.5-4.5-inch stretch mesh, 24-hour set fyke nets, and hook and line. We checked gill nets at least once every 24 hours to avoid deterioration of fish samples. We

collected a minimumof three individuals of each species per waterbody. Upon collection, we identified fish to species and sorted either into a specimen or non-target category, with all non-target species immediately returned alive into the water. All equipment was rinsed in ambient water from the waterbody being sampled prior to fish collection to remove any foreign material from the external surface. Researchers wore latex or nitrile powder-free gloves when handling fish.

Following collection, fish were weighed to the nearest gram, measured by length, and logged by species. Fish were euthanized using an American Veterinary Medical Association approved physical method per University of Minnesota IACUC-approved protocol (ID: 1803-35736A). Specimens were measured (+/- 1mm), wrapped in aluminum foil, and individually placed into sealed plastic bags. Fish were frozen at -18°C until further processing.

Whole fish were homogenized using a stainless-steel commercial meat grinder to obtain one representative fish tissue sample per species per waterbody. 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. In 2016, fish were pooled together by waterbody and then frozenuntil shipment. In 2017, fish were separated by species and waterbody for CEC analysis and then frozen until shipment to SGS AXYS. Fish were not tested for all hormones because the laboratory was unable to analyze all hormones (Lists 7 and 8 in **Supplementary Table S2**) in tissues. All water, sediment, and fish samples shipped to

SGS AXYS were packed in a cooler that keeps them at or below 4°C to ensure that the tissues did not thaw during overnight shipment.

Analytical procedures

The CECs selected for analysis were based on those in United States Environmental Protection Agency (EPA) Method 1694 (U.S. EPA, 2007) with additional compounds incorporated (see **Supplementary Table S2** for analytical lists). This expanded EPA 1694 analyte list includes selected hormones and other pharmaceuticals and personal care products (PPCPs) identified by the EPA and other SGS AXYS clients as priorities for assessment based on annual consumption, expected toxicity, and persistence. Analyses conducted by SGS AXYS under EPA Method 1694 and EPA Report EPA-820-R-10-008(U.S. EPA, 2010) are explained below with further details provided in the **Supplementary Information**.

Liquid chromatography with tandem mass spectrometry (LC-MS/MS) operated in the multiple reaction monitoring (MRM) mode was used to monitor target analytes and isotopically labeled standards. The most intense MRM transition for each analyte and labeled standard was used for quantification. For this study, supplementary standards andLC-MS/MS transitions were included for the additional compounds not in EPA Method 1694. All EPA method performance criteria (linearity, sensitivity, accuracy, precision) were validated for all target analytes. The EPA Method sorts PPCP compounds into four groups according to their optimum extraction pH (acidic: Lists 1, 2 and 4 or basic: List 3)and LC-MS/MS conditions. The additional base extractable compounds monitored in this study were added to the List 3 analysis and the acid

extractable additional compounds were collected into other LC-MS/MS runs (Lists 5, 6, 7, and 8).

Water (POCIS) processing

POCIS disks were disassembled, and the solid-phase extraction (SPE) material was removed from the POCIS disks. The hydrophilic-lipophilic balance (HLB) sampling material was collected for analysis. The HLB was spiked with a suite of isotopically labeled internal standards for all analytes, transferred to a glass chromatography column and extracted by elution with 50 mL of methanol followed by 20 mL of 1:1 acetone:methanol. The extract was concentrated and analyzed for all target analytes. Control samples (SPE spiked with target analytes) were also analyzed to demonstrate quantitative recovery of captured analytes.

Sediment and fish tissue processing

For acid extraction, an aliquot (approximately 2.5 g of wet sediment) of each sample was spiked with a suite of isotopically labeled internal standards for acid extraction, adjusted to pH 2.0 with phosphate buffer, and extracted twice by sonication with acetonitrile followed by a third extraction with acetonitrile alone. For tissue, approximately 2.5 g wasspiked with a suite of isotopically labeled internal standards, extracted by sonication in acetonitrile, followed by two extractions with aqueous pH 2.0 phosphate buffer and acetonitrile. The combined acetonitrile and aqueous solutions from each sample were concentrated to remove the acetonitrile, diluted to 200 mL in water and treated with acetate buffer and 250 mg of tetrasodium ethylenediamine

acetate dihydrate and the pH adjusted to 3.5. The solution was then extracted using an Oasis HLB cartridge.

For base extraction, another aliquot of each solid and tissue sample was spiked with a suite of isotopically labeled internal standards. Each sediment sample was adjusted to pH10 with NH_4OH and extracted twice by sonication with acetonitrile. For tissue, the sample was first extracted by sonication in acetonitrile, then adjusted to pH 10 with NH_4OH and extracted twice by sonication. The combined acetonitrile and water solutionswere concentrated to remove the acetonitrile, diluted to 200 mL in water and extracted using an Oasis HLB cartridge.

LC-MS/MS analysis

LC-MS/MS analyses were performed using a Waters 2690 or 2795 high performance liquid chromatograph (HPLC) equipped with a Micromass Quattro Ultima Mass Spectrometer and workstations running QuanLynx/Masslynx software. For quantitative analysis, data acquisition was performed in multiple reaction monitoring (MRM) mode, monitoring selected MRM transitions (precursor ion >> product ion) for each analyte andstandard. For many of the additional PPCP compounds and hormones, a second MRM transition has been added to the method; this additional data can be used to provide additional confirmation of the presence of specific analytes. Electrospray ionization (ESI) operated in the positive ion mode (ESI Positive) was used for most compounds (Lists 1, 2, 4, 5, 6, and 7) but ESI in the negative ion mode (ESI Negative) was used for the List 3 and 8 compounds. LC-MS/MS conditions and parameters for

Lists 1 through 4 compounds are described in U.S. EPA Method 1694; isotope dilution quantification was used for all compounds having an isotopically labeled analog and recovery corrected internal standard quantification for all other compounds. The analytical results were quantified using the software provided by the instrument manufacturer and then validatedby a senior chemist experienced in review of CEC data. During this validation process allavailable tools were used to confirm the presence of each detected analyte and to avoid false positives. These tools included the use of secondary MRM transitions where available and screening of low concentration results based on comparison to the procedural blanks and the effect of chromatographic noise on peak shape.

Quality assurance and control

To evaluate POCIS contamination due to sample handling, we collected a field blank for each sample period. The field blank was exposed to the air at time of POCIS deployment and retrieval. The blank was stored in an airtight container and frozen until shipment to SGS AXYS. Four of the detected chemicals were reported as detected in POCIS field blanks (androstenedione, androsterone, DEET, and desogestrel; Supplemental Table S3). We have reported herein the detected concentrations of these chemicals. Due to their detection in field blanks, the accuracy of the concentrations reported should be interpreted with caution.

Methods were validated by U.S. EPA Tier 1 procedures (U.S. EPA, 1999). All analytes were quantified by isotope dilution internal standard quantification. A lab blank was

included for each batch of samples analyzed to check for laboratory background or other external contamination. If a contaminant was detected in a lab blank, a screening limit forthat analyte was set to ten times the concentration found in the lab blank. A quality control sample was included with each batch. Recoveries of all added labeled standards were viewed to verify all analyses met regular methods specifications. Further qualityassurance and quality control information for all analyses can be found in the **Supplementary Information**.

Statistical analysis

All data analysis was performed with R Version 3.5.0 (Ihaka & Gentleman, 1996). All maximum concentrations and detection frequencies were reported across anthropogenic pressure categories (i.e., developed, undeveloped, and wastewater effluent-impacted) andmedia (i.e., water (POCIS), sediment, fish). Following a significant Kruskal-Wallis test, Dunn's test of multiple comparisons (using the *dunn.test* package (Dinno, 2017)) was performed post-hoc to explore differences among anthropogenic pressure categories. *P*- values were adjusted using the Benjamini-Hochberg method to control for the familywiseerror rate and limit the false discovery rate (Benjamini & Hochberg, 1995). A nonparametric test was required because the data do not meet the assumptions of parametric tests. Similarly, we used a nonparametric test, Fisher's exact test, to compare lipophilicity (based on the octanol-water partition coefficient, logP) among detected compounds. We assessed the relationship between high or low logP and sample media using a cutoff of 3.5 logP (U.S. EPA, 2000). The Venn diagram was created using the *VennDiagram* package (Chenn, 2018).

Due to the abundance of nondetect data, a Kaplan-Meier procedure was used to calculateconcentration means and percentiles using the *NADA* package (Lee, 2017) which is applicable to left-censored environmental concentration data (Helsel, 2010). Kaplan-Meier (KM) statistics were not calculated for water samples since our POCIS extracts provide the concentration per POCIS rather than water concentration. KM statistics werecalculated for contaminants detected in fish and sediment samples, separated by anthropogenic pressure.

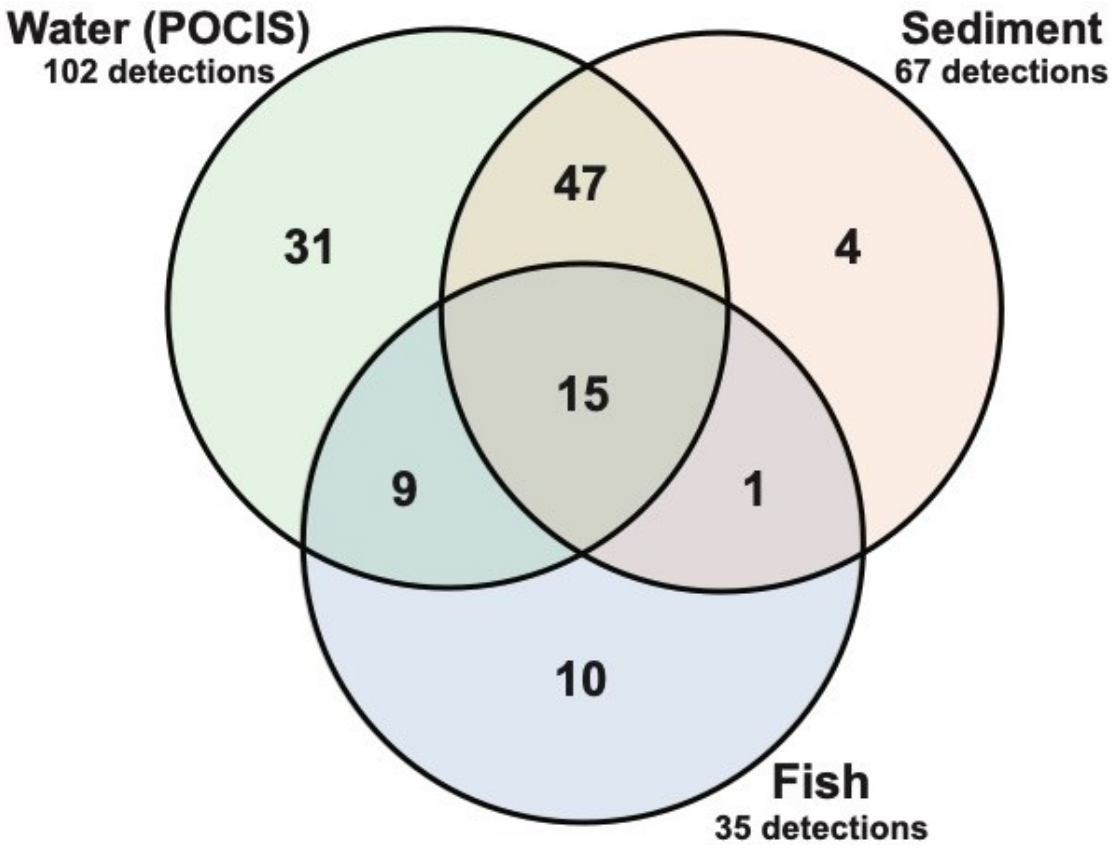

Figure 2. A Venn diagram representing the number of unique and shared contaminants in all media. The number in parentheses represents the total number of contaminants detected in each media. The following15 CECs were detected in all media: 1) 10-hydroxy-amitriptyline, 2) Amitriptyline, 3) Azithromycin, 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.

Results and discussion

Chemicals and primary use categories detected in all media

Across 28 sites in northeastern Minnesota, 117 of the 158 (74%) compounds tested were detected in water, sediment, and/or fish **(Tables 1-3)**. **Tables 1-3** report maximum concentration and detection frequency of detected compounds by media and anthropogenic category. **Tables 2-3** also report KM mean and standard deviation **(Supplemental Table S5 and S6 reports full KM statistics)**. A total of 102 CECs were detected in water (POCIS), 67 in sediment, and 35 in fish tissue **(Figure 2)**. The following 15 CECs were detected in all media: 10-hydroxy-amitriptyline, amitriptyline, azithromycin, caffeine, citalopram, cocaine, DEET, diphenhydramine, enrofloxacin, fluoxetine, metformin, miconazole, sertraline, venlafaxine, and verapamil. As anticipated,chemicals were detected more frequently and at higher concentrations at wastewater effluent-impacted sites, with up to 83 chemicals detected in a lake impacted by wastewater effluent **(Figure 3)**. However, as many as 17 chemicals were detected in an undeveloped lake, with no significant difference in the number of contaminants between developed and undeveloped sites. Many chemicals were detected in lakes at locations with little to no shoreline development.

We grouped all CECs into 23 primary use categories, ranging from pharmaceuticals to insect repellent **(Figure 4)**. Across all media, insect repellant (i.e., DEET) was ubiquitous, with some of the highest concentrations detected in undeveloped sites. The presence of DEET at undeveloped sites could be due to atmospheric deposition, as DEET has been detected in particulate matter ($PM_{2.5}$) (Cheng et al., 2006) and in rain,

Table 1. Maximum concentration and detection frequency (Detect. freq.) of all detected contaminants in POCIS extracts from wastewater effluent-impacted, developed, and undeveloped sites.

		Wastewater effluent-impacted (n = 8)		Developed (n = 14)		Undeveloped (n = 11)	
Contaminant	**Primary use**	**Maximum concentration (ng/POCIS)**	**Detect. freq. (%)**	**Maximum concentration (ng/POCIS)**	**Detect. freq. (%)**	**Maximum concentration (ng/POCIS)**	**Detect. freq. (%)**
1,7-Dimethylxanthine	Stimulant	976.0	25.0	-	-	-	-
10-hydroxy-amitriptyline	Antidepressant	1090.0	87.5	-	-	-	-
17 alpha-Estradiol	Hormone	69.6	12.5	-	-	-	-
17 alpha-Ethinyl-Estradiol	Hormone	-	-	-	-	53.7	9.1
17 beta-Estradiol	Hormone	87.8	37.5	-	-	50.1	9.1
2-Hydroxy-ibuprofen	Non-opioid analgesic	12400.0	37.5	-	-	-	-
Acetaminophen	Non-opioid analgesic	715.0	25.0	-	-	-	-
Albuterol	Bronchodilator	28.8	50.0	-	-	-	-
Allyl Trenbolone	Hormone	-	-	9.2*	7.2	2.2*	9.1
Alprazolam	Antianxiety	39.9	62.5	-	-	-	-
Amitriptyline	Antidepressant	1740	62.5	-	-	-	-
Amlodipine	Cardiovascular modulating agent	707.0	50.0	-	-	-	-
Amphetamine	Stimulant	565.0	75.0	-	-	6.0*	18.2
Androstenedione	Hormone	1700.0	87.5	41.4	78.6	42.4	100.0
Androsterone	Hormone	425.0*	12.5	1430.0*	14.3	-	-
Atenolol	Cardiovascular modulating agent	1300.0	75.0	-	-	-	-
Atorvastatin	Cardiovascular modulating agent	544.0	50.0	-	-	-	-
Azithromycin	Antimicrobial	4800.0	62.5	-	-	-	-
Benzoylecgonine	Stimulant	60.6	87.5	-	-	-	-
Benztropine	Anticholinergic	2.1*	37.5	-	-	-	-
Bisphenol A	Plastic residue	41200.0	37.5	-	-	-	-
Caffeine	Stimulant	12200.0	62.5	-	-	-	-
Carbadox	Antimicrobial	6.8*	25.0	-	-	-	-

Carbamazepine	Antiepileptic	2300.0	100.0	66.8	21.5	-	-
Cimetidine	Antacid	85.7	50.0	-	-	-	-
Citalopram	Antidepressant	8010.0	87.5	-	-	-	-
Clarithromycin	Antimicrobial	249.0	62.5	-	-	-	-
Clinafloxacin	Antimicrobial	-	-	-	-	116.0*	18.2
Clotrimazole	Antimicrobial	17.9	37.5	-	-	-	-
Cocaine	Stimulant	259.0	87.5	-	-	-	-
Codeine	Opioid analgesic	503.0	75.0	-	-	-	-
Colchicine	Antigout	-	-	10.9*	7.2	-	-
Cotinine	Nicotene metabolyte	924.0	75.0	-	-	-	-
Cyclophosphamide	Antineoplastic	2.4*	12.5	-	-	-	-
DEET	Insect repellant	238000.0	100.0	924.0	100.0	1180.0	100.0
Dehydronifedipine	Cardiovascular modulating agent	6.2	37.5	-	-	-	-
Desmethyldiltiazem	Cardiovascular modulating agent	642.0	62.5	-	-	-	-
Desogestrel	Hormone	-	-	-	-	295.0*	9.1
Diazepam	Antianxiety	37.9	62.5	-	-	-	-
Digoxin	Cardiovascular modulating agent	33.2*	12.5	-	-	-	-
Diltiazem	Cardiovascular modulating agent	829.0	100.0	-	-	-	-
Diphenhydramine	Antihistamine	4110.0	100.0	-	-	-	-
Drospirenone	Hormone	504.0	12.5	-	-	-	-
Enalapril	Cardiovascular modulating agent	29.1	50.0	-	-	-	-
Enrofloxacin	Antimicrobial	-	-	7.3*	7.2	-	-
Equilenin	Hormone	56.6	37.5	-	-	-	-
Erythromycin-H2O	Antimicrobial	1880.0	100.0	4.8*	7.2	-	-
Estrone	Hormone	726.0	100.0	16.9*	28.6	95.1	9.1
Fluoxetine	Antidepressant	2080.0	62.5	5.6*	7.2	-	-
Furosemide	Cardiovascular modulating agent	7270.0	50.0	-	-	-	-
Gemfibrozil	Cardiovascular	8970.0	100.0	7.0*	14.3	-	-

	modulating agent						
Glipizide	Antidiabetic	194.0	50.0	-	-	-	-
Glyburide	Antidiabetic	20.5	37.5	-	-	-	-
Hydrochlorothiazide	Cardiovascular modulating agent	4870.0	62.5	-	-	-	-
Hydrocodone	Opioid analgesic	449.0	62.5	7.1*	14.3	33.0	9.1
Hydrocortisone	Hormone	236.0*	12.5	-	-	-	-
Ibuprofen	Non-opioid analgesic	36000.0	62.5	-	-	-	-
Iopamidol	Contrast agent	347.0*	12.5	-	-	-	-
Lomefloxacin	Antimicrobial	-	-	-	-	10.5*	9.1
Meprobamate	Antianxiety	548.0	37.5	-	-	-	-
Mestranol	Hormone	118.0	25.0	140.0	7.2	250.0	18.2
Metformin	Antidiabetic	429.0	62.5	-	-	-	-
Methylprednisolone	Hormone	-	-	-	-	15.5*	9.1
Metoprolol	Cardiovascular modulating agent	13100.0	100.0	-	-	-	-
Metronidazole	Antimicrobial	11.2*	12.5	-	-	-	-
Miconazole	Antimicrobial	6.1*	25.0	-	-	9.3	9.1
Naproxen	Non-opioid analgesic	60100.0	100.0	118.0*	35.8	-	-
Norfluoxetine	Antidepressant	700.0	50.0	-	-	-	-
Norverapamil	Cardiovascular modulating agent	171.0	50.0	-	-	-	-
Ofloxacin	Antimicrobial	106.0	25.0	-	-	-	-
Oxazepam	Antianxiety	233.0	62.5	-	-	-	-
Oxolinic Acid	Antimicrobial	-	-	-	-	3.3*	9.1
Oxycodone	Opioid analgesic	313.0	75.0	1.4*	7.2	-	-
Paroxetine	Antidepressant	688.0	50.0	-	-	-	-
Progesterone	Hormone	55.0	25.0	4.8*	7.2	-	-
Promethazine	Antihistamine	37.8	50.0	-	-	-	-
Propoxyphene	Opioid analgesic	9.4	75.0	-	-	-	-
Propranolol	Cardiovascular modulating agent	5990.0	75.0	-	-	-	-
Ranitidine	Antacid	310.0	50.0	-	-	-	-
Rosuvastatin	Cardiovascular	2940.0	50.0	-	-	-	-

	modulating agent						
Roxithromycin	Antimicrobial	0.7*	12.5	-	-	-	-
Sertraline	Antidepressant	4210.0	75.0	-	-	-	-
Sulfadiazine	Antimicrobial	10.7	25.0	-	-	-	-
Sulfadimethoxine	Antimicrobial	4.1*	12.5	-	-	-	-
Sulfamethazine	Antimicrobial	4.5*	12.5	-	-	-	-
Sulfamethizole	Antimicrobial	4.2	12.5	-	-	-	-
Sulfamethoxazole	Antimicrobial	1770.0	75.0	-	-	-	-
Sulfanilamide	Antimicrobial	37.7*	12.5	-	-	-	-
Sulfathiazole	Antimicrobial	8.1*	25.0	-	-	-	-
Tamoxifen	Antineoplastic	2.7	12.5	-	-	-	-
Testosterone	Hormone	204.0	50.0	-	-	-	-
Theophylline	Bronchodilator	1030.0	12.5	-	-	-	-
Thiabendazole	Antimicrobial	149.0	62.5	-	-	-	-
Triamterene	Cardiovascular modulating agent	4520.0	100.0	0.7*	7.2	-	-
Triclocarban	Disinfectant	313.0	37.5	-	-	-	-
Triclosan	Disinfectant	1830.0	37.5	-	-	-	-
Trimethoprim	Antimicrobial	8510.0	62.5	-	-	-	-
Valsartan	Cardiovascular modulating agent	24600.0	100.0	-	-	8.7*	9.1
Venlafaxine	Antidepressant	17000.0	75.0	-	-	2.1*	9.1
Verapamil	Cardiovascular modulating agent	1800.0	62.5	-	-	-	-
Warfarin	Anticoagulant	21.8	62.5	-	-	-	-

*Indicates maximum concentration is greater than the detection limit but less than the quantification limit (3x detection limit)

Table 2. Maximum concentration (conc.), Kaplan-Meier mean and standard deviation, and detection frequency (Detect. Freq.) of all detected contaminants in sediment samples from wastewater effluent-impacted, developed, and undeveloped sites. KM = Kaplan-Meier. SD = standard deviation. NA = Not applicable.

