Upper Mississippi River Basin Association
Water Quality Task Force
Virtual Meeting

January 25-26, 2022

Agenda
with
Background
and
Supporting Materials

UPPER MISSISSIPPI RIVER BASIN ASSOCIATION
WATER QUALITY TASK FORCE MEETING

January 25-26, 2022

Agenda

Connection Information

- Web, video conferencing, click on the following link:
  - January 25: https://umrba.my.webex.com/umrba.my/j.php?MTID=m1cf14e7397080c96d83b47ca8c464f9a
  - January 26: https://umrba.my.webex.com/umrba.my/j.php?MTID=maac78e39f0dbd2d7987748cc02896f8b
- Dial-in number: (312) 535-8110
  - January 25 access code: 2552 037 0548
  - January 26 access code: 2554 271 3079
  - Passcode: 1234

January 25, 2022

Time	Attachment	Topic	Presenter
1:00 p.m.		**Welcome and Introductions**	***John Hoke**, MODNR*
1:05	**A1-22**	**Approval of the September 28-29, 2021 WQ Task Force Meeting Summary**	***All***
1:10		**UMRBA WQ Task Force Updates**	
		• How Clean is the River? Report	***Lauren Salvato**, UMRBA*
		• Reaches 8-9 Pilot	
1:25		**Contaminants**	
	B1-3	• Radium Study in Aquifers of North-Central Illinois	***Dr. Walton Kelly**, Illinois State Water Survey*
1:55		**Break**	
2:25		**Contaminants (Continued)**	
	B4-18	• Prioritizing Chemicals of Ecological Concern in Great Lakes Tributaries	***Steve Corsi**, USGS*
2:55		**Ecological Risk Assessments**	
		• Aquatic Life Water Quality Criteria for Toxics: Prioritization and Progress	***Dr. Kathryn Gallagher**, USEPA*
3:25		**Chloride**	
	C1-21	• Impacts of Chloride and Sulfate Ions on Macroinvertebrate Communities	***Bob Miltner**, OHEPA*
		• UMRBA Resolution	***Lauren Salvato**, UMRBA*
4:00 p.m.		**Adjourn for the Day**	

January 26, 2022

Time	Attachment	Topic	Presenter
8:30 a.m.		**Reflection**	***All***
8:35		**Citizen Science**	
	D1-2	• Plastic Pollution Campaign	***Jennifer Wendt****, MRCTI* and ***Steve Gustafson****, Partners of Scott County Watershed*
		• WQ Citizen Science Programs	***All***
9:35		**CWA Program Updates** • 305(b) and 303(d) Consultation • TMDL Updates	***All***
10:00		**Break**	
10:30		**Nutrients** • State and Federal Updates	***All***
10:55		**Administrative Items** • Future Meeting Schedule	***All***
11:00 a.m.		**Adjourn**	

ATTACHMENT A

Draft Summary of the September 28-29, 2021 WQTF Virtual Meeting

(A-1 to A-22)

Upper Mississippi River Basin Association
Water Quality Task Force Virtual Meeting

September 28-29, 2021

Draft Highlights and Action Items Summary

Tuesday, September 28

Approval of the WQEC-WQTF Draft June 8-9, 2021 Meeting Summary

The UMRBA Water Quality Task Force (WQTF) approved the June 8-9, 2021 draft highlights and action items summary pending an edit to page A-4 on the quantity of super gages funded by Illinois EPA and the Metropolitan Water Reclamation District of Greater Chicago.

UMRBA WQ Task Force Updates

How Clean is the River? Report

Erin Petty provided an update on the *How Clean is the River?* Report. The analysis was conducted using the EGRET R package to generate flow-normalized trends. Data collected were from Illinois EPA, Minnesota PCA, Wisconsin DNR, and the UMRR program, with flow gauge information from USGS and USACE from 1989 to 2018. Sixteen total sites were selected on the UMR (spanning Pool 4 to the Open River) and the La Grange reach of the Illinois Waterway. The initial findings are as follows:

- Total nitrogen (TN) and nitrate + nitrite were generally increasing
- Sulfate and chloride were generally increasing
- Conductivity was generally increasing
- Dissolved oxygen (DO) was generally increasing
- Chlorophyll-a (chl-a) was generally decreasing from Pool 4 to 13 and increasing below Pool 13
- Total phosphorus (TP) and ammonia were generally decreasing
- Zinc, aluminum, and copper were generally decreasing
- Total suspended solids (TSS) and temperature (water) were generally decreasing
- pH is generally decreasing

Next steps include writing the report and finalizing maps and graphics to communicate the results. Petty clarified that the temperature variable was for water in response to a question from Shawn Giblin. Aabir Banerji asked about confounding factors in chl-a versus turbidity. Are there ways to tease the two apart? Do we have separate turbidity measures that could be corrected for the signal for chl-a? Robert Voss replied that rivers respond differently based on the size of the photic zone. Voss would not suggest a trend analysis as the appropriate way to analyze Banerji's questions. A lot more data and a smaller scale would be a better analysis. Generally, if there are nutrient and chl-a increases on the river, but a decrease in sediment or turbidity, then that could mean that sediment is dropping out but nutrients are available for the chl-a response. Lee Ganske asked if a review process is planned between release of results and the public rollout. Voss noted that the R code and data are available on the Google Drive, but staff can share widely for review. WQTF members agreed to conduct a review.

Reaches 8-9 Pilot

Dan Kendall provided an update on the Reaches 8-9 pilot project. As a reminder, UMRBA, Missouri, Illinois, and Iowa agencies and laboratories are sampling 109 river miles from the Iowa River confluence to L&D 21 (near Quincy, Illinois).

Fixed site sampling is completed. Probabilistic summer sampling is complete for water chemistry and macroinvertebrates. Some fish sampling remains, specifically the collection of black bass for the fish consumption use assessment. There were challenges in collecting bass within the required size parameters. Laboratory analyses for water chemistry by Missouri DNR are still ongoing. Analyses for PFAS by USEPA Region 5 and cyanotoxin analysis by Iowa DNR are complete. Much of the remaining work for the pilot includes field data entry and laboratory data entry.

Kendall detailed problems and solutions encountered along the way for the Reaches 8-9 pilot.

— COVID-19 shifted the originally scheduled sampling period from December 2019-December 2020 to October 2020-September 2021.

— The public water suppliers (PWS) that were voluntarily participating in the drinking water use assessment stopped participating in March 2020. Some of the reasons were challenges collecting the monthly volume of samples requested after staff capacity was reduced. The planning committee adjusted and incorporated drinking water parameters with fixed site sampling.

— PFAS sampling was temporarily suspended due to laboratory contamination issues. Sampling resumed in January 2021 after USEPA Region 5 adjusted sampling protocols to combat the contamination issue.

— Samples were lost by FedEx in January 2021 and have not been recovered.

— Five of the 34 Hester Dendy sampling sets were lost after deployment. Reasons are currently unknown.

— The contractor who was going to identify the macroinvertebrates was no longer available, and the planning committee quickly worked to find alternatives. Rhithron will be the new contractor. They identified the macroinvertebrates collected for the Reaches 0-3 pilot.

John Olson (retired Iowa DNR) is the contractor to draft the condition assessment report. Gregg Good suggested asking Olson why we chose to use skin-on filets in the original plan. The current discussion is whether to analyze the fish tissue with skin-on or skin-off. While the recommendation of skin-on is in the field operations manual, the states use skin-off, so it would be helpful to know why that decision was made.

Harmful Algal Blooms (HAB)s

State and Federal Updates

Minnesota – Ganske said the hot dry summer in Minnesota coupled with drought led to many blooms and hotline calls. Ganske said Minnesota PCA has not dealt with suspected illness. There was one dog death near the Minnesota and Iowa border on Lake Okamanpeedan/Tuttle. Additional investigation revealed the death was not related to harmful algal blooms (HABs), although the media reported on the story ahead of the agency. There was no microcystin detection (detect) in samples collected at the lake.

Substantial algal bloom activity occurred in Lake Pepin for the first time in several years. In response to a question from Kendall about toxin levels from the summer season, Ganske replied that Minnesota PCA does little toxin monitoring so he is unaware of how many detections occurred.

Illinois– Alexandrea Terlep said Illinois EPA has had a busy HAB season. Illinois EPA staff collected around 50 samples in response to suspected HAB events. Around 250 samples were analyzed from recreational waters, and 21 of those samples had results over 20 parts pers billion (ppb). This is in line with previous years. The highest value recorded was approximately 4,300 ppb microcystin on Lake Louise in Lake County in July 2021.

Illinois EPA staff have collected 180 cylindrospermopsin samples in recreational waters and no results were above the USEPA criteria of 15 µg/L. Similarly, there were no hits for raw and finished tap water that was sampled and analyzed. Illinois EPA is now able to analyze samples for anatoxin and saxitoxin. Sixty-five samples were taken from recreational waters for anatoxin, returning only one detection at 0.16 ppb. Only one hit from 80 drinking water samples (both raw and finished water) was found. Sixty-five samples in recreational waters were collected for saxitoxin. About 25 percent or 17 samples had detections, with the highest concentration of 0.3 ppb. Terlep said staff have observed that if saxitoxin is found in raw water, saxitoxin is also detected in finished water in low concentrations.

Terlep reviewed the bloom that occurred on the Illinois River at Starved Rock State Park. In mid-June the Army Corps of Engineers (Corps) noticed a suspected bloom at the Starved Rock L&D. On June 10, Illinois EPA staff collected samples. Of the four toxins analyzed (i.e., microcystin, cylindrospermopsin, anatoxin, and saxitoxin), microcystin was the only toxin detected at 95 ppb. Illinois EPA proceeded to issue a warning to the public. About a week later, USGS and Illinois EPA staff went back to Starved Rock for follow-up sampling. The bloom was still observed, and results for microcystin were 250 ppb. Two of the three samples USGS collected and analyzed were above the recreational criteria of 8 ppb or 8 µg/L. Thankfully, subsequent rain events dissipated the bloom at Starved Rock. On June 30, a final sample was collected and there was no evidence of a toxic bloom.

There is a bloom currently in Campus Lake at Southern Illinois University Carbondale (SIUC). It is a long-lasting bloom that has been in effect since July 2021 and has closed the lake to recreation. The agency used the opportunity for species identification samples in July and in mid-September. Both sets of samples showed that microcystin was the predominant species. The July sample had results of over 30 ppb microcystin. The water looked green. The August bloom had an extremely high pH of 10.2 and the water looked bright green. Surprisingly, the microcystin concentration was just above 8 ppb. The recent September 23 sampling event is still being analyzed. The bloom is less noticeable, and the pH level dropped. SIUC contracted with a consulting firm for a solution, and aerators are currently deployed in the lake.

One case of suspected dog illness occurred near Rochester shortly after the July 4th Holiday weekend. A citizen called stating that her four large dogs experienced partial paralysis after swimming in small ponds. Illinois EPA staff talked to the veterinarian that treated the dogs and confirmed that they showed symptoms consistent with algal poisoning. The dogs were washed with a dog dish soap and treated with an anti-inflammatory. As far as Terlep is aware, the dogs made a complete recovery.

Staff were able to visit the ponds the same day as the call due to the close proximity to Springfield, Illinois. The water did not have any visual signs of a bloom. Samples collected for microcystin and cylindrospermopsin came back as non-detections (non-detect). Terlep did not observe anything under the microscope either. It is unclear what caused the dog illness, but from the prompt sampling, it was not likely caused by algal toxins.