		Wastewater effluent-impacted (n=8)			Developed (n=14)			Undeveloped (n=11)		
Contaminant	**Primary use**	**Maximum conc. (ng/g)**	**KM mean (SD) conc. (ng/g)**	**Detect. freq. (%)**	**Maximum conc. (ng/g)**	**KM mean (SD) conc. (ng/g)**	**Detect. freq. (%)**	**Maximum conc. (ng/g)**	**KM mean (SD) conc. (ng/g)**	**Detect. freq. (%)**
10-hydroxy-amitriptyline	Antidepressant	8.1	2.0 (3.1)	37.5	-	-	-	-	-	-
17 alpha-Ethinyl-Estradiol	Hormone	9.1*	9.1 (NA)	12.5	-	-	-	-	-	-
Albuterol	Bronchodilator	1.1	0.6 (0.3)	25.0	-	-	-	1.0*	1.0 (NA)	9.1
Amitriptyline	Antidepressant	122.0	33.5 (48.5)	37.5	17.3	17.3 (NA)	7.2	-	-	-
Amlodipine	Cardiovascular modulating agent	52.3	15.8 (25.9)	37.5	-	-	-	-	-	-
Amphetamine	Stimulant	14.3	3.4 (5.1)	37.5	1.7*	1.7 (NA)	7.2	3.6*	2.0 (0.6)	36.4
Androstenedione	Hormone	2.8*	2.8 (NA)	12.5	-	-	-	-	-	-
Androsterone	Hormone	31.7*	31.7 (NA)	12.5	23.3*	20.9 (2.7)	21.5	86.7*	36.4 (31.2)	
Atenolol	Cardiovascular modulating agent	7.6	1.8 (2.8)	37.5	-	-	-	-	-	-
Atorvastatin	Cardiovascular modulating agent	3.1*	3.1 (NA)	12.5	-	-	-	-	-	-
Azithromycin	Antimicrobial	31.4	13.0 (14.5)	50.0	-	-	-	-	-	-
Benztropine	Anticholinergic	0.9*	0.9 (NA)	12.5	0.4*	0.4 (NA)	7.2	-	-	-
Bisphenol A	Plastic residue	-	-	-	-	-	-	733.0*	733.0 (NA)	27.3
Caffeine	Stimulant	20.3*	19.4 (0.5)	25.0	-	-	-	-	-	-
Carbamazepine	Antiepileptic	16.4	16.4 (NA)	12.5	-	-	-	-	-	-
Cimetidine	Antacid	25.1	6.4 (11.5)	37.5	-	-	-	-	-	-
Ciprofloxacin	Antimicrobial	40.8*	30.1 (9.8)	25.0	-	-	-	7.3*	7.3 (NA)	9.1
Citalopram	Antidepressant	209.0	70.0 (84.0)	50.0	27.7	27.7 (NA)	7.2	2.8	2.8 (NA)	9.1
Clarithromycin	Antimicrobial	2.4*	1.6 (0.4)	25.0	-	-	-	-	-	-
Clotrimazole	Antimicrobial	66.1	8.8 (24.2)	62.5	2.3*	1.3 (0.4)	14.3	5.6	1.1 (1.7)	9.1

Cocaine	Stimulant	0.4*	0.2 (0.1)	25.0	-	-	-	-	-	-
Cotinine	Nicotene metabolyte	3.2*	3.2 (NA)	12.5	-	-	-	1.5*	1.5 (NA)	27.3
DEET	Insect repellant	53.5	10.8 (20.9)	50.0	11.1	2.0 (2.8)	64.3	131.0	13.3 (43.0)	9.1
Desmethyldiltiazem	Cardiovascular modulating agent	1.4	0.9 (0.4)	37.5	-	-	-	-	-	-
Diltiazem	Cardiovascular modulating agent	3.2	1.4 (1.4)	37.5	-	-	-	-	-	-
Diphenhydramine	Antihistamine	265.0	107.5 (106.6)	62.5	-	-	-	-	-	-
Enrofloxacin	Antimicrobial	-	-	-	2.8*	2.8 (NA)	7.2	-	-	-
Equilenin	Hormone	2.2*	2.2 (NA)	12.5	-	-	-	-	-	-
Equilin	Hormone	15.2*	15.2 (NA)	12.5	-	-	-	-	-	-
Erythromycin-H2O	Antimicrobial	3.3*	2.5 (0.5)	50.0	-	-	-	-	-	-
Estriol	Hormone	-	-	-	-	-	7.2	38.6*	38.6 (NA)	9.1
Estrone	Hormone	19.5	19.5 (NA)	12.5	-	-	-	-	-	-
Fluoxetine	Antidepressant	61.0	17.7 (22.6)	62.5	2.5*	2.5 (NA)	7.2	1.4*	1.4 (NA)	9.1
Furosemide	Cardiovascular modulating agent	72.0*	72.0 (NA)	12.5	-	-	-	-	-	-
Gemfibrozil	Cardiovascular modulating agent	278.0	36.8 (128.9)	25.0	-	-	-	-	-	-
Hydrocodone	Opioid analgesic	2.5*	2.5 (NA)	12.5	-	-	-	3.8*	2.2 (0.7)	18.2
Ibuprofen	Non-opioid analgesic	150.0	150.0 (NA)	12.5	-	-	-	-	-	-
Metformin	Antidiabetic	36.8	10.4 (13.9)	50.0	3.1*	3.1 (NA)	7.2	-	-	-
Metoprolol	Cardiovascular modulating agent	217.0	38.3 (84.3)	50.0	5.1*	5.1 (NA)	7.2	-	-	-
Miconazole	Antimicrobial	119.0	22.3 (61.2)	25.0	1.4*	1.4 (NA)	7.2	1.8*	1.9 (NA)	9.1
Moxifloxacin	Antimicrobial	38.2*	38.2 (NA)	12.5	-	-	-	-	-	-
Norfloxacin	Antimicrobial	-	-	-	21.6*	21.6 (NA)	7.2	-	-	-

Norfluoxetine	Antidepressant	39.3	11.5 (14.0)	50.0	-	-	-	-	-	-
Norverapamil	Cardiovascular modulating agent	9.9	1.8 (3.9)	50.0	-	-	-	0.3*	0.3 (NA)	9.1
Ofloxacin	Antimicrobial	29.9	15.9 (12.2)	37.5	-	-	-	-	-	-
Oxolinic Acid	Antimicrobial	-	-	-	-	-	-	2.1*	2.2 (NA)	9.1
Oxycodone	Opioid analgesic	-	-	-	0.6*	0.6 (NA)	7.2	-	-	-
Paroxetine	Antidepressant	38.0	38.0 (NA)	12.5	-	-	-	-	-	-
Progesterone	Hormone	1.6*	1.7 (NA)	12.5	-	-	-	2.7*	1.1 (0.7)	36.4
Promethazine	Antihistamine	3.8	3.8 (NA)	12.5	-	-	-	-	-	-
Propoxyphene	Opioid analgesic	3.0	3.0 (NA)	12.5	-	-	-	-	-	-
Propranolol	Cardiovascular modulating agent	85.9	19.5 (31.3)	50.0	-	-	-	-	-	-
Ranitidine	Antacid	0.9*	0.9 (0.0)	25.0	-	-	-	-	-	-
Rosuvastatin	Cardiovascular modulating agent	4.2*	4.2 (NA)	12.5	-	-	-	-	-	-
Sertraline	Antidepressant	417.0	72.5 (158.0)	62.5	0.5*	0.5 (NA)	7.2	0.9*	0.6 (0.1)	18.2
Sulfamethoxazole	Antimicrobial	0.7*	0.7 (NA)	12.5	-	-	-	-	-	-
Sulfathiazole	Antimicrobial	1.6*	1.6 (NA)	12.5	-	-	-	-	-	-
Tamoxifen	Antineoplastic	1.4	1.4 (NA)	12.5	-	-	-	-	-	-
Testosterone	Hormone	1.6*	1.6 (NA)	12.5	-	-	-	-	-	-
Thiabendazole	Antimicrobial	8.2	3.1 (2.8)	25.0	-	-	-	-	-	-
Triamterene	Cardiovascular modulating agent	337.0	44.2 (123.7)	62.5	0.6*	0.6 (NA)	7.2	-	-	-
Triclocarban	Disinfectant	3050.0	446.4 (1081.4)	75.0	8.4*	8.4 (NA)	7.2	12.2	5.8 (2.9)	18.2
Triclosan	Disinfectant	129.0*	80.7 (21.8)	50.0	-	-	-	-	-	-
Trimethoprim	Antimicrobial	2.9*	2.2 (0.5)	25.0	-	-	-	-	-	-
Valsartan	Cardiovascular modulating	138.0	138.0 (NA)	12.5	-	-	-	-	-	-

	agent									
Venlafaxine	Antidepressant	16.1	8.1 (5.3)	62.5	-	-	-	0.8*	0.8 (NA)	9.1
Verapamil	Cardiovascular modulating agent	6.7	2.8 (3.3)	37.5	-	-	-	-	-	-

*Indicates maximum concentration is greater than the detection limit but less than the quantification limit (3x detection limit)

Table 3. Maximum concentration, Kaplan-Meier mean and standard deviation, and detection frequency of all detected contaminants in fish tissue samples fromwastewater effluent-impacted, developed, and undeveloped sites. KM = Kaplan-Meier. SD = standard deviation. NA = Not applicable.

		Wastewater effluent-impacted (n=8)			**Developed (n=14)**			**Undeveloped (n=11)**		
Contaminant	**Primary use**	**Maximum conc. (ng/g)**	**KM mean (SD) conc. (ng/g)**	**Det. freq. (%)**	**Maximum conc. (ng/g)**	**KM mean (SD) conc. (ng/g)**	**Det. freq. (%)**	**Maximum conc. (ng/g)**	**KM mean (SD) conc. (ng/g)**	**Detection frequency (%)**
10-hydroxy-amitriptyline	Antidepressant	0.2*	0.1 (0.0)	14.3	-	-	-	-	-	-
Amitriptyline	Antidepressant	0.1*	0.1 (NA)	7.1	0.5	0.2 (0.1)	20.0	0.6	0.2 (0.1)	17.6
Azathioprine	Immunosuppressant	-	-	-	1.3*	1.3 (NA)	5.0	-	-	-
Azithromycin	Antimicrobial	1.5*	1.4 (0.0)	14.3	0.8*	0.8 (0.0)	10.0	-	-	-
Betamethasone	Hormone	-	-	-	2.2*	1.4 (0.3)	10.0	0.8*	0.8 (NA)	5.9
Caffeine	Stimulant	-	-	-	74.9	74.9 (NA)	5.0	-	-	5.9
Ciprofloxacin	Antimicrobial	-	-	-	4.6*	4.6 (NA)	5.0	7.5*	7.5 (NA)	-
Citalopram	Antidepressant	-	-	-	-	-	-	0.4*	0.2 (0.1)	11.8
Cocaine	Stimulant	0.1*	0.1 (NA)	7.1	-	-	-	-	-	-
Colchicine	Antigout	-	-	-	0.4*	0.4 (NA)	5.0	-	-	-
DEET	Insect repellant	141.0	29.6 (34.8)	100	20.7	10.3 (5.6)	90.0	2450.0	202.1 (591.8)	94.1
Diatrizoic acid	Contrast agent	11.7*	11.7 (NA)	7.1	-	-	-	-	-	-
Diphenhydramine	Antihistamine	2.9	0.9 (0.8)	14.3	-	-	-	-	-	-
Doxorubicin	Antineoplastic	13.4*	13.4 (NA)	7.1	-	-	-	11.9*	11.9 (NA)	5.9
Drospirenone	Hormone	-	-	-	12.0*	12.0 (NA)	5.0	-	-	-

Enrofloxacin	Antimicrobial	1.3*	1.2 (0.0)	14.3	-	-	-	1.2*	1.2 (NA)	5.9
Etoposide	Antineoplastic	2.2*	2.2 (NA)	7.1	1.6*	1.6 (NA)	5.0	-	-	-
Fluoxetine	Antidepressant	0.8*	0.8 (NA)	7.1	-	-	-	-	-	-
Fluticasone propionate	Hormone	0.8*	0.8 (NA)	7.1	1.8*	1.2 (0.2)	20.0	1.6*	0.9 (0.3)	17.6
Hydrocortisone	Hormone	70.5*	34.6 (12.8)	42.9	53.4*	29.5 (7.4)	35.0	43.5*	30.0 (5.1)	11.8
Iopamidol	Contrast agent	103.0*	103.0 (NA)	7.1	69.6*	49.0 (6.9)	15.0	-	-	-
Melphalan	Antineoplastic	53.7*	34.2 (7.0)	21.4	73.9*	20.0 (18.8)	15.0	47.2	47.2 (NA)	5.9
Metformin	Antidiabetic	-	-	-	-	-	-	22.5	22.5 (NA)	5.9
Methylprednisolone	Hormone	-	-	-	4.4*	4.4 (NA)	5.0	-	-	-
Miconazole	Antimicrobial	1.1*	1.1 (NA)	7.1	0.8*	0.8 (NA)	5.0	-	-	-
Prednisolone	Hormone	-	-	-	4.8*	4.8 (NA)	5.0	-	-	-
Roxithromycin	Antimicrobial	-	-	-	-	-	-	0.6*	0.6 (NA)	5.9
Sarafloxacin	Antimicrobial	7.0*	7.0 (NA)	7.1	-	-	-	-	-	-
Sertraline	Antidepressant	1.0	0.7 (0.1)	14.3	0.4*	0.3 (0.0)	10.0	0.5*	0.5 (NA)	5.9
Sulfadimethoxine	Antimicrobial	0.1*	0.1 (NA)	7.1	-	-	-	-	-	-
Sulfamethizole	Antimicrobial	0.3*	0.3 (NA)	7.1	-	-	-	-	-	-
Sulfanilamide	Antimicrobial	74.5	55.5 (7.5)	14.3	49.2	33.9 (4.8)	15.0	58.7	50.6 (2.9)	11.8
Venlafaxine	Antidepressant	0.3*	0.3 (0.0)	21.4	0.2*	0.2 (NA)	5.0	0.3*	0.3 (NA)	5.9
Verapamil	Cardiovascular modulating agent	0.1*	0.1 (NA)	7.1	-	-	-	-	-	-
Virginiamycin M1	Antimicrobial	4.4*	1.8 (0.9)	28.6	1.4*	1.4 (NA)	5.0	5.3*	2.5 (0.9)	29.4

*Indicates maximum concentration is greater than the detection limit but less than the quantification limit (3x detection limit)

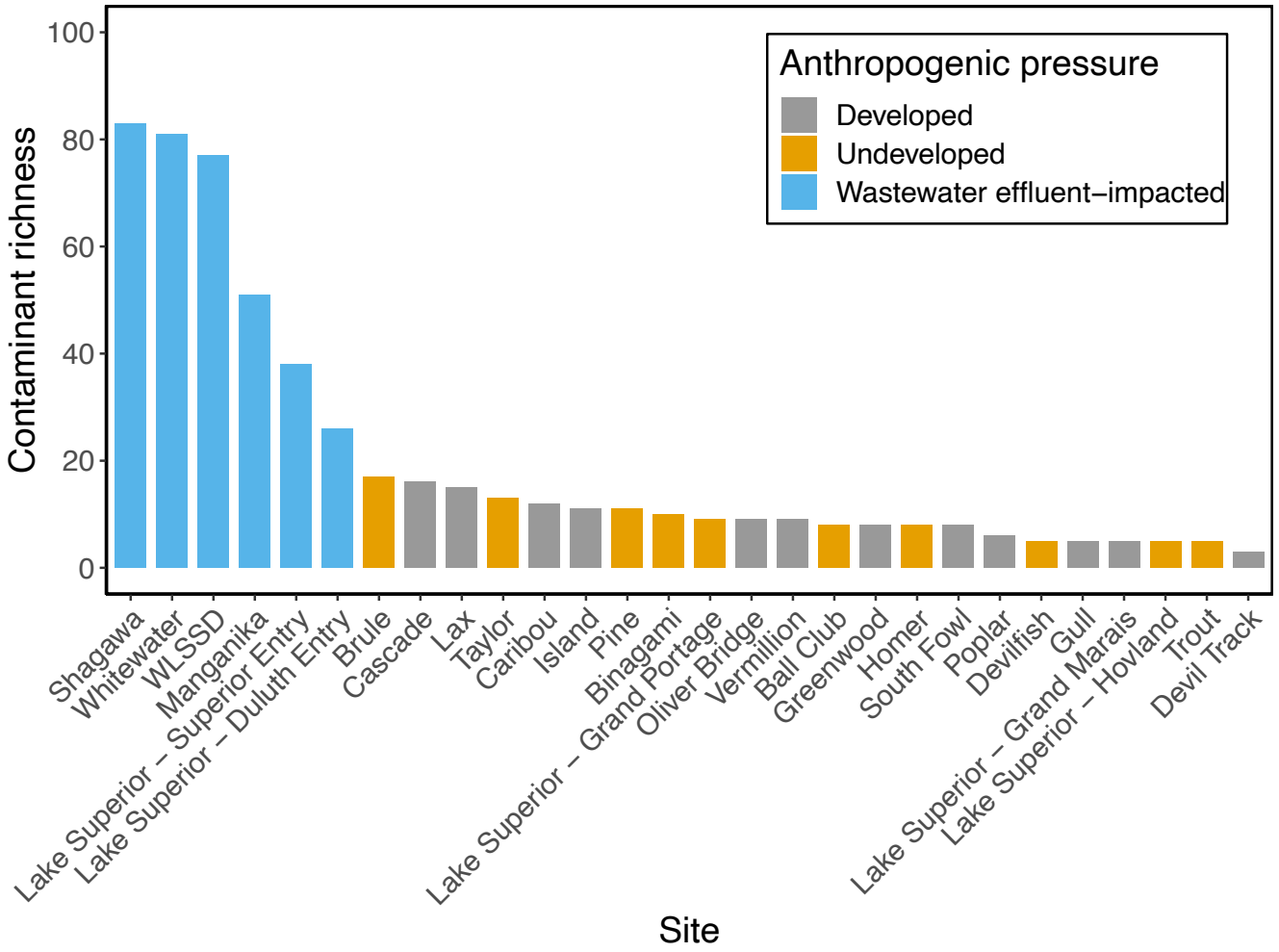

Figure 3. Number of unique contaminants across sites in northeastern Minnesota in 2016.

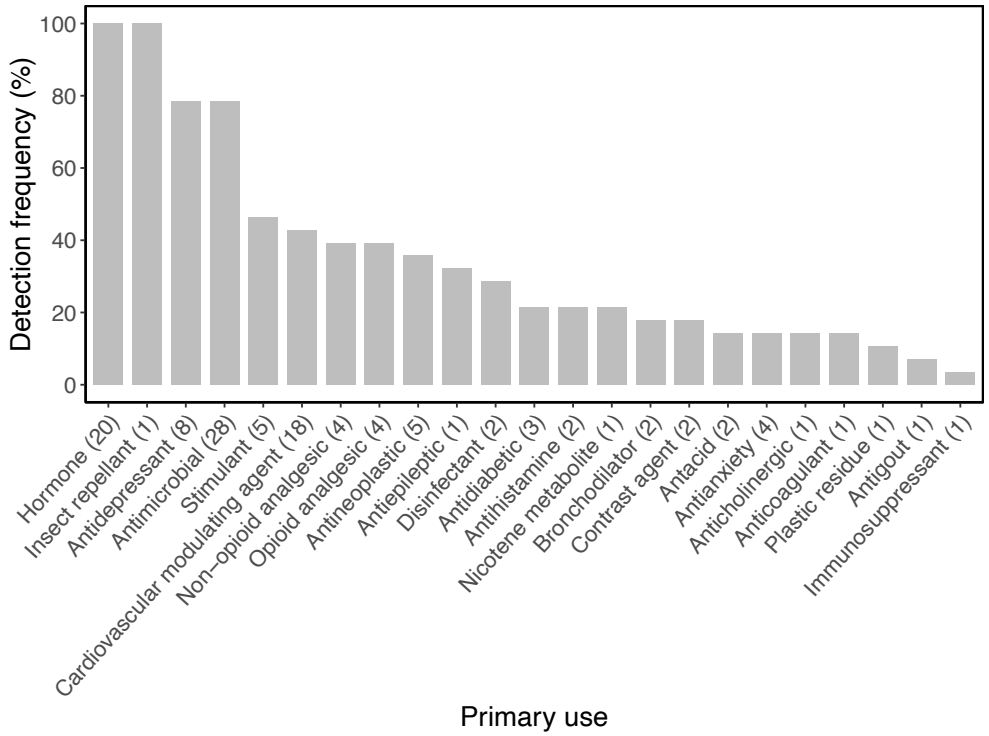

Figure 4. Frequency of contaminants detected in northeastern Minnesota in 2016 - 2018. Contaminants are grouped by primary use category. The number in parentheses represents the number of possible compounds detected in each category. If the contaminant was detected in any media at each site, it was considered a detection.

snow, and air samples in Minnesota (Ferrey et al., 2018). Hormones, a primary use category which includes endogenous hormones (e.g., estrone) and exogenous hormones, such as corticosteroids (e.g., hydrocortisone) and oral contraceptives (e.g., 17 alpha-Ethinyl-Estradiol), were also detected in all sampling sites. Antidepressants were also detected at high frequency, with all antidepressants for which we screened being detected in at leastone medium.