A dog death occurred an hour after swimming in Valley Lake in northern Illinois. Illinois EPA staff received a call from a veterinarian. The lake was experiencing a bloom at the time of the dog's death. Lake County staff collected samples for all four toxins one day later. There were signs of a bloom but all four toxins were non-detects. It is not conclusive that toxins were not present, but because a necropsy or tests were not conducted, it is impossible to know the cause of death.

Illinois EPA has had issues with the Abraxis test strips. For a bloom that occurred on the Fox River, the test kit results were 10 ppb. The laboratory sample results were 140 ppb. Follow up sampling with the kit resulted in a non-detect while the laboratory result was 12.3 ppb. Terlep said she is unsure whether the kits can be trusted or whether the batch of kits was bad. She sent a new set of kits to northern Illinois and will have staff compare the new and old kits. Terlep said she would like to know if others are experiencing the same issue. In response to a question from Albert Ettinger, Good replied that a HAB summary report will be put together at the end of 2021.

In response to a question from Giblin about a funding source to run necropsies following dog deaths, Good replied that Illinois EPA does not have any funding. A dog death that occurred a few years ago was an older dog. The University of Illinois veterinary medicine program did a courtesy autopsy and found heat stroke was the cause of death. The dog's symptoms seemed consistent with algal toxins poisoning, but there was no proof. Necropsies can range from a few hundred to thousands of dollars depending on the reason. Gina LaLiberte noted that water intoxication has similar symptoms to algal toxin poisoning, and unless veterinarians are checking for ion levels, the ailment can be misdiagnosed. A dog playing fetch in the water for hours takes in too much water. A veterinarian will also present more than one probable cause of death, but algal toxin poisoning usually gets the blame because the pet owner is not in control of that.

Iowa – Kendall said that Iowa primarily monitors at state park beaches. During the 2020 season, Iowa DNR changed its recreational criteria to USEPA's recommended 8 µg/L of microcystin. During the 2021 season, there were 24 advisories. While this was more advisories than last summer, it was overall a below average year (the average is 31 advisories). DNR staff expected the number of advisories to increase once switching from 20 µg/L to 8 µg/L. A lot of lakes turned green, but toxin production (once analyzed) was low.

Iowa DNR laboratory analyzed samples of a suspected bloom on the UMR mainstem on July 27, 2021 and follow-up samples on August 10, 2021. This was a collaborative effort between the Corps and Illinois EPA (as mentioned by Terlep). The laboratory also analyzed samples for the Reaches 8-9 pilot. Kendall skimmed the results and noted the highest value was 3 or 4 µg/L for microcystin. Generally, the values were higher than he expected.

Wisconsin – LaLiberte said there has been improved HAB awareness and reporting in Wisconsin. For the past few years, DNR has maintained an email address the public can use to report events. In 2021, staff received about 170 reports. This included lakes for which there were multiple reports, and 40 instances of reports misidentified as toxin blooms (e.g., filamentous algae). LaLiberte and staff are working on updates to both the website and online reporting before next year's bloom season.

There were about six cases that were jointly investigated with the Wisconsin Department of Health Services via the HAB surveillance program. One of those cases was a dog death, however, the dog had underlying health conditions and the death may not have been related to cyanotoxin poisoning.

A few minor blooms occurred on Lake Superior. This was good from the public health standpoint, but not from the research standpoint because USGS staff were focused on studying blooms in Lake Superior during summer 2021.

LaLiberte reviewed that Wisconsin DNR does not have a coordinated statewide monitoring effort. Staff are working on incorporating HABs into its monitoring strategy for the next five-year cycle.

Missouri – John Hoke said that Missouri DNR had 27 reports of HABs through either the emergency response hotline or DNR's online reporting form. Nineteen were confirmed as being a cyano-bloom either through photo or field test confirmation, including three drinking water supply impacts. This has caught the attention of the drinking water branch and watershed protection program. The two programs have collaborated on source water protection measures. There have been no animal illness reports. Voss added that the University of Missouri does batch analysis of algal toxin samples on lakes, but DNR has not yet seen the 2021 sampling summary.

USEPA Office of Water - Dr. Lesley D'Anglada provided an update on USEPA Office of Water's (OW) work. The first, published in July 2021, is the final technical support document for implementing USEPA's recommend recreational ambient water quality criteria for microcystins and cylindrospermopsin. The second is the recommended nutrient criteria for lakes and reservoirs. This was released in August 2021 for total phosphorus and total nitrogen to protect the following use assessments: aquatic life, recreation, and drinking water supplies. USEPA developed a statistical model that can be used with local data to develop nutrient criteria.

USEPA updated its application (app) for sanitary surveys for marine and fresh waters. The app helps identify potential pollution sources as well as monitor and share data on the potential for HABs. The survey includes a place to report a HAB bloom, including various observations and photos. The app also geolocates sites to connect to weather service information.

The Unregulated Contaminant Monitoring Rule (UCMR 4) was conducted from 2018 to 2021. More than 85,000 cyanotoxin samples were collected from 6,000 public water supply systems. Around one percent had detections in finished water for the four toxins. The data are used to inform future public health interventions to protect drinking water supplies.

The upcoming 2022 National Aquatic Resource Survey monitoring will include microcystin and cylindrospermopsin monitoring. USEPA is working to provide additional real-time reporting tools with the BloomWatch app if blooms are encountered in the field.

The Cyanotoxins Preparedness and Response Toolkit, published in March 2021, is an online interactive tool with resources for drinking water systems and water managers to prepare before a bloom event occurs. The interactive PDF also allows for an assessment after the bloom occurs. If a government unit or other entity does not have a cyanotoxin management plan, this tool can be a good starting point.

CyanoHAB story map was published in August 2021. The story map compiles incidents in freshwater reports across the nation and illustrates where HABs occurred over time since 2015.

USEPA conducted workshops and webinars in 2021. The USEPA HAB regional workshops summary report is for outcomes of the nine HAB workshops that occurred between 2015 and 2019. The USEPA Freshwater HABs newsletter for both fresh water and marine environments is now available.

OW is currently working on risk assessments for saxitoxins and nodularins. D'Anglada hopes the assessments will be ready for review at the end of 2021. They will be used to determine if there is adequate data for health advisories for drinking and recreational waters. Systematic reviews are also being conducted for microcystin, cylindrospermopsin, anatoxin-a, saxitoxins, and nodularin in fish and irrigation water.

USEPA and NOAA have developed separate, but coordinated, draft HABs and Hypoxia Event of National Significance policies for freshwaters (EPA) and marine/coastal waters (NOAA) in the U.S., as directed by the 2017 Harmful Algal Blooms, Hypoxia, Research and Control Act (HABHRCA). Once the Office of Management and Budget finishes reviewing USEPA's draft policy, it will then be released for public comment.

Nicole Manasco says the Corps is wrestling with occupational exposure to microcystin. The literature indicates it may be building overtime in our bodies and the Corps Districts do not know how to share the information responsibly with the workforce. Does USEPA have any guidance? In response, D'Anglada said that exposure to cyanotoxins is inhalation of aerosols. The issue is with available data, and there is not a lot of toxicity data for respiratory exposure. USEPA has asked the Office of Research and Development for more data to be able to provide guidance.

In response to a question about whether USEPA will ask states to adopt its nutrient lakes criteria, D'Anglada said that USEPA would like states to adopt its criteria for the protection of public health. USEPA is providing technical support and will host webinars to answer questions, and the agency may suggest other actions in the future.

USEPA Office Research and Development – Dr. Scot Hagerthey said his role with USEPA is as a coordinator for the Safe and Sustainable Waters Resources Research Program as well as the HAB and nutrient portfolios. Hagerthey overviewed the current research and what is planned for the immediate future.

The Safe and Sustainable Waters Resources Research Program's goal is to provide science and innovative technology needed to maintain public drinking water systems and protect biological, physical, and chemical integrity of the water. The Strategic Research Action Plan (STRAP) guides research for each focal area. The third iteration of STRAP will end in FY 2022. By the end of September 2022, the majority of HABs projects will wrap up and many final reports will be circulated from the third STRAP. The fourth STRAP will be in effect FY 2023 to 2026.

Hagerthey reminded participants that at the September 2020 WQTF meeting, Brenda Rashleigh detailed the ongoing HAB research. That included health effects on toxicity to humans and biota, specifically for microcystin congeners. The research pertained to toxicity studies for anatoxin-a and exposure to aerosols in waterbodies. The project is starting up again after experiencing delays due to COVID-19. Another area of research focus is on source and drinking water treatment and developing tools for treating and managing HABs in drinking water. The final focus area is related to HABs ecology to characterize bloom-impacted environments. The CyanNetwork, a collaboration with USEPA, NASA, NOAA, and USGS is used to identify HABs across the contiguous U.S. using satellite sensors. The sensors currently capture blooms in large water bodies. In 2021, ORD released the web application, and now the CyanNetwork is available as both an Android and web application. Historical data from the satellite data collection was used to produce status and trends of blooms for 2,000 lakes in USEPA's report on the environment. The data show long term trends and the number of blooms that occurred, broken down by the magnitude, duration, and extent of the events. The report is a synopsis of the national picture of HABs, but it can be broken down to small scales (e.g., individual states) for further analysis.

Hagerthey said that for the four years, the planning process is underway. Research action plans and projects related to HABs are developed in close partnership with the OW, ECOS, ERAS, and tribal partners. Hagerthey and colleagues have compiled information on key problems to address with research and how best to deliver tools and resources. The planning process is in early development. Over the next two to six months, staff will work on specific research projects and further refine the research that will be conducted. The Biden-Harris Administration's priorities will be incorporated: climate changes and HABs from an environmental justice standpoint.

Research will look at two main issues for HABs: 1) human and ecological health effects and toxicity, and 2) the management of surface waters used for irrigation and drinking water. Common priorities identified so far include: 1) in-vito and vitro studies on toxicity of HABs to humans, aquatic life, and life dependent on aquatic life that can support health advisories; 2) advancing methods to detect and collect microcystin, cylindrospermopsin, anatoxin-a, saxitoxin and nodularins; 3) emphasizing research on benthic HABs; and 4) how to evaluate the efficacy of intervention processes to remove toxins from drinking water systems. USEPA is downsizing the amount of research on the ecology of HABs and shifting toward forecasting HABs. The intent is not to have a tool, but to build science to move in that direction. If states and other entities collect data on HABs, they can coordinate with USEPA staff such as Dr. D'Anglada. The data are important to understand and validate models and approaches.

The Safe and Sustainable Waters Resources Research Program website is continuously updated, and Hagerthey encouraged participants to check the site to stay abreast of reports and other content. Dr. Amy Shields asked if regional staff could coordinate with Hagerthey on participating in the planning process. Hagerthey replied that a regional representative will be on each coordination team and those people will be identified soon.

NOAA – Dr. Tony Marshak provided a written update on the work of the Interagency Working Group on the Harmful Algal Bloom and Hypoxia Research and Control Act (IWG-HABHRCA). The IWG is a Congressionally-mandated group that is tasked with coordinating and convening Federal agencies and their stakeholders to discuss HAB and hypoxia events to develop action plans, reports, and assessments to monitor, address, limit, mitigate, and assist communities with their own resilience to HAB and hypoxia events in U.S. coastal and inland waters. In 2021, the IWG completed the 2020 Great Lakes Report to Congress. The report can be accessed with the following link: https://cdn.coastalscience.noaa.gov/page-attachments/research/FINAL_HABHRCA_GreatLakes_ProgressReport_November_2020.pdf.
In October 2021, the IWG-HABHRCA Coordinated Planning Document will be finalized. The document includes information on:

- IWG summary of activities and deliverables, including highlights of past successful coordinated activities and planned efforts
- Member agency roles and responsibilities and priority areas for enhanced Federal agency coordination, research, and response to HABs and hypoxia (including HHENS)
- Future IWG coordination strategies for continued and improved efficiencies over 2021 to 2026

Additional involvement of the IWG and outreach activities are as follows:

- Working with the National HAB Committee to update the 2005 HARRNESS (Harmful Algal Research and Response: A National Environmental Science Strategy) Report

- Participation in the three-part webinar series hosted by USEPA, NOAA, and the Sitka Tribe of Alaska on Managing HABs in Tribal Waters
- Convening of the April 2021 Interagency HAB Preparedness and Response Workshop
- Interagency participation in the May 2021 US Army Corps of Engineers HAB Research and Development Workshop
- Continued development of parallel NOAA and EPA HHENS policies for public comment in the Federal Register later this year.