Three therapeutic classes of potential environmental concern – antimicrobials, antineoplastics, and cardiovascular modulating agents (Sanderson et al., 2004) – were found at a detection frequency greater than 35%. Antimicrobials were nearly ubiquitous

across all sites, with 27 of the 28 antimicrobials detected being antibiotics. While 23 antimicrobials were detected in wastewater effluent-impacted sites, 14 were detected in developed and undeveloped sites. Ciprofloxacin and enrofloxacin were detected in rain samples in Minnesota (Ferrey et al., 2018), suggesting that atmospheric wet deposition plays a role in the presence of these contaminants in sites with minimal human impact.As antibiotic resistance genes have been detected in the aquatic environment (Bueno etal., 2019), the presence of antibiotics in our study area introduces the concern that bacterial antibiotic resistance might also be induced in these aquatic systems (Pazda et al., 2019).

We found antineoplastics and cardiovascular drugs across sites of varying anthropogenic factors. Five antineoplastic drugs – cyclophosphamide, doxorubicin, etoposide, melphalan, and tamoxifen – were detected in samples of fish, water, and/or sediment. These results are in contrast to a 2014 study of 50 Minnesota river locations in which these drugs were not detected (Ferrey et al., 2017). Cardiovascular drugs were detected in all media from wastewater effluent-impacted and developed sites in this study. Eighteen cardiovascular modulating agents were detected, including lipid-lowering agents such as gemfibrozil, diuretics such as triamterene, and antihypertensive drugs such as valsartan.

Patterns across sampling sites

Patterns in detections among primary use categories varied by medium, demonstrating the importance of testing multiple sample types. In surface water, the top five categories detected were insect repellant, hormones, antimicrobials, non-opioid analgesics, and cardiovascular modulating agents **(Figure A1)**. All primary use categories except one,

immunosuppressants, were detected in surface water. Eighteen (of the 23) primary use categories were detected in sediment **(Figure A2)**. The most frequently found categories were insect repellant, antimicrobials, hormones, antidepressants, and the disinfectants triclocarban and triclosan. In fish, 12 (of the 23) primary use categories were detected **(Figure A3)**. The most frequently detected primary use categories in fish were insect repellant, hormones, antimicrobials, antidepressants, and antineoplastics. The contrast agents iopamidol and diatrizoic acid, which are used during computed tomography (CT) scans or other tissue imaging examinations (e.g. x-rays), were detected in nearly 15% of fish samples. These contrast agents, such as iopamidol, have been detected at high frequencies in rivers in Minnesota (Ferrey et al., 2017) and with a moderate bioconcentration potential (log10 bioconcentration factor≈4, U.S. EPA, 2020) in aquatic organisms, our 15% detection rate is not unexpected.

There was a significantly higher mean number of detections in water samples from wastewater effluent-impacted sites than from both developed and undeveloped sites (P = 0.0003 and P = 0.0003, respectively) **(Table 4)**. Wastewater effluent-impacted water bodies exhibited the highest number of detected CECs, ranging from 26 to 83 contaminants **(Figure 3)**, in contrast to the contaminant detection frequency at developed and undeveloped sites, which ranged from 3 to 17 contaminants. A similar pattern emerged with fish. Fish taken from wastewater effluent-impacted sites had significantly higher mean detections than those from both developed and undeveloped sites (P = 0.0039 and P = 0.0016, respectively). Sediment from wastewater effluent-impacted sites had significantly higher mean detections than sediment from developed sites (P =

0.0033). However, no significant difference in the mean number of contaminants in sediment was observed between wastewater effluent-impacted and undeveloped sites (P = 0.1856). No differences were observed in the mean number of detections for water, sediment, and fish samples between developed and undeveloped sites (P = 0.9665, P = 0.0868, and P = 0.4886, respectively).

Table 4. Mean number of contaminants detected across sites by anthropogenic pressure and sample type

	Mean detections		
	Wastewater effluent-impacted	Developed	Undeveloped
Water (POCIS)	48.6[a]	3.7	3.7
Sediment	21.4[b]	2.3	4.4
Fish	6.9[c]	4.2	3.8

[a]Significantly higher than developed (P = 0.0003) and undeveloped (P = 0.0003)
[b]Significantly higher than developed (P = 0.0033)
[c]Significantly higher than developed (P = 0.0039) and undeveloped (P = 0.0016)

Across all media, 85 CECs were detected in wastewater effluent-impacted sites that werenot detected in developed and undeveloped sites. Twenty-two CECs were detected in developed and undeveloped sites that were not detected in wastewater effluent-impacted sites, including the following primary use categories: antidepressant, antidiabetic, antigout, antimicrobial, hormone, immunosuppressant, opioid analgesic, plastic residue, and stimulant. Among these, nine CECs were detected in water, six in sediment, and 11 in fish tissue. We would expect POCIS samplers to yield more detections of hydrophilic compounds whereas fish and especially sediment samples would be expected to contain more lipophilic organic compounds. Fish also allow the detection of pseudo-persistent compounds (rapidly metabolized, but commonly detected due to their high presence in water) that may be consumed by humans or wildlife. While we expected the distribution of the chemicals across media to conform to what can be

predicted based on physicochemical properties, such as the octanol-water partition coefficient (logP) (Amézqueta et al., 2020), logP did not influence the detection rate (Fisher's exact test P =0.304; see **Supplementary Table S4** for batch logP values). The number of hydrophilic (i.e., logP < 3.5) compounds was approximately two to four times the number of hydrophobic (i.e., logP ≥ 3.5) compounds in all media. As logP was not associated with detection patterns across the three media, it may be of limited usefulness as an *a priori* indicator in designing or interpreting CEC occurrence studies. This finding further demonstrates the importance of deploying multiple detectors for a broad assessment of CEC occurrence. More work is needed to better understand the occurrence patterns foundin this study.

The effects of the chemicals detected in this study on aquatic biota are not well understood, and an assessment of the risk they pose at the concentrations reported hereare beyond the scope of this occurrence study. However, several studies have demonstrated that very low concentrations of pharmaceuticals can affect aquatic organisms at the genetic, physiological, or behavioral level, including bivalves (Abdelhafidh et al., 2017; Binelli et al., 2009; Fong, 1998; Parolini & Binelli, 2012; Parolini et al., 2011), snails (Fong & Ford, 2014), brown trout (Hoeger et al., 2005), zebrafish (Irons et al., 2010), fathead minnow (Painter et al., 2009), and water fleas (Laëtitia Minguez et al., 2015). More chemical specific and chemical mixture work is needed with wild fish to understand the organismal to population level effects of the chemical concentrations found in this study.

Limitations and future directions

Having only POCIS concentrations, and not water concentrations, limits comparability to other studies in which water concentrations were measured directly; however, we chose this approach because it shows a more complete picture of the range of chemicals that were present in water over the entire interval of POCIS deployment, notwithstanding the unknown variability in sorption and desorption. The drawback of this approach is that the concentration data provided here are at best a rough estimate, as the amount of water that comes into contact with the POCIS over the deployment interval is unknown. Similarly, testing for CECs in whole fish samples, versus filet-only, does not provide a clear picture of which chemicals humans may be consuming. The detections reported here demonstrate the patterns of fish exposure to CECs at these locations, which may be important not only to the health of the aquatic ecosystem but also for food security. Further sampling and analyses are needed to understand the impacts of these chemicals in this ecosystem.

In order to fulfill the goals of the ESH approach initiated with this study, future work will involve a detailed ecological risk assessment of detected CECs with prioritization of these sites based on CEC concentration and mixtures of contaminants. More evaluation of the effects that CECs have on fish health is needed, as well as a further understanding of the fate of these contaminants and how they attenuate in the aquatic environment over time.

Conclusion

This study - comprising samples of water (POCIS), sediment, and fish from 28 locations -

provides insight to the spatial distribution of pharmaceuticals, personal care products, and other micro-contaminants across environmental compartments in a boreal aquatic ecosystem in northeastern Minnesota. We identified CECs in remote, undeveloped locations, which suggests that sources of contamination to surface water extend beyond the direct influence of wastewater treatment plants or septic systems. These data demonstrate that a wide variety of CECs are present in aquatic ecosystems utilized by the Grand Portage Band of Lake Superior Chippewa for subsistence. The effects of these chemicals in this ecosystem are currently unknown.

Acknowledgements

We are grateful to the Grand Portage Reservation Tribal Council and Tony Swader for their continued support of this ongoing research. We thank the participants of the initial workshop that initiated the project. Many thanks to Leila Knoll and Aidan Neher for assistance in the field. We thank Ben Blair for insight and direction on manuscript development and Dennis Helsel for assistance on Kaplan-Meier statistics. Funding support comes from University of Minnesota College of Veterinary Medicine's One Medicine One Science Signature Programs grant, the Environmental Protection Agency's Great Lakes Restoration Initiative, and the Environment and Natural Resources Trust Fund as recommended by the Legislative-Citizen Commission on Minnesota Resources.

Chapter 3

A chemical prioritization process: Applications to contaminants ofemerging concern in freshwater ecosystems (Phase I)*

*Chapter published as: **Deere JR**, Streets S, Jankowski MD, Ferrey M, Chenaux-Ibrahim Y, Convertino M, Isaac EJ, Phelps NBD, Primus A, Servadio JL, Singer RS, Travis DA, Moore S, Wolf TM. (2021). A chemical prioritization process: Applications to contaminants of emerging concern in freshwater ecosystems (Phase 1). *Science of the Total Environment,* 772(146030), 1-11. https://doi.org/10.1016/j.scitotenv.2021.146030

Accompanying (Phase II) manuscript published as: Servadio JL, **Deere JR**, Jankowski MD, Ferrey M, Isaac EJ, Chenaux-Ibrahim Y, Primus A, Convertino M, Phelps NBD, Streets S, Travis DA, Moore S, Wolf TM. (2021). Anthropogenic factors associated with contaminants of emerging concern detected in inland Minnesota lakes (Phase II). *Science of the Total Environment,* 772(146188), 1-14. https://doi.org/10.1016/j.scitotenv.2021.146188

Overview

Contaminants of emerging concern (CECs), such as pharmaceuticals, personal care products, and hormones, are frequently found in aquatic ecosystems around the world. Information on sublethal effects from exposure to commonly detected concentrations of CECs is lacking and the limited availability of toxicity data makes it difficult to interpret the biological significance of occurrence data. However, the ability to evaluate the effects of CECs on aquatic ecosystems is growing in importance, as detection frequency increases. The goal of this study was to prioritize the chemical hazards of 117 CECs detected in subsistence species and freshwater ecosystems on the Grand Portage Indian Reservation and adjacent 1854 Ceded Territory in Minnesota, USA. To prioritize CECs for management actions, we adapted Minnesota Pollution Control Agency's Aquatic

Toxicity Profiles framework, a tool for the rapid assessment of contaminants to cause adverse effects on aquatic life by incorporating chemical-specific information. This study aimed to 1) perform a rapid-screening assessment and prioritization of detected CECs based on their potential environmental hazard; 2) identify waterbodies in the study region that contain high priority CECs; and 3) inform future monitoring, assessment, and potential remediation in the study region. In water samples alone, 50 CECs were deemed high priority. Twenty-one CECs were high priority among sediment samples and seven CECs were high priority in fish samples. Azithromycin, DEET, diphenhydramine, fluoxetine, miconazole, and verapamil were high priority in all three media. Due to the presence of high priority CECs throughout the study region, we recommend future monitoring of particular CECs based on the prioritization method used here. We present an application of a chemical hazard prioritization process and identify areas where the framework may be adapted to meet the objectives of other management-related assessments.

Fish are a primary subsistence food used by the Anishinaabeg (people) of Grand Portage Band of Lake Superior Chippewa historically and presently and thus sets the context for this paper exploring potential impacts of contaminants on this culturally important resource. The Grand Portage Band is a federally recognized Indian tribe in extreme northeastern Minnesota and proudly exercises its rights to food sovereignty through subsistence hunting and fishing.

Introduction

Contaminants of emerging concern (CECs) are a diverse group of chemicals – often defined as chemicals that were previously unknown, unrecognized, or unregulated – that are generally poorly understood with respect to transport, fate, and toxicity in the environment (Nilsen et al., 2019). Many of these chemicals, including pharmaceuticals, personal care products, and hormones, are widely detected in aquatic environments and have been found at toxicologically relevant concentrations in wastewater and other areas with high human disturbance (Blair et al., 2013; Fairbairn et al., 2018; Kiesling et al., 2019) as well as in remote, less developed regions (Deere et al., 2020; Elliott & VanderMeulen, 2017; Ferrey et al., 2015; 2020). Typical wastewater treatment plant (WWTP) technologies are not designed to remove CECs; thus, most CECs remain in wastewater effluent after treatment (Rizzo et al., 2019). While WWTPs are often considered the main anthropogenic point source of CECs to surface waters, the occurrence of CECs in remote areas has only recently been investigated. Atmospheric transport of these CECs and their deposition through precipitation likely play a role in the appearance of CECs in remote locations (Ferrey et al., 2018). With growing evidence that nonpoint sources of pollution contribute to CEC presence in rural environments, assessing the effects they might have on aquatic systems is important.

While data regarding acute lethality from high concentrations of some CECs in aquatic organisms does exist (Fent et al., 2006; Santos et al., 2010), information on sublethal effects, such as neuroendocrine or immune effects, due to exposure to concentrations that are commonly detected in the environment is generally lacking (Nilsen et al., 2019). In

addition to effects from the acute or chronic exposure to single CECs, cumulative effects from exposure to mixtures are also of concern, especially when physiological effects become demographically and ecologically significant (Adeel et al., 2017; Thelusmond et al., 2018). The ability to evaluate the individual and interactive effects of CECs on fish, aquatic ecosystems, and ultimately all forms of life is critical, particularly as the frequency of their occurrence continues to rise in natural waterbodies around the world (Baker & Kasprzyk-Hordern, 2013; Battaglin et al., 2018). However, the limited availability of toxicity data leading to the lack of regulatory or screening values for most CECs makes it difficult to interpret the biological significance of occurrence data.

A study initiated by the Grand Portage Band of Lake Superior Chippewa to explore potential contaminant threats to their natural resources and subsistence species found 117 CECs in aquatic environments on the Grand Portage Indian Reservation (GPIR) and adjacent 1854 Ceded Territory in Minnesota, USA (Deere et al., 2020). Its findings raised additional questions about the sources of these chemicals and about their potential hazards on the biological systems of subsistence fish species on which the Tribe depends and to which the Tribe's culture is inextricably linked. The goal of this study is to prioritize the 117 detected chemicals by the potential hazards they pose to subsistence species and aquatic ecosystems and, thus, to the Ojibwe culture and way of life.

Minnesota Pollution Control Agency's Aquatic Toxicity Profiles (ATPs) (Streets & Dobbins, 2017) is a rapid assessment tool that incorporates chemical-specific information, including acute toxicity, endocrine activity, physicochemical properties, and

frequency of occurrence data, in the evaluation of the potential for environmental contaminants to cause adverse effects on aquatic life. Given the conservative thresholds employed, this non-regulatory screening tool conservatively estimates the potential for a chemical to be hazardous in a way that guards against type II errors (i.e., to falsely conclude no potential adverse effects). The process is primarily anchored in adverse effects at the level of the organism, which is different from other recent CEC prioritization processes based on bioactivity at the molecular level (Corsi et al., 2019). We adapted the ATP framework to 1) perform a rapid-screening assessment and prioritization of detected CECs based on their potential environmental hazard; 2) identify waterbodies in the study region that contain high priority CECs; and 3) inform future monitoring, assessment, and potential remediation in the study region. Here, priority is a relative term in which detected contaminants are ranked against one another based on available information. We hypothesized that, through this framework of prioritization, a subset of higher priority chemicals would be identified. In so doing, research and policy decisions may be more tenable. We present a research-focused application of a chemical hazard prioritization process and identify areas where the framework may be adapted to meet the objectives of other management-related assessments.

Methods

Study design

Study region, site selection, sample collection, and analytical procedures were described previously (Deere et al., 2020). Briefly, we surveyed the presence of CECs intwo regions in northeastern Minnesota, the GPIR and the 1854 Ceded Territory. Across three years

(2016-2018), we sampled water, sediment, and fish at sites categorized by anthropogenic pressure: 1) wastewater effluent-impacted, which included sites that received discharge from a wastewater treatment plant; 2) developed, which included siteswith any level of shoreline development, including human residences and business; and 3) undeveloped, which included sites with no shoreline development. Candidate sites were selected based on land use, proximity to potential point sources, presence of subsistence fish species, and importance of the location for fish harvest by tribal members. Sites were further selected based on multi-criteria decision analysis (Convertino & Valverde, 2013; Deere et al., 2020). We sampled 14 waterbodies in 2016, 19 in 2017, and 2 in 2018, leading to a total of 28 unique aquatic sampling locations across all years. We collected a total of 33 water and sediment samples each and 51 fish tissue samples. Sites included inland lakes as well as locations along the Lake Superior northwestern shore. Samples were collected using Polar Organic Chemical Integrative Samplers (POCIS) for water and grab samples for sediment. POCIS are passive samplersthat represent the respiratory and dermal exposure of aquatic organisms to dissolved chemicals over a given time period. We utilized a combination of methods to collect fishsamples, including boat-operated electrofishing and gill nets, targeting important subsistence and recreational fish species of different trophic levels. Complete site selection and sampling details are described in Deere et al. (2020).

Chemical prioritization

Complete ATP methods are described in Minnesota's Aquatic Toxicity Profiles: Methods and application (Streets & Dobbins, 2017). Briefly, the ATP process applies the assembly

of data from a combination of publicly available databases, modeling tools, monitoring data, and limited literature searches to characterize chemicals on the basis of production volume, persistence and prevalence in the environment, potential for accumulation, and biological effects such as lethality and endocrine disruption. Each of these parameters is captured categorically in a yes/no question format, where a "yes" answer receives a score of 1 and "no" answer receives a score of 0. The scores are then summed by chemical to provide an overall priority level. The ATP questions include: 1) Is the contaminant persistent in the environment?; 2) Does the contaminant have the potential to accumulate?; 3) Is the chemical toxic?; 4) Do detected concentrations exceed toxicity?; 5) Is there evidence of endocrine disruption?; and 6) Is this a high production volume chemical?

We adapted these questions to meet the objectives of the current study. ATPs look at a multitude of factors, some of which are not toxicity; therefore, we focused on utilizing ATPs for hazard identification. We created chemical profiles for CECs detected in each medium (i.e., water, sediment, and fish separately), as well as profiles for CECs detected in water, sediment, or fish ("any media" category). Some questions were adjusted based on availability of data. For example, question 4 (Do detected concentrations exceed toxicity?) could only be answered with fish tissue data and not water data. We did not have POCIS sampling rates, which are necessary to calculate water concentrations from POCIS measurements (Godlewska et al., 2020).

The priority level is based on six questions for water and fish and five questions for

sediment and "any media" (**Table 5**). Therefore, CECs detected in water and fish can have a priority level of up to six and those detected in sediment or "any media" can havea priority level of up to 5. The priority levels range from high (receiving a score of 4-6), intermediate (a score of 3), or low (scores of 0-2). Available data among prioritization questions were inconsistent; therefore, a low priority level might reflect lack of knowledge about a particular chemical rather than lack of concern. This interpretation reflects the focus of prioritizing CECs for advising management decisions rather than on identifying research needs.

Table 5. Questions answered to prioritize contaminants (adapted from the original ATP framework). If the answer to the question is "yes" it receives a score of 1; if "no" it receives a score of 0. The higher the score,the higher the priority level. $T_{1/2}$ = chemical half-life. pKa = acid dissociation constant. TSC = toxic tissue screening concentration.

Media	Question	Criteria	Data Source
All	Is the contaminant persistent in the environment?	$T_{1/2}$ water > 2 months $T_{1/2}$ sediment > 6 months $T_{1/2}$ soil > 6 months $T_{1/2}$ air > 2 day	EPI Suite
All	Does the contaminant have the potential to accumulate?	log10 $K_{OW} \geq 4$ log10 $K_{OC} \geq 3$	EPI Suite
All	Is the chemical's aqueous toxicity high?[a]	Acute toxicity value (µg/L) < 10,000 Chronic toxicity value (µg/L) < 100	ECOSAR[c]
All	Is there evidence of potential endocrine disruption?	Any active estrogen, androgen, or thyroid assays	EDSP21
All	Is the contaminant detected in more than 20% of samples?	Detected in at least 6/28 sites (any media) 7/33 samples (water and sediment) 11/51 samples (fish)	Current study; Deere et al. 2020
Water	Is the chemical neutral at the pH of the water > 10% of the time?	Ionization ratio < 1	CompTox Chemistry Dashboard
Fish	Do fish tissue concentrations exceed toxicity thresholds?[b]	Maximum concentration > TSC	ECOSAR and CompTox Chemistry Dashboard

[a]Chemical toxicity expressed as aqueous toxicity in order to provide a standardized ranking systemirrespective of the media in which it was detected.

[b]This question is typically asked of all media, but due to availability of data we answered this question forfish only, based on an adapted method.
[c]ECOSAR toxicity is based on narcosis through aqueous exposure; thus, does not represent all forms oftoxicity

Prioritization 1: Is the contaminant persistent in the environment?

To determine whether each contaminant is predicted to rapidly biodegrade or if it is persistent in the environment, we used the U.S. Environmental Protection Agency (EPA) Estimation Programs Interface (EPI) Suite (U.S. EPA, 2020a), a freely available tool containing physicochemical property and environmental fate models for organic chemicals. EPI Suite has undergone thorough review by a panel of EPA's independent Science Advisory Board (Morgan & McFarland, 2007). Persistence, in the context of this prioritization method, is described as the half-life of the chemical in water, sediment, soil, or air (Webster et al., 1998). To interpret degradation potential, we used the following half-life ($t_{1/2}$) thresholds in water, sediment, soil, and air: $t_{1/2}$ in water > 2 months, $t_{1/2}$ in sediment > 6 months, $t_{1/2}$ in soil > 6 months, and $t_{1/2}$ in air > 2 days, respectively (Streets & Dobbins, 2017). If there was evidence of half-lives exceeding anyof these thresholds or if the chemical was not predicted to be readily biodegradable in EPI Suite, the contaminant was considered persistent in the environment, thus receiving ascore of 1. Half-lives were predicted using the LEV3EPI^{TM} program that contains a level III multimedia fugacity model (Parnis & Mackay, 2021).

Prioritization 2: Does the contaminant have the potential to accumulate?

Bioaccumulation and sediment accumulation potential were determined using octanol-water and organic carbon-water partition coefficients (K_{OW} and K_{OC}, respectively). Partition coefficient values were obtained from EPI Suite. A chemical was considered to

have the potential to accumulate in biota and/or sediment if it had measured or predicted partitioning properties that exceeded the following guidelines: log10 $K_{OW} \geq 4$ or log10 $K_{OC} \geq 3$ (United Nations, 2003; OECD, 2001). If there was evidence of measured or predicted partitioning properties that exceeded either of these thresholds, the chemical received a score of 1.

Prioritization 3: Is the chemical's aqueous toxicity high?

As this method is intended to be rapid and because measured toxicity data (from peer-reviewed literature, government documents, or EPA's ECOTOX (https://cfpub.epa.gov/ecotox/)) were not available for many of the detected chemicals in this study, we used modeled toxicity values from EPA's ecological structure activity relationships (ECOSAR) to assess toxicity of each contaminant. ECOSAR is a QSAR model that uses lipid solubility (i.e., K_{OW}) to predict a chemical's acute and chronic toxicity to fish and other aquatic organisms based primarily on a narcotic mode of action (Mayo-Bean et al., 2012). Narcosis (i.e., baseline toxicity) is a common mode of action in approximately 40% of organic chemicals (Kienzler et al., 2019). ECOSAR training sets include chemicals with log K_{OW} values in the range of -3 to 8 and molecular weights less than 1000; therefore, the chemicals in this study are included in ECOSAR's domain applicability (**Tables S7 - S10**) (Mayo-Bean et al., 2012). Further, ECOSAR models for other toxicities (in addition to narcosis), including specifically acting organic chemicals causing "excess toxicity" and surface-active compounds.

Although ECOSAR is recognized as a robust quantitative structure-activity relationship

(QSAR) for aquatic toxicity, we intended for this process to be used for screening purposes; thus, we took steps to check the likelihood that our screening method was conservative (i.e., reduced the chance for a misclassification error by placing a chemical with high toxicity into a lower toxicity category). We therefore included a supplementary assessment with the toxicity estimation software tool (TEST) (https://www.epa.gov/chemical-research/toxicity-estimation-software-tool-test). Like ECOSAR, TEST is a QSAR, but it incorporates additional molecular descriptor methods for toxicity estimation including hierarchical method, nearest neighbor method, and more (Martin, 2016). We used the predicted toxicity values from the consensus method, which is an average of predicted toxicities from multiple QSAR methods.

The ECOSAR-based toxicity assessment was used for chemicals detected in any media in order to standardize the relative toxicity ranking system. Acute values were classified as "very toxic" if ≤ 1,000 "g/L or "toxic" if > 1,000 to ≤ 10,000 "g/L. Chronic values were classified as "very toxic" if ≤ 10 "g/L or "toxic" if > 10 to ≤ 100 "g/L (Streets & Dobbins, 2017). Therefore, if the acute toxicity value was ≤ 10,000 "g/L and/or the chronic toxicity value was ≤ 100 "g/L, then the chemical was classified as "toxic." Acute effects were obtained from the lowest values from fish (96-hour) or daphnid (48 hour) lethal concentration 50% (LC50) values. Chronic effects were obtained from the lowest values from fish or daphnid Chronic Values (ChV). Values used and their ECOSAR class are provided in **Table S7**.

The acute toxicity thresholds were obtained from internationally harmonized

classification systems (United Nations, 2003; OECD, 2001). Harmonized classification systems for chronic effects are not available, so the categories used for chronic values were derived from the acute categories. Chronic effects typically occur at concentrations lower than acute effects (U.S. EPA OW/ORD Emerging Contaminants Workgroup, 2008), and many studies have evaluated the ratio between acute and chronic effects. The acute-to-chronic ratio varies depending on the species and chemical tested and can encompass a wide range of values. An acute-to-chronic extrapolation of 100 has been demonstrated to be protective for greater than 90% of evaluated chemicals, while an acute-to-chronic extrapolation of 10 may only be protective for approximately 50% of chemicals (May et al., 2016). Similar results have been reported with a 90th percentile of acute-to-chronic ratios close to 100 (73-80) (Lange et al., 1998; Raimondo et al., 2007). An acute-to-chronic conversion of 100 was used in this preliminary screening.

Prioritization 4: Is there evidence of potential endocrine disruption?

The presence of contaminants in aquatic systems may disrupt the endocrine system of organisms at concentrations lower than what may cause toxic effects such as death or decreased growth (Niemuth & Klaper, 2018; Jiaying Wang et al., 2018). Therefore, we utilized the EPA's Endocrine Disruptor Screening Program (EDSP) to review any potential endocrine effects. EDSP data can be accessed through EPA's Chemistry Dashboard (U.S. EPA, 2020b); specifically, the EDSP21 section under the "Bioactivity" tab of the Dashboard. If there was any evidence of activity in the assays, it was considered evidence of potential for endocrine disruption and the chemical received a score of 1. The EDSP assesses chemicals for endocrine-related activity. The activity of a

chemical in a specific assay does not relate to whole organism toxicity, but rather the potential for the chemical to affect endocrine pathways, which may induce an adverse health outcome.

Prioritization 5: Is the contaminant detected in more than 20% of samples?

The original ATP framework prioritizes chemicals often in the absence of occurrence data; thus, it relies on the EPA (U.S. EPA, 2020c) and Organization for Economic Co-operation and Development (OECD, 2009) lists of high production volume chemicals as data sources to characterize the likelihood that a chemical will be present in the environment. Both sources identify chemicals that are produced in or imported to the U.S. at a rate of at least 1 million pounds per year. If a chemical is included on either or both of these lists, then it would receive a score of 1. To evaluate our site-specific occurrence data, we modified this question to ask: "Is the contaminant detected in more than 20% of samples?" For the "any media" priority level, we assessed detection frequency by site; if the chemical was detected in at least one medium per site, then this question was given an answer of "yes." Therefore, if a contaminant was detected in any media at six or more of the 28 sites we examined in our study, it was given a score of 1, indicating that it was detected more frequently than other contaminants. For individual medium (water, sediment, and fish) priority levels, if the chemical was detected in the respective medium samples at a frequency of at least 20%, then this question was given a score of 1 for that chemical.

If we used the original ATP question, "Is this a high production volume chemical?,"

some of the results might have changed. For example, caffeine was detected in approximately 15% of water samples, so was given a score of 0 in the modified question. However, caffeine is listed as a high production volume chemical so it would have been given a score of 1 using the original ATP question. Whereas gemfibrozil, which was detected in approximately 30% of water samples and was given a score of 1 for our chemical profile, is not listed as a high production volume chemical so would have been given a score of 0 using the original ATP question.

Prioritization 6: Is the chemical neutral at the pH of the water > 10% of the time?

The aquatic toxicity of ionizable organic compounds, such as most pharmaceuticals, is dependent on water pH (Escher et al., 2020). Chemicals that are neutral at the pH of the water containing them are more likely to be absorbed by aquatic organisms than chemicals that are not neutral because of their increased tendency to cross cell membranes (Alsop & Wilson, 2019). The acid dissociation constant (pKa) of the chemical indicates whether the chemical will be neutral at a given pH (Babić et al., 2007). To determine whether a chemical was neutral at the pH of the water, which was measured at the time of sampling, we calculated an ionization ratio (or acid/base ratio) using available pH data from the study lakes at time of sampling and pKa estimates obtained from EPA's Chemistry Dashboard (U.S. EPA, 2020b), as modeled using OPERA (Mansouri et al., 2019):

$$\frac{A^-}{AH} = 10^{pH\ -pKa}$$

where A^- is the concentration of the conjugate base and AH is the concentration of the conjugate acid. If the ionization ratio was less than 1, then the chemical was considered neutral. The estimate is conservative, as the ionization state is a continuum rather than a threshold phenomenon. This ATP question was applied only to chemicals detected in water samples. Further, pH data was available for water sampled in 2017 but not 2016 or 2018, which included 19 of 28 sites. While most of the chemicals detected in 2017 were also detected in 2016 and 2018, we were not able to assess the ionization potential for 12 chemicals either because we did not have pH data (6 chemicals) or pKa estimates (6 chemicals). Based on the available data, if a chemical was neutral at least 10% of the time, then it was given a score of 1 for this question.

Prioritization 7: Do fish tissue concentrations exceed toxicity thresholds?

Tissue toxicity thresholds for our detected CECs were limited. Therefore, to determine if fish tissue concentrations may have exceeded toxicity thresholds, we estimated tissue screening concentrations (TSC) (Dyer et al., 2000). The TSC (µg/kg) for each chemical is a product of the chronic toxicity value (µg/L) and the bioconcentration factor (BCF) (L/kg):

$$TSC = chronic\ toxicity\ value * BCF$$

BCF values (the ratio of the concentration of the chemical in fish tissue to the concentration in water) were obtained from the Chemistry Dashboard (U.S. EPA, 2020b). Bioaccumulation factor (BAF) estimates could also be used in this context (Costanza et al., 2012); however, as BAF and BCF values did not yield different rankings, we chose to use BCF values in this evaluation. Additionally, as empirical (i.e., not modeled) BCF

values are experimentally determined using standard protocols, they are more readily comparable across chemicals than BAF values.

Statistical analysis

All data analysis was performed with R Version 4.0.2 (R Core Team, 2020). Following a significant Kruskal-Wallis test, Dunn's test of multiple comparisons was performed post-hoc to explore differences between priority levels and anthropogenic pressure categories, using the *dunn.test* package (Dinno, 2017). *P*-values were adjusted using the Benjamini-Hochberg method (Benjamini & Hochberg, 1995). We assessed correlation among profile questions using Spearman's rho rank correlation coefficients ("Spearman Rank Correlation Coefficient," 2008) in the *Hmisc* package (Harrell, 2020) and created correlation plots in the *corrplot* package (Wei & Simko, 2017).

As part of the descriptive summary, we assigned all detected contaminants to primary use categories, as previously described (Deere et al., 2020). Briefly, we used the World Health Organization (WHO) Anatomical Therapeutic Chemical classification system (WHO, 1993). Chemicals were first classified based on their anatomical or pharmacological groups and then assigned into the primary use categories we created.For those chemicals not in the WHO database, we classified them according to their classification in published literature.

Results

Chemical profiles were assembled for 117 CECs detected in aquatic systems in 28

northeastern Minnesota waterbodies (lakes) (**Tables S8-S10**). Across any media (water, sediment, and/or fish), 38 chemicals were deemed high priority (**Table S11**). In water (POCIS) samples alone, 50 CECs received a high priority level (**Table 6**). Twenty-one contaminants ranked as high priority among sediment samples (**Table 7**), and seven chemicals were high priority in fish samples (**Table 8**).

Table 6. High priority contaminants detected in water samples collected through POCIS. Maximumpossible priority level = 6.

Chemical	**Primary use**	**Priority level**
Diphenhydramine	Antihistamine	6
Estrone	Hormone	6
17 alpha-Estradiol	Hormone	5
17 beta-Estradiol	Hormone	5
Atorvastatin	Cardiovascular modulating agent	5
Benztropine	Anticholinergic	5
Bisphenol A	Plastic residue	5
Citalopram	Antidepressant	5
Fluoxetine	Antidepressant	5
Gemfibrozil	Cardiovascular modulating agent	5
Hydrocodone	Opioid analgesic	5
Metoprolol	Cardiovascular modulating agent	5
Paroxetine	Antidepressant	5
Roxithromycin	Antimicrobial	5
Tamoxifen	Antineoplastic	5
Triclosan	Disinfectant	5
Verapamil	Cardiovascular modulating agent	5
17 alpha-Ethinyl-Estradiol	Hormone	4
Albuterol	Bronchodilator	4
Allyl Trenbolone	Hormone	4
Alprazolam	Antianxiety	4
Amitriptyline	Antidepressant	4
Amlodipine	Cardiovascular modulating agent	4
Amphetamine	Stimulant	4
Androstenedione	Hormone	4
Azithromycin	Antimicrobial	4
Carbamazepine	Antiepileptic	4
Clotrimazole	Antimicrobial	4
Cocaine	Stimulant	4
Codeine	Opioid analgesic	4
DEET	Insect repellant	4
Desogestrel	Hormone	4

Diltiazem	Cardiovascular modulating agent	4
Equilenin	Hormone	4
Glyburide	Antidiabetic	4
Mestranol	Hormone	4
Miconazole	Antimicrobial	4
Progesterone	Hormone	4
Promethazine	Antihistamine	4
Propoxyphene	Opioid analgesic	4
Propranolol	Cardiovascular modulating agent	4
Ranitidine	Antacid	4
Sertraline	Antidepressant	4
Sulfamethazine	Antimicrobial	4
Sulfathiazole	Antimicrobial	4
Testosterone	Hormone	4
Triamterene	Cardiovascular modulating agent	4
Triclocarban	Disinfectant	4
Trimethoprim	Antimicrobial	4
Venlafaxine	Antidepressant	4

Table 7. High priority contaminants detected in sediment samples. Maximum possible priority level = 5.

Chemical	**Primary use**	**Priority level**
Clotrimazole	Antimicrobial	5
Fluoxetine	Antidepressant	5
Triclocarban	Disinfectant	5
17 alpha-Ethinyl-Estradiol	Hormone	4
Androsterone	Hormone	4
Atorvastatin	Cardiovascular modulating agent	4
Azithromycin	Antimicrobial	4
Benztropine	Anticholinergic	4
Bisphenol A	Plastic residue	4
DEET	Insect repellant	4
Diphenhydramine	Antihistamine	4
Estrone	Hormone	4
Gemfibrozil	Cardiovascular modulating agent	4
Miconazole	Antimicrobial	4
Paroxetine	Antidepressant	4
Progesterone	Hormone	4
Promethazine	Antihistamine	4
Sertraline	Antidepressant	4
Tamoxifen	Antineoplastic	4
Triclosan	Disinfectant	4
Verapamil	Cardiovascular modulating agent	4

Table 8. High priority contaminants detected in fish tissue samples. Maximum possible priority level = 6.

Fish	Primary use	Priority level
DEET	Insect repellant	5
Azithromycin	Antimicrobial	4
Diphenhydramine	Antihistamine	4
Fluoxetine	Antidepressant	4
Miconazole	Antimicrobial	4
Roxithromycin	Antimicrobial	4
Verapamil	Cardiovascular modulating agent	4

We grouped the detected CECs into 23 primary use categories to assess the number of high priority contaminants within primary use categories **(Table 9)**. For high priority contaminants, there was more diversity among primary use categories detected in water (n=17) than in sediment (n=10) or fish (n=5).

Table 9. Number of high priority contaminants (priority level 4, 5, or 6) in each primary use category andmedia. N = the number of possible compounds detected in each category.

Primary use category	N	Water	Sediment	Fish
Antacid	2	1	0	0
Antianxiety	4	1	0	0
Anticholinergic	1	1	1	0
Anticoagulant	1	0	0	0
Antidepressant	8	6	3	1
Antidiabetic	3	1	0	0
Antiepileptic	1	1	0	0
Antigout	1	0	0	0
Antihistamine	2	2	2	1
Antimicrobial	28	7	3	3
Antineoplastic	5	1	1	0
Bronchodilator	2	1	0	0
Cardiovascular modulating agent	18	8	3	1
Contrast agent	2	0	0	0
Disinfectant	2	2	2	0
Hormone	20	11	4	0
Immunosuppressant	1	0	0	0
Insect repellant	1	1	1	1
Nicotine metabolite	1	0	0	0
Non-opioid analgesic	4	0	0	0
Opioid analgesic	4	3	0	0
Plastic residue	1	1	1	0
Stimulant	5	2	0	0
Total high priority contaminants	**-**	**50**	**21**	**7**

We detected high priority contaminants in all categories of sites we sampled (i.e., undeveloped, developed, and wastewater effluent-impacted) **(Figure 5)**. Wastewater effluent-impacted sites contained the most contaminants ranked as high priority contaminants, with sites ranging from 11 to 30 high priority contaminants. Developed and undeveloped sites contained a range of 2 to 13 high priority contaminants per site. Within all priority levels, wastewater effluent-impacted sites contained a significantly higher mean number of detections than both developed and undeveloped sites **(Table 10)**. There were no significant differences between developed and undeveloped sites within all priority levels (Kruskal-Wallis $P = 0.4208$, $P = 0.4684$, $P = 0.3535$ for high, intermediate, and low priority levels, respectively). Within developed and undeveloped sites, there was a significantly higher mean number of detections of high priority level contaminants than both intermediate and low priority contaminants **(Table 10)**. There were no significant differences among any priority levels within wastewater effluent-impacted sites ($P = 0.4130$).

Table 10. Mean number of contaminants detected across sites by anthropogenic pressure and priority level.Superscripts represent significant differences.

	Mean detections		
	Undeveloped	**Developed**	**Wastewater effluent-impacted**
Low	1.9	2.1	19.2[a]
Intermediate	1.6	1.5	17.2[b]
High	5.7[d]	5.3[e]	22.7[c]

[a]Wastewater effluent-impacted significantly higher than developed ($P = .0011$) and undeveloped ($P = .0011$) within low priority level.
[b]Wastewater effluent-impacted significantly higher than developed ($P = .0007$) and undeveloped ($P = .0007$) within intermediate priority level.
[c]Wastewater effluent-impacted significantly higher than developed ($P = .0009$) and undeveloped ($P = .0012$) within high priority level.
[d]High priority level significantly higher than intermediate ($P = .0026$) and low ($P = .0040$) priority levelswithin undeveloped sites.
[e]High priority level significantly higher than intermediate ($P = .0003$) and low ($P = .0038$) priority levelswithin developed sites.

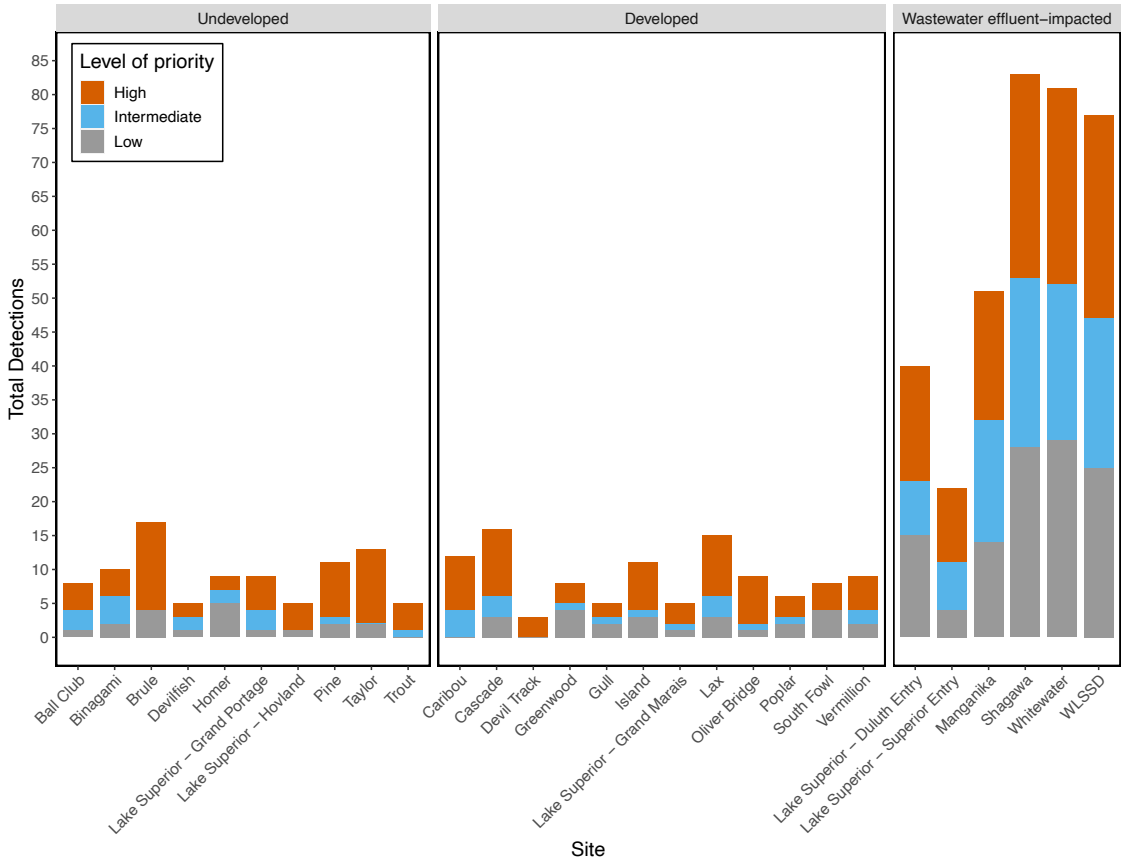

Figure 5. Total number of contaminants, and their priority level across any media, detected among undeveloped, developed, and wastewater effluent-impacted sites sampled from 2016 to 2018 in northeastern Minnesota on the Grand Portage Reservation and 1854 Ceded Territory.