Cyanotoxin Mixture Models

Dr. Vicki Christensen provided background on Voyageurs National Park for participants to understand relevance to waters of the Upper Mississippi River Basin. Voyageurs is downstream from many wilderness areas (i.e., Boundary Waters Canoe Area, Quetico Provincial Park, and Superior National Forest) and receives water inflow from pristine areas. A waterbody of concern at the Park is Kabetogama Lake. The lake acts like a backwater area. Water levels are manipulated to improve water quality with rule curves to simulate a more natural regime. Parts of the shoreline have granite outcrops and others have lower lying areas with soils high in nutrients and mineral rich, containing higher pH and specific conductance.

Algal blooms have been observed since at least 1975 (coinciding with the Park's establishment), but they were likely present before then. Some of the blooms produce multiple toxins, which raises it as a human health issue. Christensen and her co-authors collected over 120 environmental samples including water chemistry, phytoplankton identification and enumeration, toxins, and molecular assays. Molecular assays show which cyanobacteria are capable of producing a toxin. Ultimately, the conditions must be right for toxin production.

The study sought to answer two questions: 1) which variables best predict toxins and exposure risk?, and 2) is a cyanotoxin mixture model better than a microcystin-only model? The USEPA Virtual Beach software was used to run a comprehensive microcystin model and a comprehensive mixture model. The study looked at over 60 parameters at least theoretically related to cyanobacteria. Some variables lagged, e.g., total phosphorus (TP) concentration four days before a bloom may have been more relevant. One site, Sullivan Bay, had more hits of microcystin in 2016 and more with saxitoxin in 2017. Christensen reiterated the importance of collecting data over multiple years and multiple toxins.

The first comprehensive microcystin model had one false positive and one false negative. A false positive means a beach was closed unnecessarily, and a false negative means a beach was not closed when toxin levels were elevated. This is a greater public health concern. The comprehensive mixture model had many of the same parameters and matched observed and simulated conditions well. The model had a false positive, but had no false negatives.

In summary, neurotoxins are good predictors of overall risk. The toxins do not peak at the same time as microcystin and may be present without visible blooms. Water temperature, specific conductance, TP water level, wind direction, and toxin genes were all correlated to toxin occurrence. A three-toxin mixture (i.e., comprehensive mixture model) appeared to be the better model, as it produced no false negatives.

Ganske asked if the model is still used for other applications and if it the input is intensive. Christensen said quite a few people use this for bacteria, namely *E. coli* or fecal coliform at beaches. This study is the first use of the model for a mixture of cyanotoxins, which makes this model unique. Regarding sampling intensity, the harder part is setting up the model.

Environmental Factors Controlling Phytoplankton Dynamics

Giblin displayed a photo of the visual presence of algal mats and green water at L&D 4 in the middle 2000s during low flow years. The purpose was to detail how conditions on the river change related to discharge. The year 2021 was categorized as a medium discharge, although *Microcystis* and *Aphanizomenon* blooms occurred on the mainstem.

In the paper Giblin and Gerrish, 2020, the authors developed regression tree models to predict specific phytoplankton biovolumes. Each oval is a predicted biovolume of phytoplankton under different conditions, e.g., high TP and low N. The result would produce the volume of phytoplankton in comparison to low discharge conditions.

The dataset used for the regression tree analysis was from the Upper Mississippi River Long Term Resource Monitoring. The seven total sites, collected in 2009 and 2011, spanned main channel and backwaters in Pool 8 collected in 2009 and 2011. These two years offered a good mix of a high (2011) and low (2009) discharge and provided a full range of water quality conditions found along the river. Flow differences also led to significant nutrient differences: *Microcystis, Aphanizomenon,* and *Dolichospermum (anabaena)* were dominant in 2009 while *Pseudanabaena* and *Planktothrix* were dominant in 2011.

River managers should know the associated risk factors. Giblin and Gerrish produced overarching regression tree models that attempt to characterize that for river managers. The highest risks tended to be during low nitrogen and high phosphorus conditions at roughly 16 times the biovolume. Microcystin, for example, tends to be more dominant during high phosphorus and when temperatures are warm.

In a separate study, Giblin collected data in 2019 in Pools 5 and 8 to capture a combination of high and low nitrogen and phosphorus concentration across paired sites. He used a structural equation model and produced a table to address conflicting hypotheses on the drivers of cyanotoxin blooms. The model tested each driver and Giblin found that cyanotoxin and cyanobacteria problems were associated with the following conditions:

- High total phosphorus
- Low dissolved inorganic nitrogen
- High water temperature
- Low water velocity (flushing)
- High turbidity
- Low macrophyte cover
- Shallow water depth

Giblin displayed an aerial image at Trempealeau National Wildlife, which has opaque waters relative to the mainstem. The refuge is leveed off from the river and locked into a turbid, cyanobacteria-dominated state. Refuge managers have not been able to do drawdowns because of the high discharge in the mainstem, and, as a result, the aquatic bird habitat has diminished. TP has built up over time and turbidity (caused by cyanobacteria) is beyond the level of allowing for any submersed vegetation to persist. Managers are looking at how to improve conditions within the refuge by reconnecting it to the main channel to flush the refuge water with cleaner, less opaque waters.

Ettinger recalled that the levees were intended to keep the refuge isolated from silt from barge traffic and to keep carp out. He asked if Giblin had a view of the relative advantages and disadvantages of the levees. Giblin said reconnecting the refuge to the mainstem needs to be well thought out, but in his opinion the positives of reconnection outweigh the negatives. TSS values are low in Pool 6. USFWS has its own priorities that it is considering. Ettinger asked if Giblin's research is applicable in areas further south on the UMR (e.g., Swan Lake near IWW confluence). Giblin is confident that the same drivers would apply downstream but has not conducted a separate analysis.

Water Quality Monitoring Plans

Missouri - Hoke shared that Missouri is working on its next five-year water quality monitoring strategy. Missouri's ambient stream monitoring program is run in conjunction with USGS and provides the foundation for 305(b) and 303(d) assessments. Not only are CWA assessments informed, but the data meets the needs of permittees. Now the state is at crossroads and finding greater needs for data to understand Missouri's nutrient loading leaving the Missouri and Upper Mississippi River Basins. Hoke hopes to hear from the other states about their monitoring strategy development.

Iowa – Kendall said Iowa DNR's current monitoring strategy runs from 2016 to 2021. The strategy spans multiple agency programs, but also sets up the agency for additional monitoring if new sources of funding arise. For example, a few years ago, a Congressional representative had additional money and Iowa DNR was prepared to utilize the funding to monitor additional lakes and streams. The next five-year strategy will be drafted soon. Developing the strategy is a big undertaking but shows the breadth of monitoring and where data gaps exist, especially related to criteria. Hoke agreed that the biggest data gap for Missouri is also for nutrients.

USEPA Region 7 – Steve Schaff said USEPA Region 7's monitoring strategy is also being updated. He believes Region 7 is the only region that develops a strategy. The purpose is to keep all the programs coordinated and ensure that monitoring support needs are met. The Biden-Harris Administration has clear priorities that are being incorporated into the monitoring strategy. The first is environmental justice and how to not bear a disproportionate burden on communities. Schaff said that the region has been monitoring urban streams and lakes for fish tissue to establish credibility as monitoring partners. A report will be compiled to guide where monitoring is going next in terms of whether the existing monitoring network should continue, and how the data are used and analyzed. Schaff hopes the data will guide the environmental justice component of the strategy. Climate change is another priority of the Biden-Harris Administration. Additional continuous monitoring for states and tribes are being proposed. Region 7 is working to get the data analyzed and in a useable format. Ettinger noted recent literature about HABs releasing methane and suggested this is an opportunity to learn more about the effects of climate change. Schaff replied he will look into this further. Big river monitoring is an interest of Region 7. All four states within Region 7 noted that there was a gap in big river data.

The Government Accountability Office did a recent audit of USEPA's HAB program and noted that there is not an established monitoring network or protocols. Region 7 has done some work with urban HABs at a smaller scale. Schaff recently conducted field verification of the satellite data produced for the CyanNetwork to see how predicted data line up with field observations.

There is also an effort underway by Region 7 to develop a biological condition gradient (BCG) for the primary ecoregions. Region 7 states identified a data gap of biology and habitat assessments. The agency is not likely to expand on the assessment until the BCG is complete.

Hoke and Kendall reiterated the importance of big river monitoring. The UMRBA Interstate WQ Monitoring is an example of the organization's interest to conduct routine monitoring. The Reaches 8-9 pilot is helping states figure out how to work together to coordinate logistics and resources, as did the previous Reaches 0-3 pilot.

Good asked if the Region 7 monitoring strategy is its own initiative or if USEPA headquarters has made the request. Schaff said he believe Region 7 is only one with a strategy, and it is specific to Region 7's goals and objectives. In response to a question from Good about Region 7 staff available for monitoring, Schaff said the region has a Field Services Branch. The Branch is tasked with additional monitoring in the case of a TMDL needing more information. This ensures the data are collected as needed to support program work of the Region 7 states or NPDES permitting.

<u>**CWA Program Updates**</u>

<u>State Updates</u>

Missouri – Hoke said for the 2020 303(d) list, 481 waters were approved for listing, 44 water were approved for delisting, and 40 waters were not listed due to lack of data. Missouri DNR and USEPA Region 7 have been going back and forth, and a decision was made in early September 2021. Fourteen of the 40 lakes had data age issues, 17 lakes had mistakes made by Missouri DNR, and the remaining nine lakes had data collected by USEPA. DNR is working on the 2022 assessments in order to meet next year's deadline for the 50th anniversary of the CWA.

For TMDLs, the branch has been working on *E. coli* for swimming impairments as well as metals. Staff are modeling a reasonable potential analysis using BATHTUB. The model looks at a watershed level and whether permitted facilities are driving the impairment.

Minnesota – Ganske said the 2020 list has been partially approved by USEPA. The 33 sulfate listings that USEPA added on Minnesota's behalf are not approved, including two segments of the Mississippi River and a floodplain lake near Prairie Island. The 2022 list will be released in November 2021. There will be more PFAS listings, including some lakes outside of the Twin Cities Metro Area.

Lake Pepin Excess Nutrients TMDL was approved on May 19, 2021. The TMDL is several decades in the making. The first impairment listing was in 2002. Some of the major reductions mentioned in the TMDL include the following:

- Twenty percent reduction in the Mississippi River at Ford Dam (L&D 1, Minneapolis)
- Fifty percent reduction in the Minnesota River
- Twenty percent reduction in the St. Croix River (previous TMDL)
- Fifty percent reduction in the Cannon River (previous TMDL)
- Twenty percent reduction in other tributaries
- Seventy percent reduction from previously permitted loads for WWTPs
- Fifty percent reduction of resuspension in Pool 2

Other plans approved in 2021 were the following:

- Shell Rock River Watershed Restoration and Protection Strategy (WRAPs) and TMDLs
- Kettle and Upper St. Croix River WRAPs and TMDLs
- Des Moines River Basin WRAPs and TMDLs
- Lac Qui Parle River WRAPS and TMDLs
- Lake Winona (Alexandria) Excess Nutrients TMDL
- Sauk River Chain of Lakes Excess Nutrients TMDL

Wisconsin – Mike Shupryt said the 303(d) list is out for 2022 is out for public comment and closes in early October 2021. Most listings are degraded biological communities from TSS and TP. The 2022 assessment proposes to delist a few waterbodies, and for the first time there are the same amount of TSS listings as delistings.

For TMDLs, the two-year monitoring effort for the Fox River and Des Plaines River TSS TMDL is wrapping up. There is the possibility of another TMDL in the southwest part of the state, draining into the Illinois River.

Illinois – Good said Illinois EPA received full approval of the 2018 report. Staff are working on a combined 2020 and 2022 report, which will include four years' worth of data. Good said Illinois EPA staff sent a new methodology document to USEPA Region 5 a few weeks ago and are awaiting feedback.

Some of the major changes to the methodology are to chronic standards, and not fully using fish consumption advisories. It usually takes two years of data to make or remove an advisory. Now, the agency is proposing to use any new data available to impair waters, no matter the time frame. Additionally, Illinois EPA will not identify potential sources of impairments. There were challenges identifying a cause, but staff will still list potential causes of non-attainment.

Another change in the upcoming 2020/2022 combined report is the use of continuous monitoring data from USGS super gages for 12 stream segments. The stream segments will be assessed for attainment or non-attainment for aquatic life use using the pH, DO and temperature data. Good said it has been challenging to use the continuous monitoring data because standards differ across super gages, the DO standard has early life stage and normal stage criteria, and there is instantaneous, 7-day, and 30-day components to the standards. Finally, Illinois EPA has staff capacity to use R for auto generate assessment and aesthetic quality use assessment. Good hopes this will streamline assessments moving forward.

Iowa – Kendall said the 2020 list was submitted, and staff are currently working on the 2022 list. Prior to starting 2022 assessments, Iowa DNR had initial meetings with USEPA Region 7 to adjust the agency's methodology related to bacteria. The original methodology included a single sample maximum on rivers and calculating the "greater than 10 percent rule" for violations in a three-year time period. The proposed change is to move to violations in a single year. However, 10 collections are required, and there is not enough data, as staff are only able to sample up to eight times in a single year. Iowa DNR and Region 7 are trying to figure out solutions. Kendall said staff will soon be able to run their auto calculator on the 2022 list, and then move towards writing the assessment. His hope is that the assessment will be finished efficiently since most of the process is automated.

Iowa DNR recently underwent some programmatic changes. TMDLs moved to the WQ Monitoring and Assessment section, which now includes water monitoring, 303(d), and wasteload. NPDES permitting is still in a different section.

Wednesday, September 29

Nutrients

The Best Places to Tackle U.S. Farm Nitrogen Pollution

Dr. Eric Roy described the purpose of his research. Accounting for environmental losses of nitrogen (N) in cropland systems is challenging because of the multiple pathways that exist. However, there is compelling evidence that N balance can serve as a proxy for N losses to the environment, including nitrous oxide emissions and nitrate leaching. The use of N balances in policy is limited in the U.S. but has had more widespread use in Europe. Previous research has focused on calculating N balances at different scales in the U.S. but knowledge on the underlying factors that contribute to surplus N use is lacking.

The purpose of the study was to assess N balances with agronomic, environmental, social, demographic, and economic factors. Data were focused at the county level for 2011 to 2013. Roy and his team used the International Plant Nutrition Institute Use Information System (NuGIS) and Census of Agricultural data. The data are outdated, but the approach could be used as new data come online. The Fertilizer Institute is now operating NuGIS, and data for 2017 will soon be available.

Using the county level mean N flows for 2011-2013, Roy et al. created three metrics: 1) total surplus N, 2) excessive N input, and 3) potential for improvement of N balance. For the first criterion, total surplus N for roughly 1,000 counties were screened and about 36 percent had more than 1,000 metric tons/year. Criterion two, excessive input, is defined as more N going onto croplands then is required for high yields. About 25 percent of counties are where N input seems excessive based on those definitions. The third criterion, potential for improvement, is the most interdisciplinary metric. Roy and his team created a hierarchical random effects model. They were particularly interested in counties with higher N surplus then the model predicted. That was for 25 percent of the counties. The three scores were added together to identify hot spots of opportunities. Twenty hotspots account for 63 percent of surplus N but only 24 percent of cropland area across the U.S. Hot spots could be the focus of future research to better understand the links between N balance and N losses to the environment.

The analysis also included differing excess N input for different crop types, or typologies. Using a model, Roy and his team, looked at relationships between increasing N input and increasing N harvesting in crops and where the relationship starts to breakdown. The national breakpoint is 175 kg N per hectare per year or roughly 156 lbs per acre per year. The analysis was repeated for each typology, and each has a different breakpoint.

The third criterion required a statistical model to predict N balance at the county level. Factors included operating expenses, population, precipitation, soils, and more. A positive correlation was found for N balance with operating expenses, population density, precipitation, and the following typologies: corn and soybean, wheat and soybean, hay, corn and soybean, and other crops. Negative correlations with N balance include climate change belief and policy support, soil fertility based on county-level properties, and participation in USDA programs (e.g., conservation programs). The areas where is N balance is much higher than the model predicted could be "low hanging fruit" in terms of making improvements in N management.

Roy shared his concluding thoughts that the study can serve as a useful tool to guide N management policy and programs. The results themselves are not just important, but the use of the N balance to look for opportunities on the landscape to improve WQ.

Ettinger noticed the hot spot in Wyoming and asked what is grown there. Roy replied that it is fertilizer use for wheat and alfalfa groups. NuGIS tries to account for fertilizer bought in one county and used in another, but that is not always successful. However, there is a cluster in Wyoming that supports the designation as a hot spot. Roy replied that it was an inherent assumption that crops were fixed, in response to a question from Ettinger.

Bennett noticed that urban counties had high rates of N inputs and asked what could be driving the pattern. Furthermore, how would this affect the recommendations and results of your study? Roy wants to dig deeper into the result. NuGIS does not account for biosolids, so there are no different inputs. Urban counties have less agriculture, but high N inputs would still show as high as they are modeled on a per acre basis. In response to a question from Bennett about whether the data include lawn fertilization, Roy replied that the dataset differentiates between farm and non-farm fertilizer. Ettinger asked if one farm in Cook County is using too much nitrogen, would it be considered be a hot spot. Roy said it would score high for the second criterion but not necessarily for the first criterion.

Ganske asked how Roy would describe the weakest links in the dataset or where he would have liked to have better data. Roy said with an analysis like this there are inherently a lot of assumptions e.g., fixed N amount for each crop, when in reality it can vary, or fixed manure content for an animal. It is always challenging when you zoom in on a particular spot, but the study serves to broadly categorize the criteria. Roy suggested if working in Minnesota, he would plug in Minnesota specific parameters.

Constructed Wetlands are Best Protection for Agricultural Runoff into Waterways

Dr. Amy Hansen said that "best" in her presentation title *Constructed Wetlands are Best Protection for Agricultural Runoff into Waterways* means the most cost effective at simultaneously reducing nitrogen (N) and sediment loads. The paper Hansen et al., 2021 was written in collaboration of many authors and institutions, in which they linked three watershed-scale models together to evaluate portfolios of BMPs across watersheds. The NSF grant included observatory funding source to collect field data and develop models for the Minnesota River Basin.

Some of the conclusions from observational data collection point to the need to understand how near channel and in channel processes work within a watershed. 1) hydrology is the primary driver of pollutant export, 2) near channel sediment sources (bluff and bank erosion) are significant storage of sediment and sources of releases during high stream flows, and 3) wetlands and lakes are significant locations of nitrate removal and reduce peak stream flows. Hansen and her co-authors linked three watershed models together because near channel and in channel process are not included in watershed (SWAT) models in conservation management. And, wetlands and lakes are aggregated at a sub basin scale, meaning there is less resolution for size and placement of the waterbodies.

Hansen et al. developed the AgRiver modeling framework. They still used SWAT to represent what occurs on the landscape. They exported hydrology and sediment information to a river network model to capture near channel sediment (called MOSM). Hydrology and nitrate data was exported into Nitrate Network model to capture processes like denitrification as function of nitrate concentrations.

The team created candidate landscapes or entire watersheds with a portfolio of field management and near channel management actions e.g., cover crops, fertilizer management, wetlands, ravines, bluff and bank stabilization. The landscape could take hundreds of actions and each landscape was averaged over a 10 year period to determine which landscapes were optimal for reduction nitrate, suspended sediments in a cost effective manner.

The modeling framework was applied to the Le Sueur River Basin in southern Minnesota and is located within the hot spot area three in Dr. Roy's presentation. The basin was chosen due to its high suspended sediment, N, and P loads. Eighty percent of the basin is used for row crop agriculture and is extensively tile drained. For reference, the policy targets determined by the Minnesota PCA are 65 percent reduction in sediment and 45 percent reduction in N. Spending across all stakeholder groups (landowners, state, federal, and local entities) in the basin from 2004-2017 was $4.3 million per year. These numbers can serve as reference points for the scale of change being considered.

Hansen and her team found that with a typical agency budget of $500,000 per year, management targets for N and sediment are allocated to a variety of practices. If the view is broadened to all agencies working within the basin and their combined budgets, you start to see wetlands emerge as being the management choice. Hansen added that the analysis is not concluding that other practices are not effective. Ravine, cover crop, bank stabilization, and others are all good practices and are effective at sediment and N reduction, but not as cost effective as wetlands at the watershed level.

The study next evaluated the placement of wetlands and whether there is spatial dependency of N and sediment reduction results. Hansen et al. looked at clusters of wetlands that meet load reduction targets. For field management, there was no strong spatial dependency i.e., their effectiveness is not strongly dependent on their location. In contrast, for fluvial wetlands (or flow through wetlands), there are few locations that stand out where wetlands should be placed. These are highly spatially dependent within the watershed and river network.

In conclusion, wetlands appear to be the most cost effective, but the cost of wetlands is high (e.g., routine dredging). Wetland performance is highly spatially dependent. Both conclusions point to a need of coordination and collaboration across a watershed. Hansen noted the limitation that the results may not be transferrable to other watersheds. The Le Sueur is a typical Midwest watershed, so she expects N results to be similar, but not necessarily for sediment. Furthermore, there are other variables that can be evaluated.

There is follow up research being conducted to look more broadly at the Upper Mississippi River Basin. The work is a collaboration with USDA, the University of Kansas, University of Missouri, Texas A&M, and The Nature Conservancy. The team will be improving model representation of near channel processes in SWAT models, adding fish population responses as endpoint, and creating a decision analysis tool to support stakeholder decision making. A second research project Hansen is involved with is seeking to understand whether the placement and configuration of a wetland average nitrate concentration or minimize nitrate load at the outlet. There is potential synergy to target one over the other.

Giblin asked how Hansen modeled residence time of wetlands. He asked if the number was generic, because if so, performance of nitrate and sediment removal would be variable. Hansen replied that within the published model, the residence time was based on the size of the wetlands. Because SWAT was used as the base model, the wetlands were aggregated to maintain volume and surface area, then she and co-authors used flow exceedance probabilities going back 40 years to estimate residence time of a wetland based on what flow conditions would be.