The proportion of high priority contaminants out of the total number of detections by site exemplifies the magnitude of high priority CECs across all anthropogenic pressure categories. This relationship can be seen spatially, with many undeveloped locations containing more than 50% high priority contaminants out of the total CECs detected **(Figure 6)**, particularly on and near the GPIR. Note that 108 of the 117 detected CECs in this study are predicted to readily biodegrade, so the presence of high priority contaminants in remote areas is not simply due to persistence.

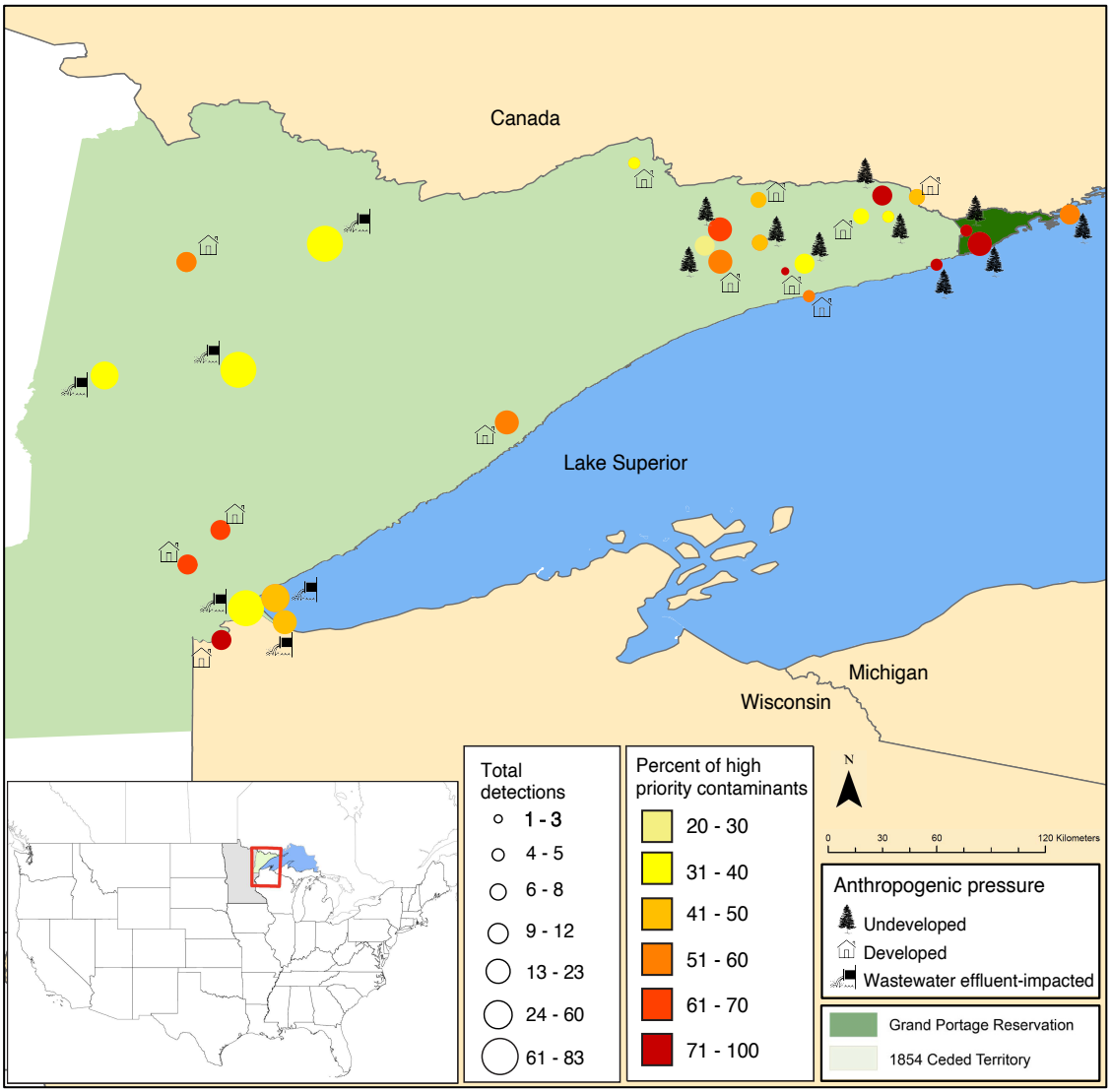

Figure 6. Number of detections relative to percent of high priority contaminants, across any media, per sites sampled from 2016 to 2018 in northeastern Minnesota on the Grand Portage Reservation and 1854 Ceded Territory. There were 38 high priority contaminants detected across any media. Sites are offset for visual representation. The size of the circle indicates the number of detections. The color of the circle represents the percent of high priority contaminants (priority level 4, 5, or 6) in any media. The symbol next to each site symbolizes the respective anthropogenic pressure category: developed, undeveloped, or wastewater effluent-impacted.

We explored how choices in the methodology of the hazard prioritization method used here might impact priority levels and provided results of these assessments in the **Supplementary Information**. Based on data availability, only one to four criteria could

be evaluated for 12 contaminants; thus, these chemicals were ranked as low or intermediate priority and should be interpreted with caution: 10-hydroxy-amitriptyline, 2-hydroxy-ibuprofen, clinafloxacin, desmethyldiltiazem, drospirenone, equilin, fluticasone propionate, lomefloxacin, moxifloxacin, norfluoxetine, norverapamil, virginiamycin. To highlight which chemicals might be ranked differently based on the availability of data, we normalized the scores according to the number of questions that could be answered. For water, four chemicals moved to a high priority level when scores were normalized: 10-hydroxy-amitriptyline, drospirenone, norfluoxetine, and norverapamil **(Table S12)**. Equilin was the only chemical that changed priority level, from intermediate to high, when sediment contaminant scores were normalized **(Table S13)**. For fish, 10-hydroxy-amitriptyline and fluticasone propionate changed from low to intermediate priority when scores were normalized **(Table S14)**. No chemicals changed in priority level from a higher to a lower level after normalization.

We performed a sensitivity analysis on the question "Is the contaminant detected in more than 20% of samples?" by changing the detection frequency threshold to 15% and 25% and determined how this affects priority levels. Thirty-four chemicals changed priority levels (either up or down) when the detection frequency in water samples was adjusted (**Table S15**). When detection frequency in water samples was 15%, 23 chemicals move up a priority level and when detection frequency was 25%, 11 chemicals move down a priority level. For sediment, 12 chemicals changed priority levels: eight chemicals moved up a priority level when the detection frequency was 15% and four chemicals moved down a priority level when detection frequency was 25% (**Table S16**). Three chemicals

changed priority levels when the detection frequency in fish samples was adjusted; all moving up a priority level when the detection frequency was 15% (**Table S17**). Changing the detection frequency to 25% did not affect the priority levels of and chemicals detected in fish.

To ensure individual questions that made up the profiles were not highly correlated, we assessed correlation coefficients among profile questions and found little correlation (**Figures A4 - A6)**. Among significant relationships, correlation coefficients between water profile questions ranged from 0.24 - 0.30; for sediment profiles, correlation coefficients ranged from 0.28 - 0.39; for fish, correlation coefficients ranged from 0.39 - 0.47.

To assess the conservative nature of our screening process, we compared TEST toxicity value predictions to the ECOSAR values used in the final profiles (see **Table S7** for TEST toxicity values in comparison to ECOSAR values). There were six chemicals that would have been high priority if we would have used TEST values instead, so we have flagged these chemicals for more follow-up actions (marked with an asterisk in **Tables S8-S10**).

Discussion

We report a case study applying the Minnesota Pollution Control Agency's ATP framework to a research study with the goal of a rapid-screening assessment for the prioritization of chemical hazards detected in freshwater ecosystems relied on for

subsistence. We identified high priority chemicals across all media, sites, and varying primary use categories, ranging from pharmaceuticals to insect repellent. Remote, undeveloped lakes often contained a larger proportion of high priority contaminants than developed and wastewater effluent-impacted sites. Due to the presence of high priority contaminants throughout the GPIR and 1854 Ceded Territory, we recommend future monitoring, rigorous evaluation of biological effects, and if warranted, the development of a risk assessment to better understand the risk posed by the high priority compounds we have identified.

The hazard profiles presented here address the potential for exposure and biological effects through the incorporation of available data including detection frequency, persistence, endocrine disruption, toxicity, and bioaccumulation of detected chemicals. While some of the questions that make up the chemical profiles may be correlated (e.g., detection frequency and persistence), they encompass persistence, bioaccumulation, and toxicity, which are common factors in many hazard assessments (Arnot & Mackay, 2008). Given their distance from known CEC point sources, we would predict that remote regions would contain a larger percentage of high priority CECs than low priority CECs as a result of the former chemicals generally being more persistent; however, all CECs detected across all sites (except nine unknowns because of data limitations) were persistent. The identification of mostly persistent chemicals is important, as a greater emphasis might be given to highly persistent CECs in chemical assessments and decision making (Cousins et al., 2019). Since most of our high priority CECs were persistent and often detected in more than 20% of the study sites, bioaccumulation, potential endocrine

disruption, and potential toxicity in water were the deciding factors in whether a chemical would be classified as high priority or not.

All of the high priority contaminants identified in fish have been shown to affect aquatic biota at the genetic, physiological, or behavioral level: fluoxetine, diphenhydramine, azithromycin, roxithromycin, miconazole, verapamil, and N,N-Diethyl-m-toluamide (DEET). For example, several studies have demonstrated that the selective serotonin reuptake inhibitor and antidepressant fluoxetine can alter reproductive and antipredator behaviors in freshwater fish (Dzieweczynski et al., 2016; Fursdon et al., 2019; Martin et al., 2017, 2019; Pelli & Connaughton, 2015) at environmentally relevant concentrations (De Abreu et al., 2014). Additionally, diphenhydramine, an antihistamine, can be toxic to aquatic organisms (Berninger et al., 2011), is often detected in the environment (Burket et al., 2020; Du et al., 2016; Ramirez et al., 2009; Scott et al., 2019; Wang & Gardinali, 2012), and has been detected in marketed fish filets (Foltz et al., 2014). Antimicrobials, including antibiotics and an antifungal medication, were also among those chemicals found to be high priority in fish in the current study. Antibiotics can be toxic to aquatic biota (Liu et al., 2014; Yan et al., 2019) and have the potential for transfer and biomagnification within aquatic food chains (Ding et al., 2015). Importantly, antibiotics in the environment may also lead to the increased abundance and diversity of antibiotic resistance genes or antibiotic resistant bacteria in the environment, affecting aquatic ecosystem (Bueno et al., 2019; Pazda et al., 2019; Reichert et al., 2019; Szekeres et al., 2018). Similarly, antifungal drugs, such as miconazole, which was found to be toxic to common water fleas (Minguez et al., 2016) and inhibits fungal cytochrome P450

enzymes (Beijer et al., 2018), can lead to the development of antifungal drug resistance (Assress et al., 2020).

Although we made some modifications to the ATP framework to accommodate our dataset, there are notable limitations. While toxicity reference values based on water column concentration data are somewhat available, apical effect data are more limited regarding sediment and fish tissue values, restricting our evaluation to a subset of detected CECs. Having only POCIS detections, and not water concentrations, also limits the extent of the current prioritization. Further, due to the weight-of-evidence approach used to create chemical profiles, a low priority level might reflect a lack of information rather than truly indicating low impact. For example, the chemical 10-hydroxy-amitriptyline, a metabolite of the antidepressant amitriptyline, has the potential to bioaccumulate, was neutral at water pH at least 10% of the time, and was present in more than 20% of samples. Therefore, it received a priority level of 3 for its water chemical profile. However, data were not available regarding its toxicity, biodegradation, or endocrine disruption potential, so this chemical was flagged as a contaminant warranting further evaluation. The question "Is the chemical neutral at the pH of the water > 10% of the time?" was added to the chemical profiles developed in this study because water chemistry data, which plays an important role in the toxicity of chemicals (Alsop & Wilson, 2019; Escher et al., 2020), was available for most of the study locations. While this evaluation employed a cutoff of 10% to conservatively prioritize chemicals and sites, this approach could mask effects at sites that are uniquely threatened. Therefore, if ranking individual sites instead of chemicals is the primary objective, the approach may

need some modification. Lastly, the growing abundance of high-throughput bioactivity data (e.g., EPA's Toxicity Forecaster (ToxCast)) may be used to further sort the currently detected chemicals by their potential to exert specific biological activity as in Corsi et al. (2019); although, such approaches also contain uncertainties, such as a somewhat unknown relevance to apical outcomes in the variety of species present in freshwater ecosystems.

This study utilized a screening-level tool that is conservative and likely to avoid type II error (i.e., false negatives); however, it is possible that some low or intermediate priority chemicals were misidentified and are of potential concern. We chose to use ECOSAR toxicity values but acknowledge that other available *in silico* toxicity models (Melnikov et al., 2016) or measured data could have led to different conclusions. For example, we explored Toxicity Estimation Software Tool (TEST) (Martin, 2016) values post hoc and noted some differences in toxicity values that would impact the answer to the toxicity question. Based on this post-hoc assessment, six chemicals would move to high priority using TEST toxicity values so they were flagged for further follow-up, such as a literature search or ToxCast evaluations. We used this method to increase the range of applicable domains and decrease the chance for misclassification of a chemical into a lower priority bin, with the ultimate goal of increasing the odds that our method is conservative while maintaining its rapid pace. While the purpose of this paper was not to compare methods, it is important to note that priority levels are dependent, in part, on the adopted prioritization method.

This study resulted in a prioritized list of chemicals that guided Phase II of this project,an investigation of anthropogenic factors associated with the detection of high priority CECs in northeastern Minnesota (Servadio et al., 2021). Determining key sources of spillover into and transport through the aquatic environment is critical to the mitigation of these high priority chemical contaminants. Further, the identification ofhigh priority chemicals on tribal lands provides information to natural resource managersand stakeholders developing best management practices for water pollution and wastewater treatment processes. However, we note that an important next step should include a risk-based assessment of the prioritized contaminants.

Conclusion

This study adds to the understanding of the potential hazards of 117 CECs detected in northeastern Minnesota and prioritizes chemicals for further study or mitigation, particularly in a region that is important for sustaining indigenous culture through subsistence fishing. We performed a rapid assessment of the detected chemicals in order to prioritize further research and management efforts in the region. Where universal standards, benchmarks, or individual toxicity assessments for CECs are lacking, chemical profiles provide a broad understanding of the potential hazards these chemicals pose to aquatic ecosystems and highlight the need for more research in these areas.

Acknowledgements

We thank the Grand Portage Reservation Tribal Council and Tony Swader for their continued support of this ongoing research. Funding support comes from University of

Minnesota (UMN) College of Veterinary Medicine's Population Systems Signature Program, UMN Agriculture Experiment Station Research Funds (MIN-62-061), UMN MnDRIVE Global Food Ventures, UMN Informatics Institute MnDRIVE, the Environmental Protection Agency's Great Lakes Restoration Initiative, and the Environment and Natural Resources Trust Fund as recommended by the Legislative-Citizen Commission on Minnesota Resources (M.L. 2017, Chp. 96, Sec. 2, Subd. 04g). This work is not a product of the U.S. Government or the U.S. Environmental Protection Agency, and the author (M.D.J.) is not engaged in this work in any governmental capacity. The views expressed are those of the author only and do not necessarily represent those of the U.S. Government or the EPA.

Chapter 4

Health of wild fish exposed to contaminants of emerging concern in freshwater ecosystems utilized by a Minnesota Tribal community

Overview

Fish play a critical role in the aquatic food web and are sensitive indicators of environmental contamination. Fish are particularly susceptible to contaminants of emerging concern (CECs) - chemicals such as pharmaceuticals, hormones, and personal care products - as uptake can occur via both dermal and gill surfaces, orally through the diet, or via the transfer of contaminants through eggs, either maternally or environmentally. Laboratory and *in vitro* studies have documented links to adverse effects in fish exposed to these chemicals. However, these studies are limited in the diversity of species examined, the effects of specific contaminants and other water chemistry interactions, data on population-level effects, and the long-term effects of low-level contaminant exposures. Therefore, *in situ* studies complement experimentally controlled research in overcoming some of these limitations. The first goal of this study was to evaluate the health of wild fish exposed to CECs in waterbodies across northeastern Minnesota with varying anthropogenic pressures: waterbodies with no human development along their shorelines, those with development, and those directly impacted by wastewater effluent. Then, we compared the utility of three different approaches that could be used to evaluate the health of fish exposed to CECs: a refined fish health assessment index (rFHI), a histopathological index, and high-throughput

(ToxCast) *in vitro* assays. We also mapped adverse outcome pathways (AOPs) associated with identified ToxCast assays to determine potential impacts across levels of biological organization within the aquatic system. These approaches were applied to subsistence fish collected within the Grand Portage Indian Reservation (GPIR) and 1854 Ceded Territory. In two years (2017 and 2019), 24 CECs were detected in fish tissues. The health of fish in undeveloped sites was as poor, or sometimes poorer, than fish in developed and wastewater effluent-impacted sites. While we could not determine a direct causal link between fish health and CEC exposure, the combined implementation of these tools revealed that subsistence fish exposed to CECs had histological and macroscopic tissue and organ abnormalities. AOPs demonstrated potential hazardous pathways to fish, including the presence of the antifungal miconazole leading to impaired fertility and decreased population growth. A better understanding of how the health of wild fish that are harvested for consumption is affected by CECs helps prioritize risk management research efforts and can ultimately be used to guide fisheries management decisions.

The Grand Portage Band is a federally recognized Native American tribe in extreme northeastern Minnesota and proudly exercises its rights to food sovereignty through subsistence hunting and fishing. Fish are a primary subsistence food used by the Anishinaabeg (people) of Grand Portage Band of Lake Superior Chippewa, which sets the context for this paper exploring the health of this culturally important resource in the area in which they live.

Introduction

Fish play an important role in the aquatic food web and are sensitive indicators of environmental contamination (Łuczyńska et al., 2018; Whitfield & Elliott, 2002). At an individual level, fish are particularly susceptible to contamination as uptake can occur via both dermal and gill surfaces, orally through the diet, or via the transfer of contaminants through eggs, either maternally or environmentally (Corcoran et al., 2010; Latif et al., 2001). Fish also have a lower capacity to metabolize xenobiotics compared with mammals (Wolf & Wolfe, 2005). Specific contaminants and complex mixtures of contaminants can affect the structure and function of biological systems in fish, which can cause molecular, biochemical, histological, and behavioral changes at the individual level before the population is affected (Giang et al., 2018). While some individual-level effects from contaminant exposure have been documented, such as the impact of industrial effluent on metabolic pathways (Levesque et al., 2002), evidence of the effects of environmental contaminants on fish health is limited.

Environmental contaminants also have population-level effects and can alter aquatic community structure (Culp et al., 2003). A seven-year exposure to a synthetic estrogen led to a near local extinction of the fathead minnow (*Pimephales promelas*) population (Kidd et al., 2007). Experimentally designed exposure studies have documented links to adverse effects, including altering behavior and reducing reproductive fitness, in exposed fish (Brodin et al., 2013; Martinović et al., 2007). However, these are limited in the diversity of species examined, the effects of specific contaminants and other water chemistry interactions, population-level effects, and the long-term effects of low-level

contaminant exposures (Martinović et al., 2007). Therefore, *in situ* studies complement experimental research in overcoming some of these limitations. As compounds bioaccumulate in fish (Grabicova et al., 2015; Viana et al., 2018) and have the potential to biomagnify up the food chain (Ali & Khan, 2018; de Wit et al., 2020), fish can be used as bioindicators of aquatic ecosystem health (Van Der Schalie et al., 1999). Understanding how fish are being affected by contaminant exposure can inform the potential risk of contaminants to human health.

For many years, fish biologists have used population surveys to evaluate the health of fish populations (Pope et al., 2010); however, such surveys are not specific to health-related population declines and could be the result of predator-prey relationships, levels of fish harvest, and poor recruitment of fish due to weather conditions or timing of plankton blooms (Rypel et al., 2018). Further, population surveys often miss more subtle impacts of contaminant exposure and data on effects at the organism and tissue-levels in field-based settings is lacking.

A small number of tools have been developed to evaluate the health of wild fish in their natural environments (Adams, Brown, and Goede 1993; Lang et al., 2017) and a combination of methodologies have been used to assess the health of wild fish *in situ* (Bailey et al., 2018; Jorgenson et al., 2018; Muttray et al., 2021; Tetreault et al., 2011), but there is no standardized tool for field-based health assessments. The health assessment index (HAI) described by Adams et al. (1993), which was an extension of one of the original field necropsy methods (Goede & Barton, 1990), is a quantitative health

index that allows statistical comparisons of fish health assessed in natural settings. These methods have recently been expanded further in a necropsy-based wildfish health assessment (Blazer et al. 2018; published after the start of this study). A limitation of necropsy-based assessments is the inability to definitively diagnose the presence and type of neoplasia or other abnormalities observed grossly or miss pathologies that cannot be seen grossly. Therefore, tissues can also be collected for histopathology, allowing for the identification of pathologies that cannot be seen macroscopically.

As observed gross and/or histopathological changes are not necessarily specific to contaminant exposure, the biological relevance of measured chemical concentrations in aquatic ecosystems can also be determined using the U.S. Environmental Protection Agency's programs ToxCast and Tox21 (hereafter, ToxCast). ToxCast is a database of chemical-biological interactions that contains chemical-specific high throughput *in vitro* biological activity data (Dix et al., 2007; Kavlock et al., 2012). ToxCast has been used for prioritizing environmental chemicals across a variety of studies, including Great Lakes tributaries (Corsi et al., 2019), bald eagles (Elliott et al., 2019), and fish plasma (Malev et al., 2020). ToxCast data can be combined with another database, adverse outcome pathways (AOPs; https://aopwiki.org) to link pathway-specific biological activities with potential adverse biological effects, both at the individual and population level (Ankley et al., 2010; Fay et al., 2018).

In this study, we investigated fish health and a particular group of environmental contaminants - contaminants of emerging concern (CECs) - in northeastern Minnesota,

within the Grand Portage Indian Reservation (GPIR) and 1854 Ceded Territory. Previous research in this region has shown the ubiquitous and persistent nature of CECs in water, sediment, and fish (Deere et al., 2020, 2021) and anthropogenic factors associated with detection of CECs (Servadio et al., 2021). CECs include chemicals such as pharmaceuticals, personal care products, and hormones and may have a variety of adverse impacts on freshwater ecosystems. Understanding the impact that CECs have on fish health in northeastern Minnesota also has important cultural significance; the Grand Portage Band of Lake Superior Chippewa rely heavily on fish species found in Lake Superior and its surrounding waterbodies for subsistence. However, concerns for the impact that these contaminants may have on the health of fish populations and people that consume them raise questions about the safety and security of subsistence foods. Thus, assessing the health status of this ecosystem is crucial for the Anishinaabeg culture and way of life.

The first objective of this study was to evaluate the health of wild fish exposed to CECs in waterbodies across northeastern Minnesota with varying anthropogenic pressures: waterbodies with no human development along their shorelines, those with development, and those directly impacted by wastewater effluent. Then, we compared the utility of three different approaches that could be used to evaluate the health of fish exposed to CECs: a refined fish health assessment index (rFHI), a histopathological index, and high-throughput (ToxCast) *in vitro* assays. We hypothesized that fish exposed to elevated numbers or concentrations of CECs would be less healthy than those present in less-contaminated waters. We also expected that the three different approaches of evaluating

fish health in this study will vary in their ability to detect potential health concerns. Results from this study will provide a better understanding of how the health of wild fish is affected by CECs, help guide decisions related to approaches used to evaluate fish health in polluted waters, and help prioritize risk management research efforts that will ultimately be used to guide fisheries management decisions.

Methods

Study design and sample collection

Detailed description of the study site and sampling design are presented in Deere et al. (2020; see also Deere et al. 2021). Briefly, we assessed fish health and surveyed presence of CECs in subsistence fish species in northeastern Minnesota on the Grand Portage Indian Reservation (GPIR) and the 1854 Ceded Territory. We targeted Tribally important subsistence fish species of different trophic levels for sampling. In 2017, we sampled walleye (ogaa in Ojibwe; *Sander vitreus*) and yellow perch (asaawens; *Perca flavescens*) from inland lakes and cisco (odoonibiins; *Coregonus artedi*) and lake trout (namegos; *Salvelinus namaycush*) from Lake Superior sites. In 2019, we collected yellowperch from inland lakes only. We sampled a total of 20 waterbodies, including sites along Lake Superior and inland lakes that have value as fish harvesting locations for Tribal members. We categorized sites by anthropogenic pressure: 1) wastewater effluent- impacted, 2) developed, and 3) undeveloped. Full site selection criteria were described previously (Deere et al., 2020). In 2017, we sampled six undeveloped, five developed, and six wastewater effluent-impacted waterbodies. In 2019, we sampled 2 lakes from each anthropogenic pressure category. Five lakes were sampled in both 2017 and 2019. In

2017, four locations were sampled along Lake Superior, whereas only inland lakes were sampled in 2019.

In 2017 and 2019, fish were collected as previously described (Deere et al., 2020). Briefly, we used multiple fish collection methods according to appropriate waterbody and target species, including gill nets and boat-operated electrofishing. In 2019, we only used boat-operated electrofishing. Upon collection, we identified fish to species level and sorted either into a specimen or non-target category, with all non-target species immediately returned alive into the water. We rinsed all equipment in ambient water from the waterbody being sampled prior to fish collection to remove any foreign material from the external surfaces. Researchers wore latex or nitrile powder-free gloves when handling fish.

Following collection, we weighed fish to the nearest gram, measured fork length, and logged by species. Fish were euthanized using an American Veterinary Medical Association approved physical method per University of Minnesota IACUC-approved protocol (ID: 1803-35736A). We separated fish randomly for either health assessment or contaminant assessment. For health assessments, we immediately necropsied fish on the shores of the lakes using appropriate equipment. We conducted a gross pathological assessment of the whole fish (rFHI; see below) on 546 fish collected in 2017 and 120 fish in 2019. We also assessed the latter using a histopathological scoring of the liver, spleen, gonad, and gills (histopathological index; see below).

Concurrently, we collected a separate set of fish samples to analyze chemical-biological interactions using *in vitro* activity data (ToxCast; see below). For contaminant assessment, we collected a minimum of three individuals ofeach species per waterbody. Fish were immediately wrapped in aluminum foil, individually placed into sealed plastic bags, and frozen at -18°C until further processing.We homogenized whole fish using a stainless-steel commercial meat grinder to obtain one representative fish sample per species per waterbody (2017 mean sample weight = 2.4 grams; 2019 mean sample weight = 1.2 grams). 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, then rinsed in acetone to dry.We sent samples to SGS AXYS Analytical (Sidney, British Columbia, Canada) for chemical analysis. SGS AXYS analyzed 34 composite samples in 2017 and six samples in 2019 using liquid chromatography with tandem mass spectrometry (LC-MS/MS). Detailed analytical procedures and quality assurance and control methods were explained previously (Deere et al., 2020).

Refined fish health index (rFHI)

We refined the HAI developed by Adams and colleagues (1993) to best align with the study design used here. The refined fish health index (rFHI) contains eight gross pathology variables that can be used to quantitatively and systematically compare the health of fish sampled across different environments. To employ this rFHI we necropsied fish in the field immediately after collection and assigned field designation codes based on gross morphology to individual fish for each variable described in **Table 11**. Field

code designations were then transcribed to a numerical value for analyses. For each variable, rFHI values range from 0-30, zero denoting normal condition and 30 denoting the most severe abnormality. The overall rFHI score was calculated for each fish by summing scores from each gross pathology variable, with a higher score indicating a fish in poorer health. A total of 546 fish (182 from developed, 195 from undeveloped, and 169 from wastewater effluent-impacted sites) were assessed from July - September 2017 and 120 fish (40 from developed, 40 from undeveloped, and 40 from wastewater effluent-impacted sites) from June - July 2019.

Table 11. Refined fish health assessment index (rFHI) adapted from Adams et al. (1993). Original field designation was used for field record. rFHI values were substituted for analyses.

Variable	Variable Condition	Original field code	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
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
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

Histopathological index

In 2019, we sampled selected organs (i.e., liver, spleen, gonad, and gills) from 120 yellow perch for histopathological analysis. We standardized samples to size and site within the organ. We stored the organs in 10% neutral buffered formalin in individual containers coded for collection time, fish number, and location. In the lab, these tissue samples were processed for histopathological analysis by brightfield microscopy. We cut two sections from each organ at 3-5 micrometers and stained them with hematoxylin and eosin (H&E). We stained a subset of samples with PAS, Grocott, Acid Fast, Giemsa, Pearl's blue, and Alcian blue, and with immunohistochemical stains including PCNA (proliferating cell nuclear antigen), Factor VIII, and SMA (smooth muscle actin). A veterinary pathologist examined slides using a blinded code. Grading of histopathological lesions included two numerical score sets, one with all lesions, and a second which only included toxicopathic lesions. Scores were also separated by organ as some samples were lost during processing resulting in uneven sample sizes (gills = 68; gonads = 98; livers = 120; spleens = 112).

ToxCast and Adverse Outcome Pathways (AOPs)

The ToxCast database is a publicly accessible database containing high-throughput

screening data for thousands of chemicals. ToxCast chemicals were tested in a consistent nd standardized set of assays and data were analyzed with a uniform analysis pipeline (Filer et al., 2017), greatly increasing the comparability of data between assays (Richard et al., 2016). The database contains chemical-biological interactions from chemical-specific high throughput *in vitro* biological activity data, which provides a means to assess biological relevance of measured concentrations (Corsi et al., 2019; Kavlock et al., 2012). We used the ToxCast database (U.S. EPA, 2019) to sort detected chemicals in fish tissues by their potential to exert specific biological activity.

Tissue concentrations were compared against activity concentration at cutoff (ACC) values obtained from the ToxCast database to calculate exposure-activity ratios. Exposure-activity ratios (EARs = sample concentration/bioactivity effect concentration) are the ratios of a measured concentration and a concentration that was determined to cause some activity in a specified ToxCast assay ("endpoint" concentration) (Blackwell et al., 2017). The bioactivity effect concentration used here was the ACC, which is an assay- specific concentration at which the model first reaches the cutoff value for the data-seriesto be considered active (Filer et al., 2017; Judson et al., 2010). Biological activity data forchemicals of interest were analyzed in R (v4.0.2; R Core Team 2020) using the *toxEval* package (DeCicco et al., 2020). ToxCast comprises data from multiple assay platforms that perform differently (Kavlock et al., 2012), so we filtered the bioactivity data to exclude some assays using the default settings in *ToxEval* (see Corsi et al. 2019 for more on default settings).

After we identified ToxCast assays and associated biological targets for the chemicals detected and calculated EARs, we also assessed chemical mixtures identified in fish tissues using the *ToxMixtures* package (DeCicco et al., 2021). Through *ToxMixtures*, endpoints were linked to adverse outcome pathways (AOPs) to provide additional context for the potential effects of the chemicals and chemical mixtures we identified. AOPs depict linkages between molecular initiating events (i.e., the interaction of chemicals with biological targets) and the subsequent responses across individuals, populations, and communities (Ankley et al., 2010). We mapped ToxCast endpoints to key events in AOPs using the AOP-wiki (Fay et al., 2018; Pittman et al., 2018). AOPs are continually being created and revised by the science community; AOPs mapped here are as of June 2021.

EARs based on *in vitro* effect concentrations do not always translate directly to *in vivo* apical responses; thus, to assess relevance of a given ToxCast assay to apical outcomes in fish, we used the US EPA Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS v5.1; https://seqapass.epa.gov/seqapass/) tool to contextualize the significance of the EAR-based analyses relative to potential fish-relevant hazards. SeqAPASS provides data on the relevance of ToxCast assays across species by assessing protein sequence/structural similarity across taxonomic groups as a means to predict relative intrinsic susceptibility (Lalone et al., 2018; LaLone et al., 2016). We mapped ToxCast assays and associated gene targets of the detected chemicals in fish tissues to the relative protein accessions. The protein accessions were then used as a query protein sequence in SeqAPASS to predict chemical susceptibility across species. We filtered the resulting data by taxonomic group “actinopteri,” which includes the infraclass teleosts, in

which the species in this current study belong. A susceptibility prediction of "yes" indicated the protein target is conserved in fish; thus, ToxCast assays and AOPs associated with proteins that had a susceptibility prediction of "yes" were includedin analyses.

Results

Of the 24 chemicals detected in fish tissues, *N,N*-Diethyl-*meta*-toluamide (DEET) was detected at the highest concentrations, with one walleye sample (i.e., one homogenized sample from approximately three fish) in an undeveloped lake having a concentration of 2450 ng/g **(Figure 7)**. The other samples ranged from 1.93 - 515 ng/g. The primary useof chemicals detected included: antidepressant, antidiabetic, antihistamine, antimicrobial, antineoplastic, cardiovascular modulating agent, contrast agent, exogenous hormone, insect repellant, non-opioid analgesic, and stimulant. We detected 14 chemicals in Lake Superior sites and 18 chemicals in inland lakes. The following seven chemicals were detected in both inland and Lake Superior sites: DEET (insect repellant), enrofloxacin (antimicrobial), hydrocortisone (exogenous hormone), miconazole (antimicrobial), sulfanilamide (antimicrobial), venlafaxine (antidepressant), virginiamycin M1 (antimicrobial). See Deere et al. (2020) for more details on CECs detected in fish, along with water and sediment.

The distribution of rFHI scores varied across species and anthropogenic pressure **(Figure 8)**. Trends in Lake Superior species were as expected, with wastewater effluent-impacted sites having significantly higher mean rFHI scores than undeveloped sites for both cisco

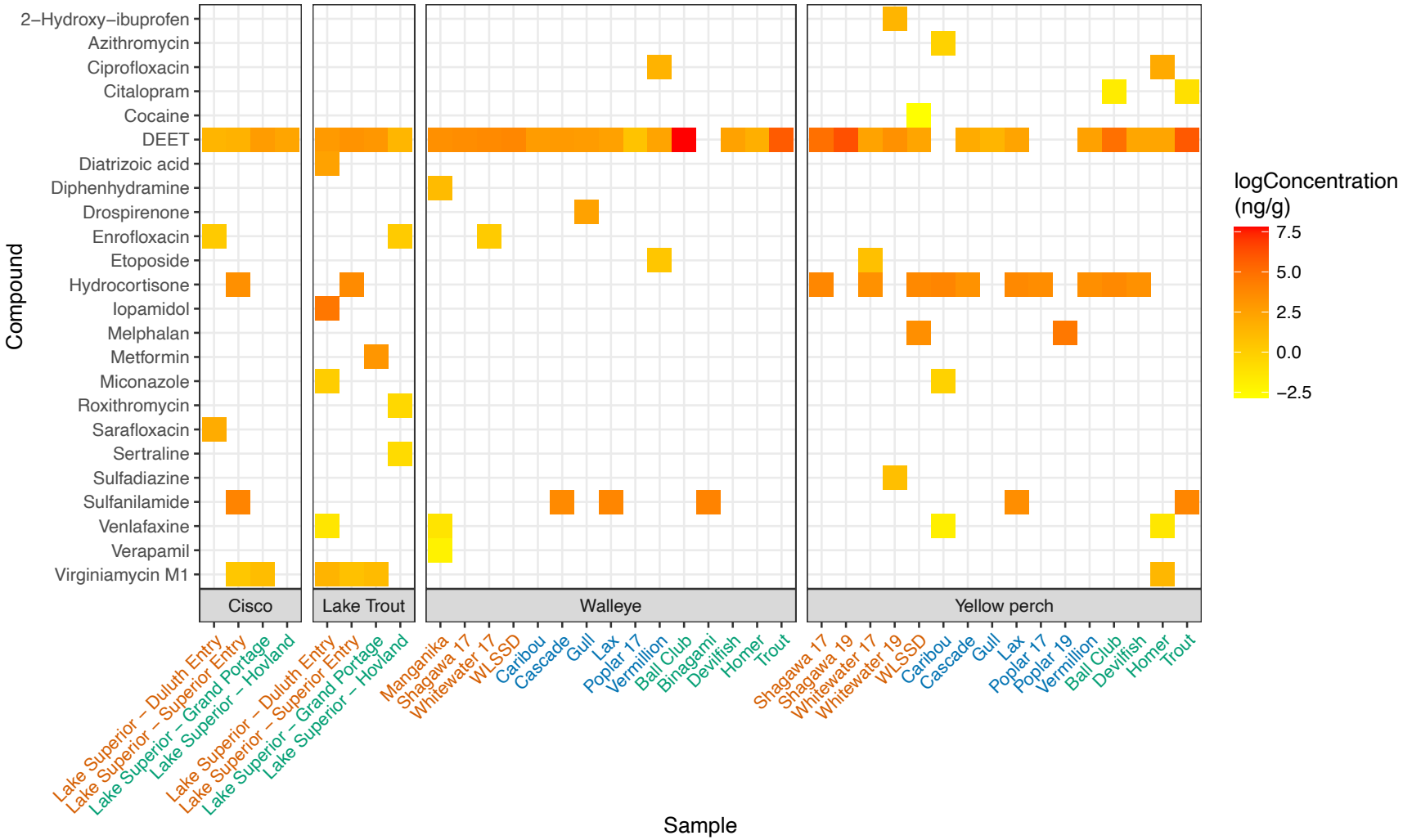

Figure 7. Heatmap of log-transformed chemical concentrations (ng/g) detected in subsistence fish species samples from Lake Superior sites (cisco and lake trout) and inland lakes (walleye and yellow perch) sampled lakes in northeastern Minnesota in 2017 and 2019. Lakes are categorized by anthropogenic pressure: wastewater effluent-impacted (red), developed (blue), and undeveloped (green).

and lake trout (Kruskal-Wallis $P = 0.0021$ and $P = 0.0002$). However, there was less consistency between walleye and yellow perch from inland sites. Surprisingly, walleye in wastewater effluent-impacted sites had lower mean rFHI scores than both developed and undeveloped lakes ($P = 0.0080$ and $P = 0.0053$, respectively). There was no significant difference in mean scores between developed and undeveloped lakes ($P = 0.4118$). For yellow perch assessed in 2017, there were no significant differences in mean scores between wastewater effluent-impacted and developed or undeveloped sites ($P = 0.0859$ and $P = 0.0628$, respectively). However, developed sites had a significantly higher mean score than undeveloped sites ($P = 0.0009$). Yellow perch assessed in 2019 did not follow the same patterns as in 2017. Mean rFHI scores in wastewater effluent-impacted were significantly higher than both developed and undeveloped sites ($P = 0.0010$ and $P = 0.0018$, respectively). There was no significant difference between developed and undeveloped lakes ($P = 0.4865$). Overall, cisco and lake trout had higher scores than walleye and yellow perch.

To investigate what might be driving the patterns we saw in rFHI scores **(Figure 8)**, we explored liver scores more closely, as the liver variable contributed the most to the overall score and the liver plays an important role in chemical metabolism and detoxification of environmental contaminants. We grouped liver scores by normal (i.e., no macroscopically visible aberrations), nodular (i.e., cysts or nodules, likely parasitic), or discolored (i.e., "fatty," general, focal, or mottled discoloration) **(Figure 9)**. Livers from Lake Superior species were more discolored than nodular, with lake trout having no livers with nodules visible.While livers from inland lake species were still discolored,

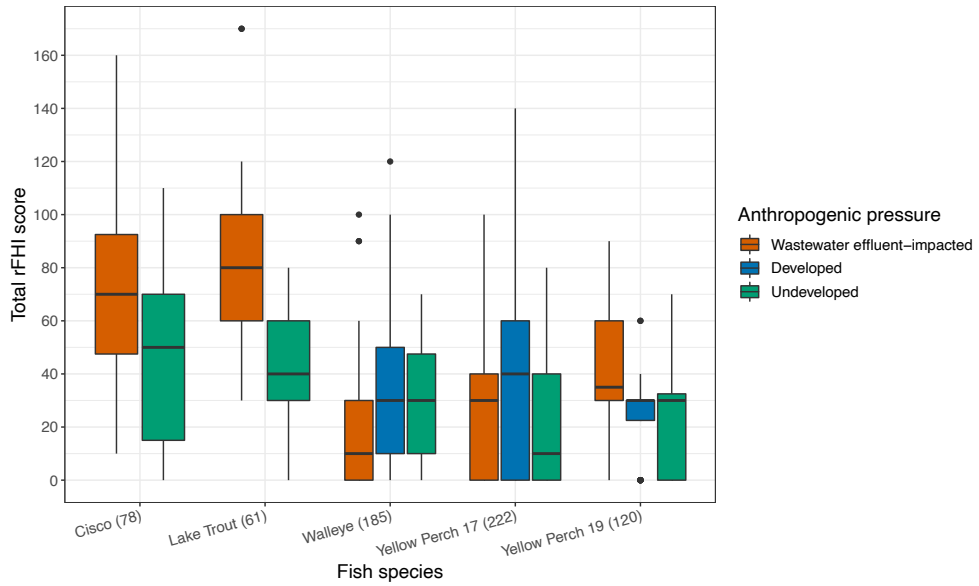

Figure 8. Distribution of total rFHI scores of subsistence fish species across northeastern Minnesota in 2017 and 2019. The number in parentheses represents the sample size of each species. We collected cisco and lake trout from Lake Superior sites and walleye and yellow perch from inland lakes. Yellow Perch were sampled in 2017 and 2019.