In response to a question from Ettinger whether cost effectiveness is based on the total cost to the operator or the total societal cost, Hansen replied total cost. The analysis did not break out who was paying. For example, with wetlands, the paper tracked lost revenue from taking land out of production, averaged over a ten-year period. The cost would be felt annually by the landowner, but an agency cost was also included e.g., dredging and maintenance. Ettinger asked if stakeholders would need to transfer money to aggregate costs in practice so that the operator would install a wetland. Hansen replied that the question is outside of her expertise, and she is unsure how much money would pass hands. Hansen has observed that near channel environments may be optimal areas for wetlands are not being farmed anyway.

Ganske asked about transferability of the work to other watersheds. The Le Sueur has particularly active bluff and bank erosion. Is that a significant factor in this model? Hansen agreed that bank and bluff erosion is worse. However, there is a significant amount of incision and erosion throughout region. Hansen would not expect near channel sediment to be as dominant as over-field erosion in other watersheds. Publications show near-channel sediment sources could account up to 50 percent of its sources.

Karen Hagerty recalled that other studies show that legacy sediments are a primary course of with channel sediment. Did Hansen's study evaluate this? Hansen said the analysis is coarse in the analysis of the mobilization of bed sediment. It prompted her research team to incorporate an annual analysis of sediment load. Hoke noted his appreciation for the study because states often make decisions how best to spend limited funds. Heather Golden appreciated Hansen's presentation and cited her previous research looking at wetlands in the uplands and how they affect nitrate across the UMRB. Golden would like to incorporate cost analyses in future research. The article can be accessed via the following link: https://iopscience.iop.org/article/10.1088/2515-7629/ac2125/pdf

State and Federal Updates

Minnesota – Ganske reminded participants that Minnesota's State Nutrient Reduction Strategy report was published in August 2020. Over the next few years, Dave Wall will lead the effort to update the strategy, with a focus on water storage and multiple benefits. Key groups include renters, crop advisors, and food consumers.

The public notice for the Albert Lea wastewater treatment facility permit reissuance ends on October 4, 2021. The facility discharges to the Shell Rock River, and the permit includes phosphorus limitations. The 2021 drought year provided the opportunity to evaluate wastewater phosphorus limits as it relates to DO impairments in the Lower Minnesota River Basin.

Ganske reiterated that a lot of the nutrient reduction work is tied back to major watershed monitoring, assessment, and strategy development approach. Minnesota PCA organizes information around nutrient reduction needs and practices around the major watersheds and evaluates processes to see how projects align or do not align with downstream goals.

Illinois– Good said the third Illinois Nutrient Loss Reduction Strategy was released on September 16, 2021. Some highlights of the report include the contributions of the agricultural community. Approximately $27 million has been spent on non-federal or state cost share and $6.9 million on agriculture research. The point source (PS) community has implemented a lot of capital improvement projects. While there is a lot of activity going on in the state, nutrient levels are not being reduced. The baseline for nitrogen and phosphorus is from 1980 to 1996. The recent calculations indicated that between 2015 and 2019, nitrate-nitrogen increased 13 percent and phosphorus increased 35 percent, primarily driven by high discharge.

Illinois EPA secured additional funding to keep its eight USGS super gages running. Leftover USGS funds cover the gages until 2022, and Illinois EPA funding continues the gages until 2024. The gage at the lower Illinois River basin is being funded by the USGS NGWOS program and includes monitoring for phosphate. The other seven sites are funded by Illinois EPA until 2024 for nitrate and turbidity but the parameters DO, pH, specific conductivity, and temperature were removed to reduce costs of operating.

Section 106 grants awarded in 2020 and 2021 were used to monitor downstream of the PS to determine "risk of eutrophication" and whether the PS is contributing. Illinois EPA evaluates as DO, pH, and chlorophyll data and if it appears there is a risk for eutrophication, then a Nutrient Assessment and Reduction Plan is added to the PS's NPDES permit. Illinois IEPA contracted with the Illinois State Water Survey to conduct the work for 16 sites.

Iowa – Adam Schnieders said Iowa has a new dashboard for reporting nutrient progress: https://nrstracking.cals.iastate.edu/tracking-iowa-nutrient-reduction-strategy. The dashboard can be used as a communication tool to the public and allows for the NRS authors to update the dashboard with new data as it is available. Schnieders appreciated the cross collaboration as Minnesota and other states served as resources for Iowa's dashboard.

The WWTP optimization efforts continue. The project includes looking at the existing design capacity to activated sludge treatment, which can lend itself to achieve nutrient reduction and energy savings by creating anoxic conditions within treatment process. The association of Iowa wastewater treatment operators and Iowa State University are partners. USEPA has also been a partner. The agency funded a six-week training for operators for Region 7 states.

Iowa continues to develop agriculture-urban partnerships to allow for cities to invest in watersheds. MOUs have been set up with Cedar Rapids, Ames, and Storm Lake, and an MOU is in progress for the City of Muscatine.

Iowa DNR and the Practical Farmers of Iowa received a USEPA farmer to farmer grant to develop cover crop seeds for public lands. Schnieders is happy that WWTP are being built and operated. The effort is a result of policies put in place in the state in 2015.

Finally, Schnieders says Iowa is awaiting more direction on the process to receive Bipartisan Infrastructure Law. He is excited to have more resources available for WQ. DNR staff are also trying to figure out how cities and counties will invest American Rescue Plan Act dollars. Schnieders would like to hear how other states are planning to utilize the funding.

Ettinger asked if Iowa is looking to adapt USEPA nutrient lake criteria, which was in part based off Iowa data. Schnieders replied that Iowa DNR is planning to set up a process to review the criteria, as they did with cyanotoxins. The scientific review will take place over the next year.

Wisconsin – Shupryt reviewed that in Wisconsin's latest update to its nutrient reduction strategy, the trends are similar: decrease in TSS and increase in nitrogen. There are Wisconsin DNR groups looking at tackling the nitrogen issues. There is a Pre-criteria group, which is tasked with assessing the state's readiness to pursue nitrogen criteria. Staff are looking at monitoring data to see if we are in position to start the process. A concurrent group is looking at regulatory procedures for voluntary or regulatory efforts down the road. The state is making a concerted effort to address nitrogen leaving the state.

Missouri – Hoke described Missouri's nutrient focused projects. Contractor Barr Engineering helped Missouri DNR quantify nutrient reductions from agriculture BMPs (e.g., cover crops, grassed waterways, ponds, and control basins) at a HUC-8 scale, using a control design. Missouri DNR will also use the information to guide a WQ trading framework.

NLRS revisions were originally published in 2014. In the current revision, DNR would like to develop PS and nonpoint source (NPS) baselines. The original strategy proposed total phosphorus effluent limits for PS. The proposed rulemaking for WWTPs is for major permitted facilities that output more than 1 million gallons per day (MGD) for domestic and 1 MGD or 80 percent reduction of TP loading over the most recent five year set of data for industrial facilities. Hoke said that TP limitations work, as evidenced the improved WQ from permit limitations of 0.5 mg/L in the southwest part of state. Hoke believes a statewide effort will further improve WQ.

Now that Missouri is past court challenges for its lake criteria, DNR staff have moved on to full implementation of TMDLs. Hoke said his staff are looking at the watershed level to understand what reductions may be necessary for impairments that do not have a PS.

Schnieders asked if the P and N limits are a technology-based approach. Hoke confirmed the P limits are technology based, but DNR has not looked at nitrogen yet. Nitrogen is being addressed in some parts of the stat with TMDLs e.g., James River Basin. Regionalization could help improve WQ for WWTP. Hoke said the state is also excited about ARPA funding to help with those upgrades. Schnieders suggested a merged technology approach. It is more affordable in the long-term for WWTP to redesign for N and P simultaneously, especially for ammonia. Hoke agreed and appreciated the suggestion.

USEPA Region 5 – Bennett said at the regional level, the Region 5 states are involved in a project related to lake nutrient criteria to help predict dissolved organic carbon (DOC) across Midwest lakes. DOC is an input for several models, and the projects wants to improve how to define and select representative DOC values (as there is typically limited data).

Nationally, USEPA OW is working on a nutrient memo to highlight priorities.

Sydney Weiss shared updates on nutrient optimization training at WWTPs. Region 5 has solidified funding for optimization trainings. So far trainings have been held in Wisconsin in November 2020 focused on small mechanical plants, in Ohio in April 2021, in the Red River in June 2021, and Illinois in August 2021. Around 100 to 300 folks joined each session. Next steps are to bring remaining sates and tribes similar trainings, pending interest and funding. More information can be found at the following link: https://www.epa.gov/sites/default/files/2021-06/documents/blenker-sherry_potw_may_2021.pdf

Weiss said there has been general success from the workshops. The optimization is relatively low cost to the operator, and mostly experimental to look at changing existing practices to improve outputs. Schnieders endorsed the operator trainings and said the workshops were formatted and exceeded expectations. He encouraged others to take the opportunity. Weiss asked for input on what would be helpful to states following the workshops, such as how to build off of and improve upon the training. Schnieders said Iowa is looking to build off the momentum and expand the training with the state. DNR staff are reaching out to operators to ask them to engage with regulators, which can be a culture change. WWTP may default to following permit guidelines, but DNR wants to encourage them to thinking differently about treatment.

Chloride Technical Workgroup

Weiss introduced the Chloride Technical Management Workgroup (CTMW), which was formed to help states address their challenges meeting chloride related water quality requirements. Weiss framed the issue with chloride. It is a persistent pollutant, used ubiquitously, and perceptions and habits increase its use. For example, people drive during snowstorms and expect to see bare streets to feel safe commuting. States have different programs and limited resources. They also have different approaches and ways of permitting, which makes the problems hard to tackle. The challenge we face is how to share the strengths of each program to enhance solutions at the state and local level.

The workgroup brings together a variety of agencies and organizations to pool resources and share info, innovate, hold each other accountable, as well as leverage skills and experience to help each other. CTMW looks at different ways to address the issue with metrics, subgroups, partnering with academics, and holding brainstorm sessions. The group is looking to expand at the state or tribal level, and to bridge the gap from state and federal level to communities.

One current initiative is the Chloride Reduction Resources Clearinghouse. The mockup, housed by USEPA Region 5, would serve as a single source of chloride information with links to information and tools. An example of a tool is the Smart Salting Tool created by Minnesota PCA. The tool looks at ways to reduce salt use, and could be further expanded to water softeners, industrial, and agriculture practices. Weiss encouraged participants to contact her if they are interested in getting involved with the workgroup at weiss.sydney@epa.gov.

Ettinger commented that chloride standards have not been reviewed since 1989. Tim Elkins replied that USEPA HQ are working to update 304(a) criteria, and the workgroup Weiss co-leads is a creative approach to work on other chloride related issues. Good asked why chlorides are increasing everywhere. In response, Weiss said ultimately chloride is persistent in the environment. She reflected on the last salt symposium in which participants discussed salt application outpacing urbanization rates, lawsuits are on an upward trend, which translates to increased application to avoid liability. Additionally, as water softeners age, they become less efficient and use more salt. There has also been expansion in the use of lake houses, not historically used in winter. Other factors include climate change, more extreme weather events, and temperature flux. The State of New Hampshire provided an example of COVID-19 leading to less travel and a reduced use of road salt.

<u>Hazardous Spills Strategic Planning</u>

Mark Ellis coordinates the UMR Hazardous Spills group and works with USEPA Region 5 to develop the Inland Sensitivity Analysis for spill response planning. He provided an overview of the strategic planning process for the Hazardous Spills group. The group developed goals and objectives, including notification updates that could benefit the WQTF. The group maintains an email listserv used to notify members of spill events. This listserv could be used by the WQTF to spread word about HAB events, or model one the Task Force could set up for its own purposes. This may be one way to alert water intake operators about HAB events or other water quality concerns. Ellis said this offers a segue into the discussion topic to follow about HAB events and the challenges of alerting the public.