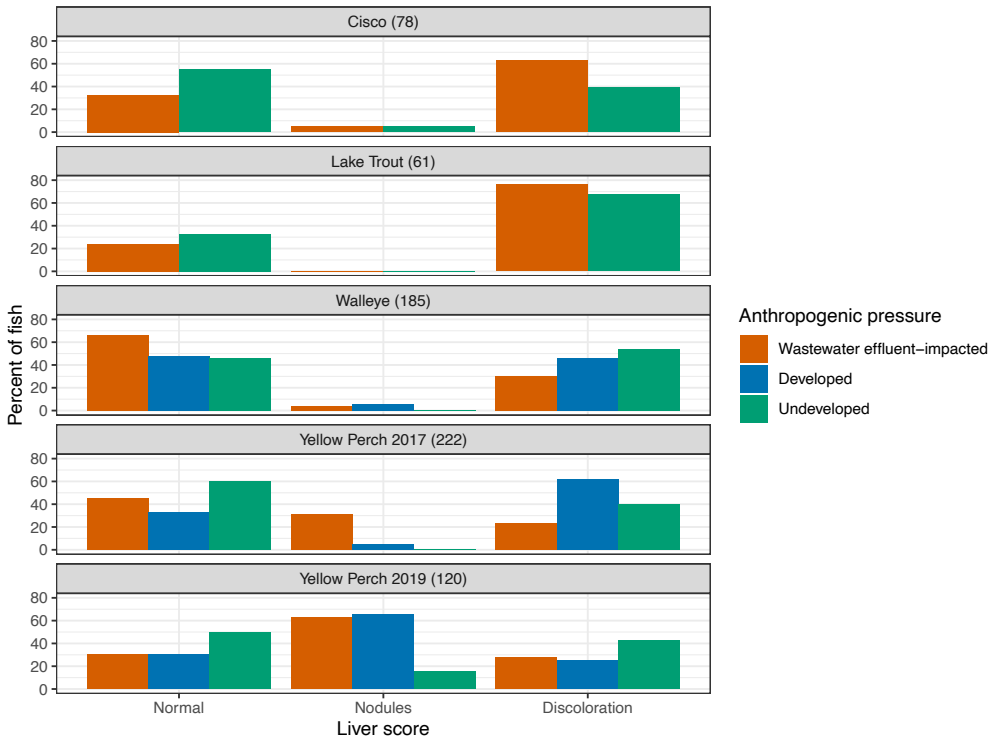

Figure 9. Percent of subsistence fish with normal, nodular, or discolored livers in waterbodies across northeastern Minnesota in 2017 and 2019. Normal indicates there were no macroscopically visible aberrations. Nodules represents visible cysts or nodules, likely parasitic. Discoloration includes livers considered "fatty," focal or general discoloration, and other (i.e., mottled). The number in parentheses represents the sample size of each species. We collected cisco and lake trout from Lake Superior sites and walleye and yellow perch from inland lakes. Yellow Perch were sampled in 2017 and 2019.

there were many more nodular livers, especially for yellow perch in 2019.

For yellow perch collected in 2019, the patterns of overall histopathological index scores differed according to tissue **(Figure 10)**. Fish in undeveloped lake sites had higher gill histopathological scores than those in developed or wastewater effluent-impacted sites; although, the differences in means were not significant ($P = 0.1383$). Fish in undeveloped lakes had significantly higher gonad scores than those in developed lakes ($P = 0.0035$). The difference in scores between undeveloped and wastewater-effluent lakes was not significant ($P = 0.0653$). There were no significant differences across liver or spleen histological scores across sites ($P = 0.4159$ and $P = 0.9983$, respectively). One fish in a wastewater effluent-impacted lake presented with hepatocellular carcinoma and vascular cancer **(Figure 11)**. Additional stains confirmed further pathologies. In two fish from an undeveloped lake, PAS stain confirmed uneven glycogen distribution possibly indicating early focus of cellular alteration, which is a preneoplastic change. In a fish from an undeveloped lake, B&H stain confirmed two spore-compatible organisms, possibly microsporidia. In two fish from two different undeveloped lakes, iron stain confirmed diffuse hepatocellular staining and more intense staining within melanomacrophage centers (MMCs). However, these were single incidents from different lakes, so there was low prevalence of hepatic aberrations.

ToxCast assays were used to screen chemicals for biological activities **(Table S18)**. Eleven chemicals detected in fish were associated with 26 AOPs that were identified as relevant to biological activities (ToxCast assays) that could be seen in fish **(Table S19)**.

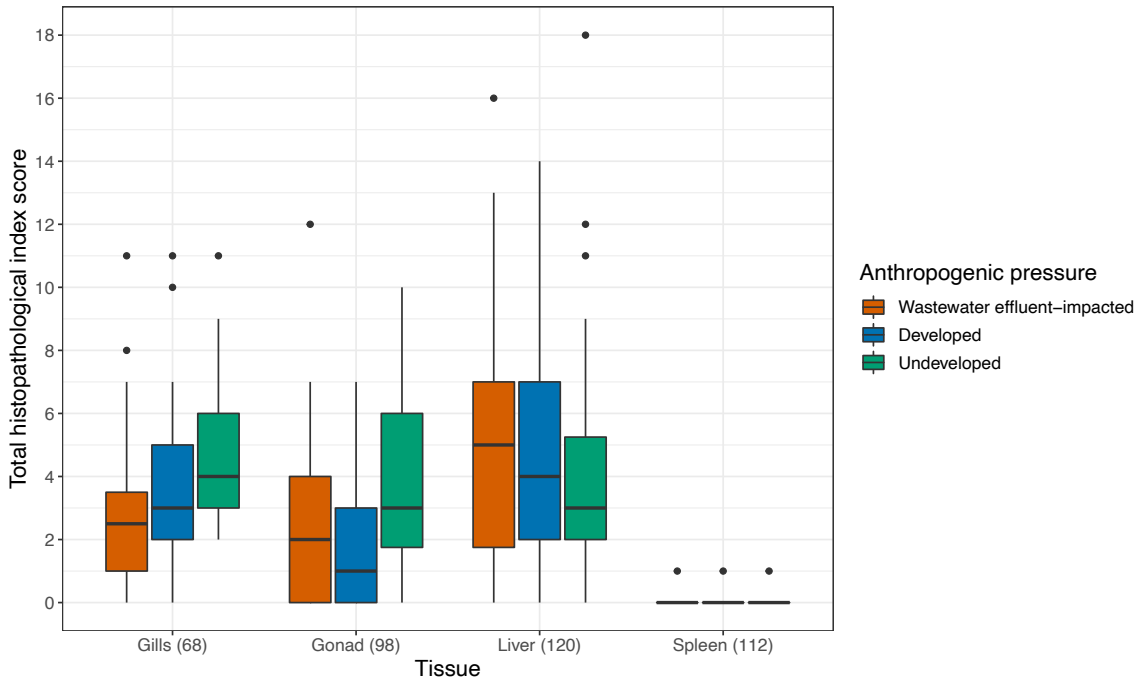

Figure 10. Distribution of total histopathological index scores of 120 yellow perch across northeastern Minnesota in 2019. The number in parentheses represents the sample size of each tissue.

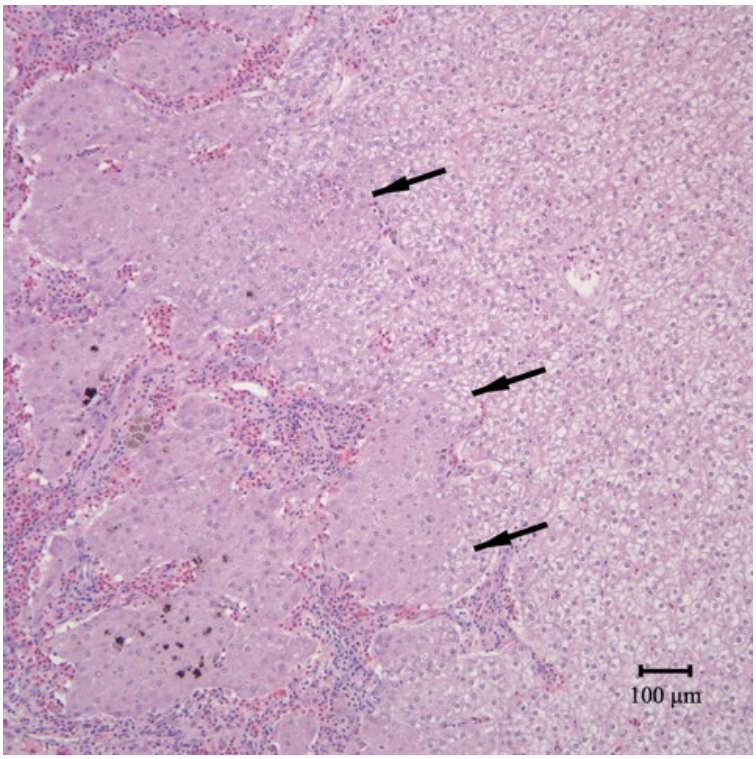

Figure 11. Microphotograph of H&E-stained liver tissue from a yellow perch collected from a wastewater effluent-impacted lake in 2019. Combined hepatocellular carcinoma and vascular cancer is indicated to the left of the arrows.

For example, miconazole was detected in lake trout and yellow perch and was associated with four AOPS that have events along their pathways that can adversely affect fish, including impaired fertility and decreased population growth **(Table 12)**.

We ranked sites using the different health metrics. The ranking of sites was not consistent across methods or species, especially across inland sites **(Table 13)**. Rankings based on total EAR values for each site identified Gull (developed) as the most affected site for walleye but the least affected site for 2017 yellow perch. The least affected site for walleye was Homer (undeveloped) and the most affected site for yellow perch was Caribou (developed). Shagawa (wastewater effluent-impacted) was the most affected lake for 2019 yellow perch, and it was also a top affected lake for 2017 yellow perch. For Lake Superior sites, Superior Entry (wastewater effluent-impacted) ranked highest for cisco and lake trout. The least affected site for cisco was Hovland (undeveloped), but the least affected site for lake trout was Grand Portage (undeveloped). Rankings based on mean rFHI at each site identified Caribou (developed) as the most affected site for walleye and Vermillion (developed) for 2017 yellow perch. The least affected for walleye and 2017 yellow perch were Shagawa and Cascade (developed), respectively. Shagawa was the most affected site and Trout (undeveloped) the least affected for 2019 yellow perch. The mean histopathological index scores for 2019 yellow perch also ranked Shagawa as the most affected site. The least affected site was Poplar (developed). Superior Entry also ranked highest for rFHI scores in cisco and lake trout. Grand Portage was the least affected site for both cisco and lake trout.

Table 12. Adverse outcome pathways associated with the detection of the antifungal miconazole that include key events and adverse outcomes that can potentially negatively affect fish. The ToxCast Assay associated with all four AOPs is Tox21_Aromatase_Inhibition and the gene is CYP19A1. The type of events are molecular initiating events (MIE), key events (KE), and adverse outcomes (AO).

AOP ID	AOP title	Type	Title
7	Aromatase (Cyp19a1) reduction leading to impaired fertility in adult female	MIE	Reduction, ovarian granulosa cells, Aromatase (Cyp19a1)
		KE	Reduction, Plasma 17beta-estradiol concentrations
		KE	Reduction, 17beta-estradiol synthesis by ovarian granulosacells
		AO	Impaired, Fertility
		AO	Irregularities, ovarian cycle
25	Aromatase inhibition leading to reproductive dysfunction	MIE	Inhibition, Aromatase
		KE	Reduction, Plasma 17beta-estradiol concentrations
		KE	Reduction, Vitellogenin synthesis in liver
		KE	Reduction, Vitellogenin accumulation into oocytes andoocyte growth/development
		KE	Reduction, 17beta-estradiol synthesis by ovarian granulosacells
		KE	Reduction, Cumulative fecundity and spawning
		KE	Reduction, Plasma vitellogenin concentrations
		AO	Decrease, Population trajectory
122	Prolyl hydroxylase inhibition leading to reproductive dysfunction via increased HIF1 heterodimer formation	MIE	Inhibition, Prolyl hydroxylases
		KE	Increased, HIF-1 heterodimer
		KE	Decreased, Aromatase (Cyp19a1) mRNA
		KE	Reduction, 17beta-estradiol synthesis by ovarian granulosacells
		KE	Reduction, Plasma 17beta-estradiol concentrations
		KE	Reduction, Vitellogenin synthesis in liver
		KE	Reduction, Plasma vitellogenin concentrations
		KE	Reduction, Vitellogenin accumulation into oocytes andoocyte growth/development
		AO	Reduction, Cumulative fecundity and spawning
		AO	Decrease, Population trajectory
123	Unknown MIE leading to reproductive dysfunction via increased HIF-1alpha transcription	MIE	Modulation, Unknown
		KE	Increased, HIF-1 heterodimer
		KE	Increased, HIF-1 alpha transcription
		KE	Decreased, Aromatase (Cyp19a1) mRNA
		KE	Reduction, Plasma 17beta-estradiol concentrations
		KE	Reduction, 17beta-estradiol synthesis by ovarian granulosacells
		KE	Reduction, Vitellogenin synthesis in liver
		KE	Reduction, Plasma vitellogenin concentrations
		KE	Reduction, Vitellogenin accumulation into oocytes andoocyte growth/development
		AO	Reduction, Cumulative fecundity and spawning
		AO	Decrease, Population trajectory

Table 13. Ranking of sites using number of chemicals and fish health rankings of walleye and yellow perch collected from inland lakes and cisco and lake trout collected from locations along the Lake Superior shore in northeastern Minnesota in 2017 and 2019. "No. chem." is the number of chemicals detected at each site. ToxCast rankings are based on total exposure-activity ratio values for each site. Refined fish health index (rFHI) rankings are based on the mean rFHI scoreat each site. Yellow perch in 2019 are also ranked by histopathological index ("histo. index") scores, which are based on the mean score at each site. ToxCast, rFHI, and histopathological rankings are ordered from least affected (1) to most affected (highest number). A hyphen (-) indicates species that were not sampled for the respective site or method. Asterisks indicate locations along Lake Superior.

		Species sampled from inland lakes										Species sampled from Lake Superior sites					
		Walleye 2017			Yellow perch 2017			Yellow perch 2019				Cisco 2017			Lake Trout 2017		
Anthro. pressure	Sites	No. chem.	Tox-Cast	rFHI	No. chem.	Tox-Cast	rFHI	No. chem.	Tox-Cast	rFHI	Histo. index	No. chem.	Tox-Cast	rFHI	No. chem.	Tox-Cast	rFHI
Wastewater effluent-impacted	Manganika	4	7	2	-	-	-	-	-	-	-	-	-	-	-	-	-
	Shagawa	1	12	1	2	12	9	1	2	6	6	-	-	-	-	-	-
	Whitewater	2	13	6	3	5	6	3	1	4	2	-	-	-	-	-	-
	WLSSD	1	10	12	4	10	4	-	-	-	-	-	-	-	-	-	-
	Duluth Entry*	-	-	-	-	-	-	-	-	-	-	3	3	2	6	2	3
	Superior Entry*	-	-	-	-	-	-	-	-	-	-	4	4	4	3	4	4
Developed	Caribou	1	4	13	4	13	11	-	-	-	-	-	-	-	-	-	-
	Cascade	2	5	3	2	4	1	0	-	3	4	-	-	-	-	-	-
	Gull	2	15	-	1	1	-	-	-	-	-	-	-	-	-	-	-
	Lax	2	3	9	3	9	8	-	-	-	-	-	-	-	-	-	-
	Poplar	1	6	4	1	8	5	1	3	2	1	-	-	-	-	-	-
	Vermillion	3	8	11	2	7	12	-	-	-	-	-	-	-	-	-	-
Undeveloped	Ball Club	1	14	7	3	11	2	-	-	-	-	-	-	-	-	-	-
	Bingami	1	11	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Devilfish	1	2	10	2	6	7	-	-	-	-	-	-	-	-	-	-
	Homer	1	1	5	4	2	10	-	-	-	-	-	-	-	-	-	-
	Trout	1	9	8	3	3	3	0	-	1	3	-	-	-	-	-	-
	Elbow	-	-	-	-	-	-	0	-	5	5	-	-	-	-	-	-
	Grand Portage*	-	-	-	-	-	-	-	-	-	-	2	2	1	3	1	1
	Hovland*	-	-	-	-	-	-	-	-	-	-	1	1	3	4	3	2

Discussion

We assessed the health of subsistence fish species collected across 20 sites in northeastern Minnesota and evaluated chemicals detected in fish tissues that were collected simultaneously. Our results suggest that the health of fish is adversely affected in lakes across a spectrum of anthropogenic pressures - from remote, undeveloped wilderness to lakes directly impacted by wastewater effluent. Indicators measured in this study revealed potentially concerning health effects at the organism level and the presence of some CECs could lead to potential population level effects. However, our hypothesis - that fish exposed to more contamination would be less healthy than those present in less-contaminated waters - was not well supported by the findings of this study as some patterns showed that the health of fish in undeveloped sites was as poor, or sometimes poorer, than fish in developed and wastewater effluent-impacted sites.

While we cannot causally link fish health effects observed here to CEC exposure, we can place contaminant results into context through assessing indicators of fish health at the organism, tissue, and bioactivity levels. The three different approaches of evaluating fish health varied in their ability to detect potential health concerns. The rFHI, while helpful in some circumstances, can be relatively subjective and may lack sensitivity, particularly in situations where differences in environmental conditions may be subtle, so results must be interpreted with caution. Gross lesions identified by necropsy-based methods are generally not pathognomonic for CECs and necropsy-based methods might miss effects that are not visible macroscopically. The histopathological index provided further diagnostic evidence of disease that could not be identified through the rFHI alone. For

example, swelling observed through the rFHI could indicate neoplasia, or it could be a parasite, inflammation, edema, or hyperplasia. The cause of swelling can be confirmed through histopathological analysis.

Organism-level indicators, such as the rFHI, have been used in many monitoring programs (Blazer et al., 2014, 2018; Lang et al., 2017), and while not as sensitive as histopathological or molecular endpoints, they provide useful information about species health. We observed several differences in scores across species and between lake type (i.e., inland vs Lake Superior). Overall, Lake Superior fish followed the expected trend of wastewater effluent-impacted sites having higher scores than undeveloped sites, whereas inland lake species were more inconsistent. When we took a deeper dive into liver scores, we observed fewer parasitic nodules in Lake Superior fish, which reduced rFHI scores overall. Parasitic nodules contributed to scores in inland species which resulted in higher rFHI scores in developed and wastewater effluent-impacted sites. Several factors could explain these differences. There are ecological differences between the lakes that could affect disease exposure or transmission. Lake Superior, the largest of the Great Lakes in North America and the largest by surface area of freshwater lakes in the world, is much bigger than the inland lakes we sampled; therefore, inland lake species are likely more densely populated and interact with intermediate hosts more often than species in Lake Superior. Additionally, eutrophic lakes are more likely to have a diversity of parasites compared to oligotrophic lakes like Lake Superior (Shah et al., 2013).

ToxCast data provided a link from contaminants detected in fish tissues to apical adverse

outcomes. AOPs were used to predict potential biological effects from fish exposed to CECs. Several of the AOPs evaluated here indicated reproductive disfunction as a potential adverse outcome for fish, which aligns with a previous study that used ToxCast to prioritize detected CECs (Corsi et al., 2019). Other field studies also found reproductive disfunction in fish exposed to contaminants, such as the disruption in gonadal development of wild roach living in rivers impacted by sewage effluent (Jobling et al., 2002) and reduced fecundity in fish directly exposed to wastewater effluent (Cavallin et al., 2016). While these results do not directly indicate that the concentrations detected in this study will lead to ecological effects, they provide evidence for driving further monitoring and evaluation.

Adverse outcome pathways revealed possibly troubling biological effects for fish living in aquatic systems across northeastern Minnesota. Several pathways included reduction of 17β-estradiol synthesis by ovarian granulosa cells. The gonads are often the major source of circulating estrogens in vertebrates, including fish (DeFalco and Capel, 2009), so if 17β-estradiol synthesisis reduced, we would expect fish plasma estradiol (E2) concentrations to decrease as well (Park et al., 2010), which can ultimately lead to decreased population growth. Additionally, some AOPs indicated that detected chemicals affect vitellogenin synthesis, which is a commonly used biomarker for endocrine-disrupting chemicals as it is the major egg yolk precursor protein, and it is important for developing embryos and larvae (Sun & Zhang, 2015). Vitellogenin presence in males can be indicative of exposure to exogenous estrogens (Mills et al., 2003; Schultz et al., 2013). Another potentially impactful key event along an AOP relevant to the chemicals detected

in this study is the metabolism of aflatoxin B_1 (AFB1), a liver carcinogen known to induce hepatocellular carcinoma in fish (Tilton et al., 2005; Whitham et al., 1982; Wogan et al., 2012), This coupled with the diagnosis of liver cancer in this study highlights the importance of understanding the full effect CECs are having in aquatic ecosystems.

The ranking of sites was not consistent across methods or species indicating that multiple lines of evidence tell different stories, which is consistent with another study that used a similar method (Jorgenson et al., 2018). However, it must be recognized that we ranked sites based on evidence in fish only. Future studies including water concentration data as well could reveal further insights.

There are some limitations in this study that are important to acknowledge. Fish gills are an important variable to observe for necropsy-based assessments as they play a role in absorption of contaminants, but we had to collect some fish via gill nets in this study. Therefore, we were not able to include gill scores in our final rFHI scores. For histopathology index scores, we were missing gill results from approximately half of the fish.

The failure to identify and understand the effects that complex mixtures of environmental contaminants have on fish and aquatic systems may result in adverse cultural, ecological, economic, and recreational consequences. Here, we provided a baseline assessment of fish health and the biological effects of CEC exposure in northeastern Minnesota. Identifying adverse impacts on fish health is integral for addressing management

strategies for healthy fish populations. While field- and laboratory-based confirmation studies would often provide information on cause-effect relationships, they should involve testing conditions that reflect the sites under which the chemical mixtures occur in order to fully understand *in situ* observations and how they relate to chemical exposures. Thus, the approaches used here provide a means to begin to understand processes that might be occurring in the aquatic systems themselves, which is particularly important for an Indigenous culture that relies on subsistence fishing.

Chapter 5

Conclusion

The direct and indirect coupling between aquatic ecosystems and human impacts on those systems cannot be ignored. The ubiquitous presence of CECs in the aquatic environment presents a significant ecosystem health issue. The challenge now is placing the detected concentrations and effects of mixtures into context of their biological and ecosystem-level impacts. The population-level effect of sublethal exposure to concentrations is complex and confounded by other environmental stressors, such as the presence of legacy contaminants, pathogens, or other water quality issues. As the ability to detect chemicals in the aquatic environment and other wildlife continues to grow, so will the importance of understanding the implications of such findings.