<u>HAB Notification at Recreational Sites</u>

Gregg Good shared the timeline of the UMR Pool 13 HAB event that occurred during summer 2021. In late July 2021, Corps staff were in the field near Savanna, Illinois in upper Pool 13. Staff noticed green water, obtained a sample, and results were 2.98 µg/L for microcystin and non-detection from cylindrospermopsin. On August 10, 2021, the water in Pool 13 was still green. The sample results were 14.1 µg/L for microcystin. Good said that in Illinois there is typically a county health department or lake association that can make notifications and place signs. At Starved Rock for example, there is a joint press release through Illinois EPA and the Illinois Department of Public health. In the Pool 13 example, Good was not aware of a local entity to contact or notify. Terlep noticed there is public sandbar called Santa Fe Beach located just upstream of where the detection occurred. How do you notify the recreators? Good did not think an Illinois EPA news release would reach the right audience. In the future when detections occur, where are the local recreation sites, who are the right people to contact, and how can we get a hold of them? The UMRBA HAB manual includes a communication list of state, federal agencies, public water suppliers, etc. There is not a comprehensive list of sites up and down the river. The Corps has a document as well with select contacts and site information found via the following link: https://www.mvr.usace.army.mil/Missions/Recreation/Mississippi-River-Project/Recreation/.

Leo Keller recalled previous conversations with John Sullivan (retired Wisconsin DNR), who asked how to keep people safe and who was monitoring the river for HABs. While recreating Sullivan noticed many potentially toxic blooms in backwater areas and on the mainstem. Keller said in the case of the Pool 13 bloom, a HAB was noticed coincidentally, a sample collected, but now what? Who is going to take action, how do we keep the public safe, and who is responsible? The Corps has some campgrounds to post notifications, but that is the extent for the Corps. If it is not otherwise connected to WQ work of the Corps, then staff are limited unless already doing field work in the area.

Good said the notification of HABs via listserv that Ellis mentioned was a good idea. Ellis said checking email is a limitation, e.g., an event reported on Saturday afternoon and email isn't checked until Monday morning. Ellis suggested this group generate a list of publicly known recreation sites and build on the informal sites overtime. Furthermore, how widespread is the knowledge of the reporting tools? Good doubted that awareness is widespread, and liked Ellis' suggestion of building the list.

Good asked other states if their agency or sister agency has contact information for the river. Giblin said the recreation is so widespread, and there are numerous informal sites in a particular pool. In Wisconsin a lot of notification goes to the local health counties. LaLiberte said it is essential to reach out to the local public health agencies before there is an incident. However, many are very small and have a limited capacity to respond. She has seen cases for inland lakes in which DNR sends a report over, and the agency does have a response plan in place. Wisconsin DNR and the Department of Public Health Services offers support for social media messaging, templates for guidance, and closure signage.

Kendall said for Iowa a list of local sites would come from the county conservation board. Iowa's protocol is to post signage, but not to close beaches. For county or city beaches, posting notices falls into local jurisdiction. DNR works with the localities but cannot post DNR signage. Ellis asked who has the authority to keep river users out of an area. For oil spills, Ellis said the group relies on law enforcement. Good echoed that Illinois EPA also leaves postings to localities. Like with a fish consumption advisory, you can provide information but cannot prevent someone from consuming fish.

Kendall asked who will continue to sample when a bloom is found. It worked out that the Corps was out in the field to take a sample. Good mentioned that no follow up sampling was conducted after the August 10, 2021, sampling event. It is near impossible for Illinois EPA staff to conduct follow up sampling. That is why Illinois EPA has opted to build partnerships up and down the river, and to provide training and sample bottles to entities. Kendall said similarly for Iowa DNR, the Des Moines office staff would have trouble getting to river. The Bellevue field station could help, but an event may not be in close proximity.

Ganske said similarly to Wisconsin, Minnesota finds it challenging to target particular recreation areas. He said that the air quality index is monitored daily by Minnesota PCA, and the information has been utilized by media and other entities. Is there anything between site-specific monitoring advisories and general education that could forecast heightened risk or concern, and hope that over time it becomes something that gets updated daily so media and others could pick up the information? On the note of state programs and public information, Manasco suggested HAB education in boating classes to target people using the waterways. Then educators could point to resources within the state to check water conditions. Good suggested the Illinois DNR annual fishing resources as a way to reach recreators. The annual report also publishes fish advisories, and HAB information could be included. Manasco also suggested a weather service-like notification system that when a phone is in a specific area, they will receive a message through the national broadcast system.

Manasco asked if there is any way to get a survey out for field staff for each organization in the field. She understands the schedule will change, but there should be a way out figure out who is available to collect samples. We can work together as a comprehensive team.

The WQTF and participants agreed that next steps are to compile a list of recreation sites along the river, including the contact information of the local jurisdiction.

Administrative Items

Hoke welcomed topic ideas for future WQTF meetings. Giblin suggested an emerging contaminants presentation. He will forward USGS staff Steve Corsi's contact information and recommended tying Corsi's research with a staff person from USEPA's Office of Research and Development. That way the presentations can contain both research and regulatory components.

Hoke congratulated Good on retirement and thanked him for his contributions to the WQTF. Good said the WQTF has been fantastic to work with and thanked the group for collaboration. Good will return to Illinois EPA as a contractor in winter 2022 for a 75-day assignment.

Future Meetings

- The next WQTF meeting will be convened virtually January 25-26, 2022.

Attendance

Anna Belyaeva	Illinois Environmental Protection Agency
Gregg Good	Illinois Environmental Protection Agency
Tara Norris	Illinois Environmental Protection Agency
Alexandrea Terlep	Illinois Environmental Protection Agency
Daniel Kendall	Iowa Department of Natural Resources
Adam Schnieders	Iowa Department of Natural Resources
Lee Ganske	Minnesota Pollution Control Agency
John Hoke	Missouri Department of Natural Resources
Erin Petty	Missouri Department of Natural Resources
Robert Voss	Missouri Department of Natural Resources
Coreen Fallat	Wisconsin Department of Agricultural, Trade and Consumer Protection
Shawn Giblin	Wisconsin Department of Natural Resources
Gina LaLiberte	Wisconsin Department of Natural Resources
Mike Shupryt	Wisconsin Department of Natural Resources
Karen Hagerty	U.S. Army Corps of Engineers, Rock Island District
Leo Keller	U.S. Army Corps of Engineers, Rock Island District
Nicole Manasco	U.S. Army Corps of Engineers, Rock Island District
Aabir Banerji	U.S. Environmental Protection Agency, Office of Research and Development
Micah Bennett	U.S. Environmental Protection Agency, Region 5
Glenn Curtis	U.S. Environmental Protection Agency, Region 7
Lesley D'Anglada	U.S. Environmental Protection Agency, Office of Science and Technology
Peg Donnelly	U.S. Environmental Protection Agency, Region 5
Wendy Drake	U.S. Environmental Protection Agency, Region 5
Heather Golden	U.S. Environmental Protection Agency, Office of Research and Development
Scot Hagerthey	U.S. Environmental Protection Agency, Office of Research and Development
Ann Lavaty	U.S. Environmental Protection Agency, Region 7
Megan Maskimowicz	U.S. Environmental Protection Agency, Region 7
Tanya Nix	U.S. Environmental Protection Agency, Region 7
Chelsea Paxson	U.S. Environmental Protection Agency, Region 7
Steve Schaff	U.S. Environmental Protection Agency, Region 7
Amy Shields	U.S. Environmental Protection Agency, Region 7
Sydney Weiss	U.S. Environmental Protection Agency, Region 5
Aleshia Kenney	U.S. Fish and Wildlife Service, Iowa-Illinois Field Office
Vicki Christensen	U.S. Geological Survey, Upper Midwest Water Science Center
Jim Duncker	U.S. Geological Survey, Central Midwest Water Science Center
Kelly Warner	U.S. Geological Survey, Central Midwest Water Science Center
Jeff Houser	U.S. Geological Survey, Upper Midwest Environmental Science Center
KathiJo Jankowski	U.S. Geological Survey, Upper Midwest Environmental Science Center
Ingrid Gronstal	Iowa Environmental Council
Albert Ettinger	Mississippi River Collaborative
Doug Daigle	Lower Mississippi River Sub-Basin Committee
Jennifer Terry	Des Moines Water Works
Meghan Arpino	Stone Environmental
Jacob Mitchell	Stone Environmental
Amy Hansen	University of Kansas
Eric Roy	University of Vermont
Mark Ellis	Upper Mississippi River Basin Association
Kirsten Wallace	Upper Mississippi River Basin Association

ATTACHMENT B

Contaminants

- **Radium Study in Aquifers of North Central Illinois (9/20/2021)** *(B-1 to B-3)*
  [Link to full report is available here: https://www.ideals.illinois.edu/handle/2142/110351]

- **Prioritizing Chemicals of Ecological Concern in Great Lakes Tributaries (5/2019)** *(B-4 to B-18)*

Prairie Research Institute News

Researchers study radium in aquifers of north-central Illinois

SEP 20, 2021 9:00 AM BY LISA SHEPPARD

WATER SURVEY

CHAMPAIGN, Ill., 9/16/21**:** Walt Kelly, Illinois State Water Survey (ISWS) groundwater geochemist, answered questions about the findings of his recent study on radium levels in groundwater of the St. Peter Sandstone aquifer, with a study area in north-central Illinois. Radium levels are above the drinking water standard in many community water supply wells open to the aquifer.

What was the purpose of this study?

This study was part of a larger study undertaken to evaluate water supplies in the Middle Illinois Region. We were interested in what the water quality of the major aquifers was, and what the water chemistry could tell us about the evolution of groundwater in the deep aquifers.

Who was the report intended for?

The report was primarily for the scientific community, especially those interested in radioactivity in sandstone aquifers. But it was also intended for those communities and industries that use these aquifers, to help them understand and manage their water quality.

Why is it important to study radium in groundwater?

Drinking or cooking with water that contains too much radium can pose a hazard to human health. Drinking water is required to have no more than 5 picocuries per liter of radium. Being a radioactive element, it can also give us clues about what reactions are occurring within the aquifers and between them.

What are the environmental factors that affect the levels of radium in groundwater, particularly for north-central Illinois?

Radium is formed as uranium and thorium in the rock decay radioactively. There are many factors that can affect whether the radium remains in the water or is removed to solid phases, including the rock and water chemical characteristics.

What role do uranium and thorium play in the water levels of radium?

They are the “parents” of radium. They are formed as the uranium and thorium, which are found in the rocks, decay. The radium further decays, eventually forming non-radioactive lead. One form of radium also decays to radon, a carcinogenic gas. The more uranium and thorium in the rock, the more potential for radium and radon to be found in the groundwater.

What were the major results from the water sampling and analysis?

It’s been known for a long time that there are elevated radium levels in these aquifers. Our work has helped us understand the sources and transport of radium in this enormously complex hydrogeological and geochemical system. One thing we have been able to do is track the movement of Pleistocene meltwater into the aquifers and learn how they have mixed with brines and affected radium and uranium.

Where were the highest levels of radium in the study area and why?

The highest concentration we measured was 17.6 pCi/L. Earlier sampling north of our study region had many higher values, as high as 37 pCi/L. Differences in radium concentrations can be attributed to different amounts of the uranium and thorium and differences in solid and water chemistry that affect whether the radium remains in solution.

What are the implications of this study?