The goal of this thesis was to determine the presence of CECs within Tribal lands and adjacent territory in northeastern Minnesota and evaluate their potential impact on the health of subsistence fish species. To assess the role anthropogenic influence may be playing in this relationship, we investigated sites along a spectrum of pressures: waterbodies with no human development along their shorelines, those with human development, and those directly influenced by wastewater effluent. Disrupted aquatic ecosystems could threaten the ability of Indigenous communities to maintain a subsistence lifestyle; thus, this thesis aimed to help communities with subsistence lifestyles understand the potential effects chemical contamination may have on their

cultural well-being.

In **Chapter 2**, we surveyed the occurrence of CECs in water, sediment, and fish to determine the spatial distribution of their presence. We detected 117 chemicals - including hormones, antimicrobials, antidepressants, insect repellant, and more - in water, sediment, and/or fish. These chemicals were found in remote, undeveloped locations, which suggests that sources of contamination to surface water extend beyond the direct influence of wastewater treatment plants or septic systems. The breadth of contaminants found across 28 locations within the GPIR and 1854 Ceded Territory highlights the importance of protecting food sovereignty for Indigenous communities.

To prioritize the 117 detected CECs for management actions, we performed a rapid-screening assessment of detected CECs based on their potential environmental hazard in **Chapter 3**. This work stemmed from Minnesota Pollution Control Agency's Aquatic Toxicity Profiles framework (Streets & Dobbins, 2017), a tool that incorporates chemical-specific information - such as acute toxicity, endocrine activity, physicochemical properties, and detection frequency data - to evaluate the potential for environmental contaminants to cause adverse effects on aquatic life. We classified 50 contaminants in water, 21 in sediment, and seven in fish as high priority. This chapter adds to the understanding of the potential hazards of 117 chemicals detected in a region that is important for sustaining Indigenous culture through subsistence fishing. The high priority contaminants identified in Chapter 3 were further evaluated by Servadio and colleagues (2021) to determine whether anthropogenic or environmental factors were

associated with detection of chemicals. That study found a strong connection between impervious surface and contamination, even among undeveloped lakes, and found an association with greater numbers of buildings near lakes, indicating that anthropogenic activities are associated with CEC presence. Taken together, these studies can facilitate the selection of high priority chemical hazards for temporal monitoring at locations of high concern.

Chapter 4 aimed to evaluate the impact CECs might be having on fish health. Results from this study revealed potential biological effects of the chemicals identified, as well as macroscopic and microscopic tissue and organ abnormalities of subsistence fish harvested for fish consumption. The health of fish was adversely affected in lakes across a spectrum of anthropogenic pressures: undeveloped, developed, and wastewater effluent-impacted. We found that the health of fish in undeveloped sites was as poor, or sometimes poorer, than fish in developed and wastewater effluent-impacted sites.

There are already several next steps in motion for this study system. We are surveying CECs in subsistence species, wildlife, and wild rice to further understand the spatial distribution of chemicals throughout the environment in this region. Thus far, we have detected CECs in beaver, deer, grouse, hare, moose, and wild rice. We plan to expand collection to include macroinvertebrates, more fish, amphibians, reptiles, other mammals, birds, fungi, and plants. Additionally, due to a recent discovery of the chemical that causes unexplained acute mortality in coho salmon in the Pacific Northwest (Tian et al., 2021), our team plans to characterize the tire rubber-derived chemical, *N*-(1,3-

dimethylbutyl)-*N*'-phenyl-p-phenylenediamine (6PPD), and its potential impact in northeastern Minnesota. Further, we are evaluating metagenomic data collected from yellow perch feces to analyze potential associations with CEC data. Other important future directions should include the collection of water concentration data and lab-based studies to further assess the impact CECs have on fish health.

We now know that CECs are present in environments that we once thought were pristine wilderness. Collectively, this thesis provides evidence for the presence of CECs across northeastern Minnesota, including in remote, undeveloped areas; prioritizes their potential chemical hazards; and reveals the potential impact on fish health, which could ultimately lead to negative impacts at the ecosystem level. The continued longevity of Ojibwe culture and way of life depends on the protection of natural resources; thus, it is imperative to mitigate CEC spillover into these environments and protect the waters in which the Ojibwe are intrinsically linked.

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Appendix

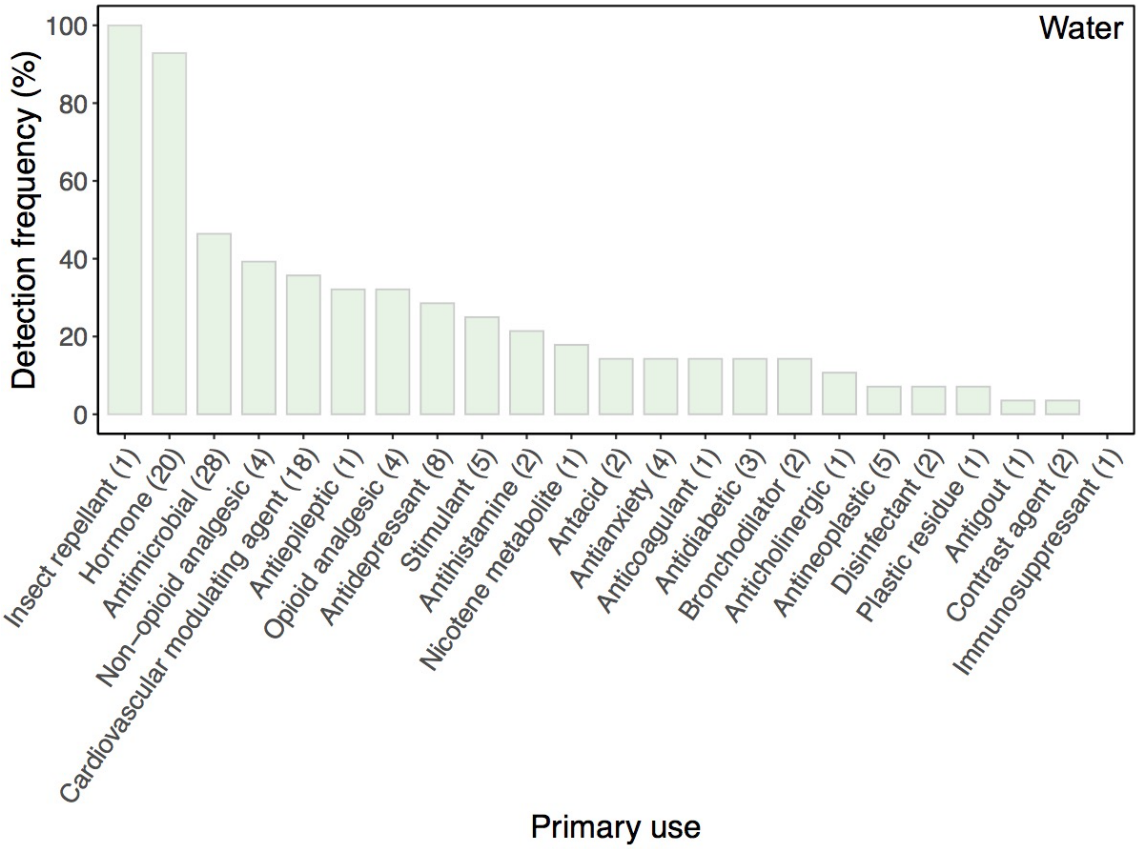

Figure A1. Frequency of contaminants detected in water (POCIS) samples in northeastern Minnesota in 2016-2018. Contaminants are grouped by primary use category. The number in parentheses represents the number of possible compounds detected in each category. If the contaminant was detected in any media at each site, it was considered a detection.

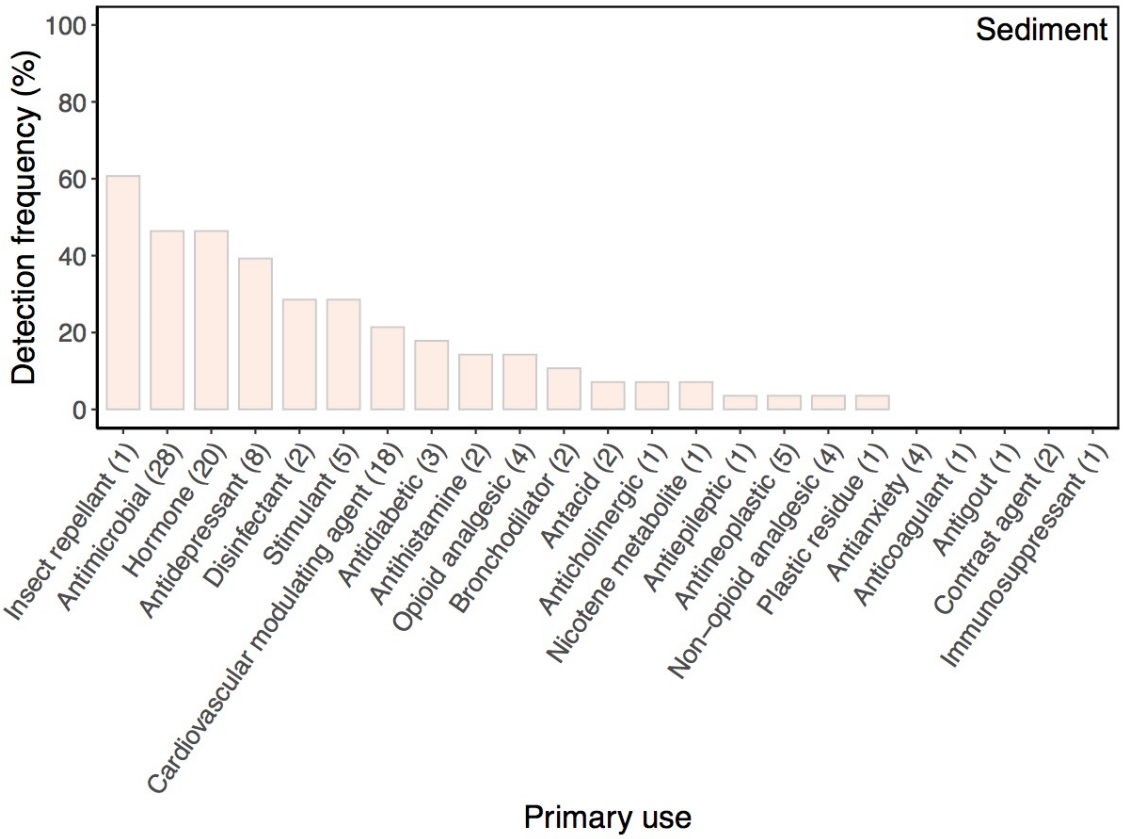

Figure A2. Frequency of contaminants detected in sediment samples in northeastern Minnesota in 2016-2018. Contaminants are grouped by primary use category. The number in parentheses represents the number of possible compounds detected in each category. If the contaminant was detected in any media at each site, it was considered a detection.

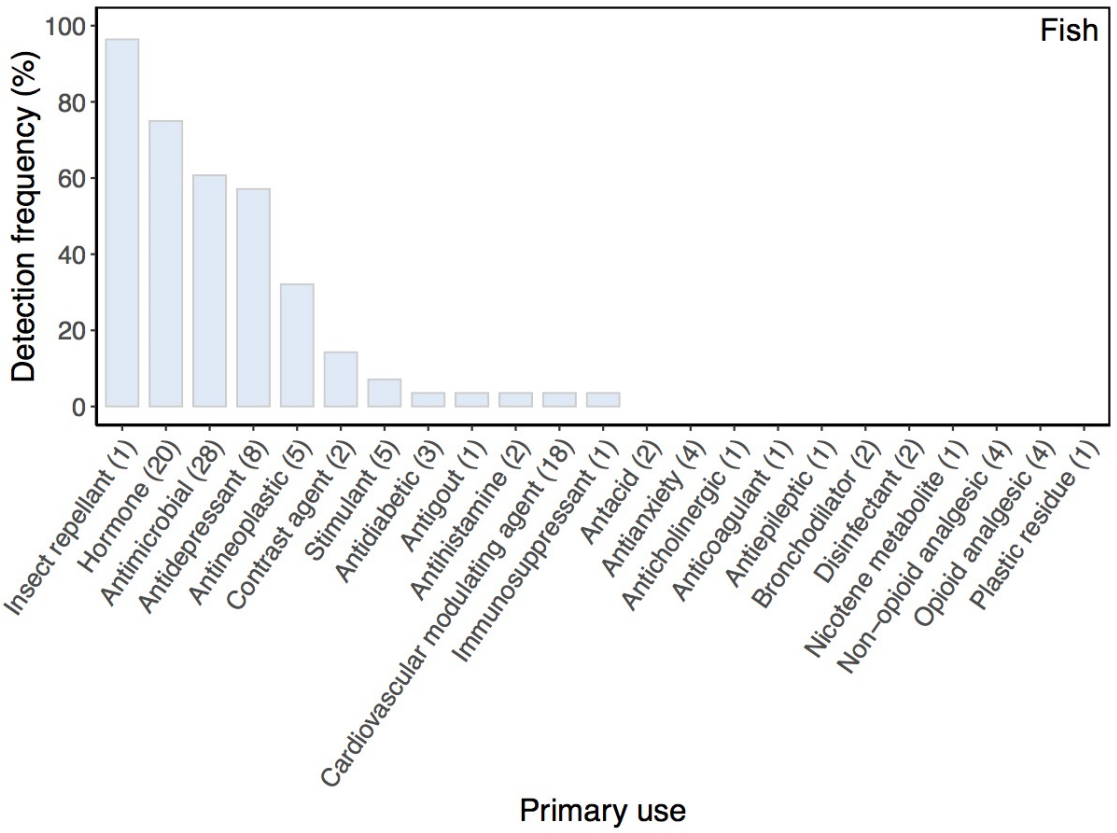

Figure A3. Frequency of contaminants detected in fish tissue samples in northeastern Minnesota in 2016-2018. Contaminants are grouped by primary use category. The number in parentheses represents the number of possible compounds detected in each category. If the contaminant was detected in any media at each site, it was considered a detection.

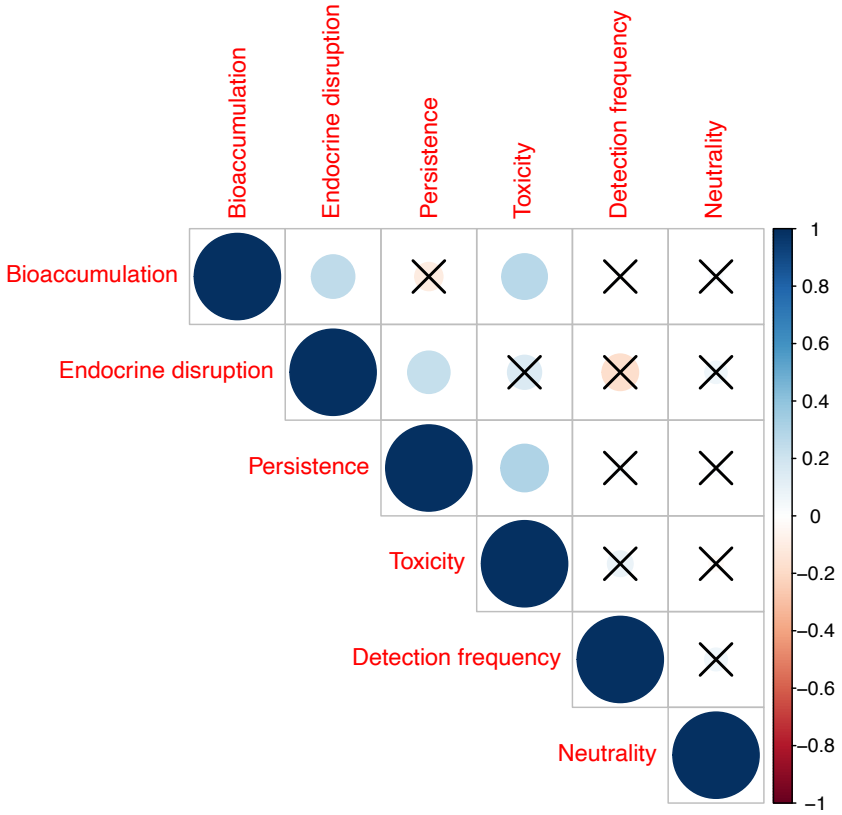

Figure A4. Correlogram displaying Spearman Spearman's rho rank correlation among profile questions for chemicals detected in water. Insignificant (based on a p-value > 0.05) correlations are marked with an "X." Blue indicates positive correlations and red demonstrates negative correlations. Color intensity and size of the circle are proportional to the correlation coefficients.

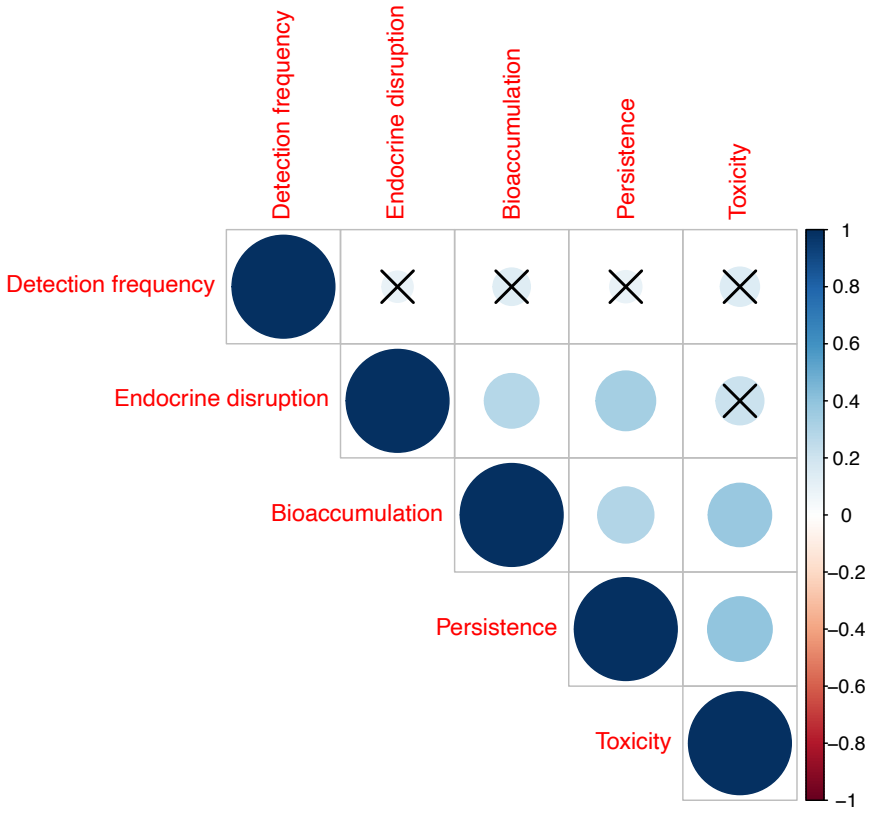

Figure A5. Correlogram displaying Spearman Spearman's rho rank correlation among profile questions for chemicals detected in sediment. Insignificant (based on a p-value > 0.05) correlations are marked with an "X." Blue indicates positive correlations and red demonstrates negative correlations. Color intensity and size of the circle are proportional to the correlation coefficients.

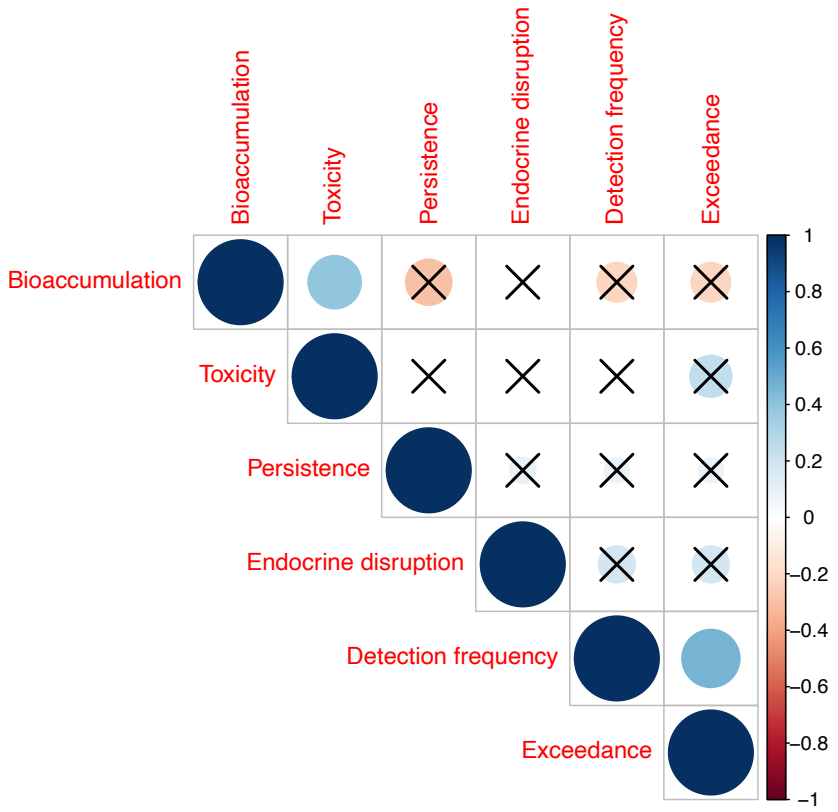

Figure A6. Correlogram displaying Spearman Spearman's rho rank correlation among profile questions for chemicals detected in fish. Insignificant (based on a p-value > 0.05) correlations are marked with an "X." Blue indicates positive correlations and red demonstrates negative correlations. Color intensity and size of the circle are proportional to the correlation coefficients.