Communities using these aquifers will always have to deal with radium, because it is naturally occurring and is continually being produced. There may be several options, including various treatments or blending. One thing to remember in treatment is that the waste stream is radioactive, and its handling may be regulated by the Illinois Environmental Protection Agency.

What are the plans for future studies?

We have no concrete plans, but we are interested in looking more closely at rock cores to learn more about the association of uranium, thorium, and radium within the solid phase.

The report detailing the study, Hydrogeological and Geochemical Controls on Radium and Uranium in the St. Peter Sandstone Aquifer in the Middle Illinois Water Supply Planning Region, is available in the University of Illinois IDEALS depository (http://hdl.handle.net/2142/110351). Co-authors include Samuel Panno, Keith Hackley, Daniel Hadley, and Devin Mannix.

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Science of the Total Environment 686 (2019) 995–1009

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Prioritizing chemicals of ecological concern in Great Lakes tributaries using high-throughput screening data and adverse outcome pathways

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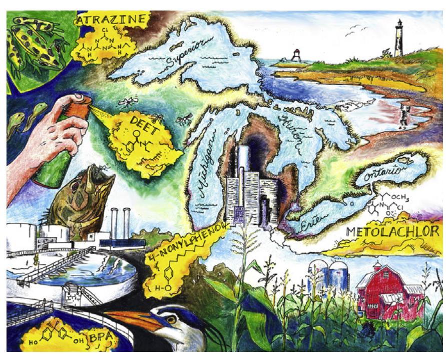
HIGHLIGHTS

- Potential for ecological impacts of 67 organic contaminants was evaluated.
- Use of ToxCast data increased the number for which bioeffects could be assessed.
- Chemicals and sites with greatest potential for adverse bioeffects were identified.
- Adverse outcome pathways linked ToxCast responses to potential adverse outcomes.
- Mixture effects were predicted using ToxCast exposure-activity-ratios and AOPs.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 2 May 2019
Received in revised form 28 May 2019
Accepted 30 May 2019
Available online 5 June 2019

Editor: Damia Barcelo

Keywords:
Surface water
Organic contaminants
Chemical mixtures
Bioeffects
High-throughput screening
Adverse outcome pathways

ABSTRACT

Chemical monitoring data were collected in surface waters from 57 Great Lakes tributaries from 2010 to 13 to identify chemicals of potential biological relevance and sites at which these chemicals occur. Traditional water-quality benchmarks for aquatic life based on in vivo toxicity data were available for 34 of 67 evaluated chemicals. To expand evaluation of potential biological effects, measured chemical concentrations were compared to chemical-specific biological activities determined in high-throughput (ToxCast) in vitro assays. Resulting exposure-activity ratios (EARs) were used to prioritize the chemicals of greatest potential concern: 4 nonylphenol, bisphenol A, metolachlor, atrazine, DEET, caffeine, tris(2 butoxyethyl) phosphate, tributyl phosphate, triphenyl phosphate, benzo(a)pyrene, fluoranthene, and benzophenone. Water-quality benchmarks were unavailable for five of these chemicals, but for the remaining seven, EAR-based prioritization was consistent with that based on toxicity quotients calculated from benchmarks. Water-quality benchmarks identified three additional PAHs (anthracene, phenanthrene, and pyrene) not prioritized using EARs. Through this analysis, an EAR of 10^{-3} was identified as a reasonable threshold above which a chemical might be of potential concern. To better understand apical hazards potentially associated with biological activities captured in ToxCast assays, in vitro bioactivity data were matched with available adverse outcome pathway (AOP) information. The 49 ToxCast assays prioritized via EAR analysis aligned with 23 potentially-relevant AOPs present in the AOP-Wiki. Mixture effects at monitored sites were estimated by summation of EAR values for multiple chemicals by

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https://doi.org/10.1016/j.scitotenv.2019.05.457

individual assay or individual AOP. Commonly predicted adverse outcomes included impacts on reproduction and mitochondrial function. The EAR approach provided a screening-level assessment for evidence-based prioritization of chemicals and sites with potential for adverse biological effects. The approach aids prioritization of future monitoring activities and provides testable hypotheses to help focus those efforts. This also expands the fraction of detected chemicals for which biologically-based benchmark concentrations are available to help contextualize chemical monitoring results.

1. Introduction

Tributaries of the Great Lakes are influenced by a diverse set of stressors that may cause adverse ecological effects. Consequently, deciding how to most effectively allocate limited resources for management and monitoring activities can be a challenge. When mixtures of contaminants are detected, it can be difficult to determine which contaminants are of greatest concern and in turn what potential source mitigation or remediation strategies could most effectively lower risk. For example, in addition to legacy contamination with compounds such as polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethane (DDT), Great Lakes tributary ecosystems are influenced by current-use contaminants from sources such as urban and agricultural runoff, treated and untreated wastewater, and atmospheric deposition (Custer et al., 2016; Furdui et al., 2007; Guo et al., 2017a, 2017b; Lepak et al., 2015). Contaminants from these sources include polycyclic aromatic hydrocarbons (PAHs), pesticides, pharmaceutical and personal care products, flame retardants, plasticizers, detergents, and degradation products of these contaminants, among others (Baldwin et al., 2016; Elliott et al., 2017, 2018a).

During 2010–13, 709 surface-water samples were collected in 57 Great Lakes tributaries and analyzed for 67 organic compounds that represent 15 classes of chemicals based on their functional use or chemical properties (Baldwin et al., 2016). Watersheds included in this effort represent a broad range of urban, agricultural, forested, and water and wetland land cover attributes (Table SI-1), and samples were collected to capture spatial, seasonal, and hydrologic variability. Understanding the potential significance of these contaminants on aquatic ecosystem health can be a challenge. For example, a comprehensive search for US and Canadian water-quality benchmarks for acute and chronic exposure to aquatic life for the 67 target analytes resulted in information for only 34 of them from 10 chemical classes (Baldwin et al., 2016). Considering the limited number of traditional, in vivo-based water-quality benchmarks available for the diverse range of contaminants, we aimed to expand the scope of our screening-level characterization of potential biological significance by leveraging the growing database of chemical-specific high throughput in vitro biological activity data generated via the ToxCast and Tox21 (hereafter referred to as ToxCast) programs (Dix et al., 2007; Kavlock et al., 2012; Tice et al., 2013).

The ToxCast database contains information on biological activities of thousands of chemicals. The information comes from several hundred high-throughput assays that cover a range of cell responses and approximately 300 signaling pathways (Kavlock et al., 2012; Tice et al., 2013). A number of other sources of potential chemical-biological interaction data also exists, including PubChem (Kim et al., 2016), ChEMBL (Gaulton et al., 2017), ToxNet (Fonger et al., 2000), TG-GATEs (Igarashi et al., 2015), the Comparative Toxicogenomics Database (Davis et al., 2013), and the Connectivity Map (Lamb et al., 2006; Subramanian et al., 2017). However, most of these sources aggregate chemical-biological interactions from the peer-reviewed literature where the use of different experimental designs, assay systems, testing conditions, and analytical/statistical approaches can confound quantitative comparisons of potency. In contrast, ToxCast chemicals were tested in a consistent and standardized set of assays and resultant data were analyzed with a uniform analysis pipeline (Filer et al., 2016). This allows for more reliable comparison of the relative potency of the tested chemicals thereby supporting the type of comparative prioritization analysis that was the aim of the present study.

Using in vitro bioeffects data in this manner is consistent with the long-range vision for toxicity-testing described in the National Research Council (NRC) report "Toxicity Testing in the 21st Century—a vision and strategy" (Krewski et al., 2010). This NRC report envisioned more efficient and effective strategies for toxicity-testing given advances in our understanding of biology and the emergence of modern testing technologies. Considering the complex mixture of chemicals that often occur in environmental waters impacted by multiple different sources and land uses, evaluation of potential biological effects could benefit from this 21st century approach to toxicity evaluation. While information from these techniques is commonly targeted at human health impacts, recent studies have explored it for use in evaluation of potential adverse impacts in nonmammalian species as well including evaluation of potential impact on aquatic life (Blackwell et al., 2017, 2019; Heiger-Bernays et al., 2018; Schroeder et al., 2016) and avian species (Elliott et al., 2018b). For this type of prioritization, exposure-activity ratios (EARs = sample concentration/bioactivity effect concentration) have been identified as a valuable calculation (Blackwell et al., 2017; Elliott et al., 2018b); however, it is currently unclear what EAR threshold to consider as a level of concern and whether the threshold is relatively constant or changes substantially as different chemicals are evaluated, and an evaluation is needed to determine which ToxCast assays and related adverse outcome pathways are ecologically relevant.

The objectives of the current study were to couple the contaminant surveillance data from 57 monitored Great Lakes tributaries with high-throughput assay results from ToxCast to (1) assess whether the EAR approach appears to be valuable in this context, (2) if so, determine what EAR level(s) warrant concern based on comparison with evaluations using traditional water quality benchmarks, (3) identify chemicals of concern, (4) screen for potential adverse biological effects that may be associated with those chemicals, through linking bioactivity data to adverse outcome pathways (AOPs) for ecologically-relevant species, and (5) prioritize tributaries with respect to potential biological effects.

2. Methods

2.1. Sampling design

The Great Lakes tributaries were sampled from September 2010 through September 2013 and included sites draining to each of the five Great Lakes (Fig. SI-1, Table SI-1). A total of 709 water samples were collected. The study was designed to provide a general indication of contaminants in Great Lakes tributaries on spatial, temporal, and seasonal scales. Thirty-eight of the sites were sampled only one or two times to provide background information on a large spatial scale and identify potential concern for future studies. These sites were thought to be less likely to have large numbers of contaminants present than sites sampled more frequently given their history and land use composition. The remaining 19 sites were sampled more frequently with 7–64 samples that added to the spatial extent of the study and served to represent temporal and seasonal variability. Drainage areas ranged from 101 to 16,400 km^2, with mean annual flows from 2.58 to 219 cubic meters per second (October 2010–September 2013). Watershed land cover varied from dominantly urban (up to 92% of watershed) to agricultural (up to 84%) to forest and wetland (up to 93%). Watershed population densities ranged from 1.3 to 964 people/km^2 with 0% to 47% mean annual streamflow contributions from wastewater treatment plants. Details of the site selection, sampling design, sampling protocols, and quality assurance sample results have been previously published (Baldwin et al., 2016).

Whole water samples were analyzed for 67 organic waste compounds at the U.S. Geological Survey National Water Quality Laboratory in Denver, CO. Compounds were extracted using continuous liquid-liquid extraction and methylene chloride solvent, then determined by capillary-column gas chromatography/mass spectrometry (Zaugg et al., 2006). Four analytes were omitted from the final data set due to contamination in blank sample results (phenol, bis(2 ethylhexyl) phthalate (DEHP), acetophenone, and triethyl citrate). Analytes were categorized into 15 chemical classes based on their functional use or chemical properties (Table SI-2) and all analytical data have been previously published (Baldwin et al., 2016).

2.2. Screening values

2.2.1. ToxCast

Results from the ToxCast database (US EPA, 2015) were used to evaluate potential biological activities that may be associated with chemicals detected in the water samples. The ToxCast program evaluates individual chemicals for interactions with, or effects on, cells, proteins, DNA, RNA, mitochondria, receptors, and enzymes, etc. (Judson et al., 2016). For most of the ToxCast assays, annotations describing the intended biological targets are available in the database download (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data) or the U.S. Environmental Protection Agency (US EPA) CompTox dashboard (https://comptox.epa.gov/dashboard). The ToxCast data analysis pipeline provides several summary metrics modeled from chemical dose-assay response curves: the activity concentration at cutoff (ACC), the half maximal activity concentration (AC50), and the top value (T), or efficacy (Filer et al., 2016). The ACC is an assay-specific metric determined as a multiplier of the baseline median absolute deviation of measured activity in the assay that provides an indication of the concentration at which the bioactivity measured first exceeds the baseline concentration. More thorough descriptions of its derivation are provided elsewhere (Filer et al., 2016; Judson et al., 2009). Because this metric is indexed to a standard response threshold and is not dependent on chemical-specific activity, its use has been favored in recent applications of the ToxCast data over the other metrics (Blackwell et al., 2017; Fay et al., 2018). Thus, the ACC (parameter name modl acc in the ToxCast database) was used as the final endpoint for comparison with water quality data.

For this study, data from all assay platforms (Kavlock et al., 2012) available in the ToxCast database were considered, with the exception of BioSeek, which had several endpoint values that were anomalously low compared to those from other sources. Considering the nature of the assays and the associated reliability/quality for detecting gain or loss of signal, Attagene "gain" endpoints and Novascreen "loss" endpoints were used, while Attagene "loss" endpoints and Novascreen "gain" endpoints were removed because these assays were not optimized or designed to report for the given assay direction (Blackwell et al., 2017). Chemical/assay combinations that did not result in a significant test response (a "hit call") were also removed from consideration.

Data quality remarks were included with the reported metrics for the ToxCast test data. Results with the following data quality flags were removed from consideration for the present study: "Borderline active", "Only highest conc above baseline, active", "Gain AC50 < lowest conc & loss AC50 < mean conc", and "Biochemical assay with <50% efficacy". All results from in vivo zebrafish embryo assays (NHEERL_PADILLA) matched at least one of these filtering criteria, and so were removed. Dose-response curves for chemical-assay combinations remaining after this selection process were also examined manually. Eleven of these dose-response curves were found to be of questionable quality based on anomalous values or lack of response and removed from analysis: NVS_ENZ_hPTEN for nonylphenol, NVS_ENZ_rMAOBP for Diethyl phthalate, TOX21_p53_BLA_p3_viability for Cotinine, TOX21_p53_BLA_p5_ratio for Metolachlor, TOX21_p53_BLA_p3_ratio for Carbazole, TOX21_p53_BLA_p3_ratio for Triphenyl phosphate, TOX21_p53_BLA_p3_ratio for Tris(2 chloroethyl) phosphate, TOX21_p53_BLA_p3_ratio for Fluoranthene, NVS_NR_hPPARg for Triphenyl phosphate, CLD_CYP1A1_6hr for bisphenol A (BPA), CLD_CYP1A2_6hr for BPA. In addition, the Tanguay_ZF_120hpf_ActivityScores were removed since this endpoint is an aggregation of activity from other zebrafish assays that are all represented separately in the ToxCast data set. With these various exclusions, 255 total ToxCast assays were suitable for computations for the chemical data set in the present study (Table SI-3).

Of the 65 chemicals detected from the tributary samples, 54 (83%) were represented in ToxCast (i.e., tested in at least one ToxCast assay). Among those 54, 48 had measurable effects (i.e., at least one active hit call) within the range of concentrations tested. The number of active hits per chemical ranged from 1 to 92 assays (Table SI-4).

2.3. Data analysis procedures

2.3.1. Screening for potential pathway-based effects

To screen the water chemistry data for potential bioactivity, chemical concentrations were compared to effect concentrations in ToxCast assays by computing EAR values as the quotient of the measured concentration and the ACC for each assay-chemical combination. The EAR is analogous to a hazard quotient, but rather than relying on a concentration at which adverse health effects like impaired survival, growth, or reproduction are detected, one instead uses the concentration required to elicit a response in a pathway-based assay. An EAR of 1 or higher indicates that the measured concentration in a water sample was greater than the ACC in the assay medium. Extrapolating this information for application to environmental concentrations is complex. For example, current information does not include correction for chemical partitioning in the assay system (e.g., free versus bound chemical in a test well), so actual biological activity in vitro or in situ may differ from this. However, it does provide a value that effectively normalizes for relative concentration detected in the environment and relative potency to elicit a specific biological effect. Thus, the EAR value is suitable for relative ranking and prioritization. Still, it is recognized that not all ToxCast assays used in this analysis are likely to be relevant to ecological species even though many of them target biological activities that are conserved among species. A complete evaluation of ToxCast assays for ecological relevance is currently not available, so the EAR approach used here is conservative in this respect. The AOP analysis described below does include evaluation of ecological relevance but is not considered to be comprehensive, since AOP information is not yet defined for all biological targets represented in ToxCast.

For chemical concentrations reported as below the level of detection, EAR values were assumed to be zero for this analysis. To test the impact of this, EAR values were computed as a ratio of the detection limit for each compound and all available ACC values (Fig. SI-2). Results indicated that there was potential for pathway-based bioactivity at the detection limit for some chemical-assay pairs. EAR values from the minimum ACC for each compound ranged from $<10^{-4}$ (multiple compounds) to 0.19 (assay = OT_ERa_EREGFP_0120 for 4 Nonylphenol, Branched). This suggests that methods used in the current study could result in underestimation of EAR values in cases where concentrations below the analytical detection limits could still elicit a significant biological effect (i.e., near the ACC). Hence, further evaluation may be needed to determine whether these chemicals may be of concern.

Results were examined using several different EAR summations (Table 1a), and different combinations of chemical occurrence and EAR magnitudes (Table 1b). Each summation has value for addressing

Table 1a
Definition of several different EAR summations used for analysis and interpretation of results.

Summation	Abbreviation	Description
Exposure-activity ratio	EAR	The quotient of the measured concentration of a chemical in a sample and the activity concentration at cut-off (ACC) for a given assay-chemical combination. $\text{EAR} = \frac{\textit{measured concentration in sample } (\mu M)}{\textit{ACC for chemical}-\textit{assay pair } (\mu M)}$
EAR by chemical	EAR_{Chem} $EAR_{SiteChem}$	The sum of EAR values from all assays for an individual chemical in a given sample. The number of active assays and magnitude of individual assay-chemical EAR values combine to provide a means to prioritize chemicals by potential for effect: $EAR_{Chem} = \sum EAR_{[i]}$, where i = assays relevant for each individual chemical. Maximum EAR_{Chem} for an individual site
EAR by assay endpoint	$EAR_{Mixture}$ $EAR_{SiteMixture}$	The sum of EAR values for each individual assay endpoint across all chemicals for a given sample. $EAR_{Mixture}$ provides an estimate of cumulative bioactivity for an individual assay from a mixture of chemicals, assuming additivity of effects. This is consistent with previous use of $EAR_{Mixture}$ for individual assays (Blackwell et al., 2017), and is analogous to a "toxic unit" approach for summing adverse effects for similar-acting chemicals present in complex mixtures (Nirmalakhandan et al., 1994). $EAR_{Mixture} = \sum EAR_{[i]}$, where i = chemicals associated with an individual assay endpoint. Maximum $EAR_{Mixture}$ for an individual site
EAR by adverse outcome pathway (AOP)	EAR_{AOP} $EAR_{SiteAOP}$	The sum of EAR values for each assay—chemical combination associated with an individual AOP. Using the subset of assays that are currently associated with AOPs, this summation accounts for the fact that more than one ToxCast assay may align with key events along a specific AOP. If all relevant EARs for each chemical were included, the effect of an individual chemical might essentially be "double counted". To avoid this, the summation considers only the maximum EAR for each individual chemical that is associated with an AOP: $EAR_{AOP} = \sum \max(EAR_{[i]})_{[j]}$, where i = chemical, j = an assay relevant to a given AOP. Maximum EAR_{AOP} for an individual site
Toxicity quotient	TQ TQ_{max}	The quotient of the measured concentration of a chemical in a sample and the water quality benchmark for a given chemical. $\text{TQ} = \frac{\textit{measured concentration in sample } (\mu g/L)}{\textit{Water quality benchmark for chemical } (\mu g/L)}$ Maximum TQ at a site for all water quality benchmarks available for a given chemical. 1

different questions. For example, the EAR-by-chemical (EAR_{Chem}) is useful for ranking and prioritizing among chemicals for a given sample with regard to their potential to elicit a biological effect. The EAR-by-assay endpoint ($EAR_{Mixture}$) is used to estimate the potential cumulative impact of a mixture of chemicals on a given assay response assuming additivity. Yet another summation, the EAR_{AOP} is used to consider the potential for a group of chemicals to contribute to a common adverse pathway/outcome (detailed further below). The frequency of occurrence of chemicals and exceedance of EAR or TQ thresholds were then used to help prioritize chemicals, ToxCast assays, and chemical mixtures to identify a manageable number of situations for which there is potential for adverse impacts (Table 1b). Similarly, identification of chemicals of concern and EAR and TQ levels of concern are defined for use with the intention to compare among the different chemicals, chemical mixtures, assays, and sites within the current study. Results are not intended for regulatory actions, but for consideration by stakeholders for further investigation and validation at specific sites.

Where appropriate, these different EAR summations were calculated for each of the 709 individual samples. The number of samples collected per site was variable, so the maximum EAR value per site was determined to provide a means to weight each site equally. This resulted in three additional EAR variables that were ultimately used in the current study: $EAR_{SiteChem}$, $EAR_{SiteMixture}$, $EAR_{SiteAOP}$ (Tables 1a and 1b).

Computations and analyses were primarily carried out using functionality of the R package *toxEval* (De Cicco et al., 2018) with additional custom calculations and visualizations developed using the R packages *dplyr* (Wickham et al., 2018), *ggplot2* (Wickham, 2016), and base R functionality (R Core Team, 2017). The *toxEval* package was developed within the current study to facilitate analysis and visualization of EAR and toxicity quotient (TQ) values for complex chemical concentration data sets that represent multiple chemicals, sampling locations, and samples per location. Version 2 of the ToxCast database was included as the default set of benchmark concentrations within *toxEval* with EAR values as the default evaluation parameters. Additionally, user-selected chemical-biological interaction benchmark data, such as the water quality benchmarks used in the current study, can be used for computation and analysis of TQs within *toxEval.*

2.3.2. Water quality benchmarks

Water quality benchmarks for 27 of the measured chemicals were reported by (Baldwin et al., 2016). For the present analysis, water quality benchmarks for an additional seven chemicals were added to the list. These include a broad range of toxicity benchmarks from regulatory and non-regulatory entities that are not necessarily enforceable limits but provide guidance on concentrations that represent a potential hazard for aquatic organisms (Table SI-2). The benchmark values were used to assess how the EAR-based approach compared with more traditional methods focused on apical adverse effects. Chemical-specific TQs were computed as the ratio of the measured concentration and the associated water quality benchmark. The minimum water quality benchmark and

Table 1b
Description of methods used for prioritization of chemicals, ToxCast assays, and chemical mixtures using EAR and TQ values.

Type of Prioritization	Parameter	Description
Chemical prioritization	TQ_{Max}	Chemical occurrence at ten or more sites and $TQ_{max} > 10^{-3}$ for five or more sites
Chemical prioritization	$EAR_{ChemSite}$	Chemical occurrence at ten or more sites and $EAR_{max} > 10^{-3}$ for five or more sites
ToxCast assay prioritization	$EAR_{SiteMixture}$	$EAR_{SiteMixture} > 10^{-3}$ for ten or more sites
Chemical mixture prioritization	$EAR_{SiteAOP}$	Samples that had chemical mixture combinations with $EAR_{SiteAOP} > 10^{-3}$ and chemicals that contributed at least 1% of $EAR_{SiteAOP}$ in samples from 5 or more sites

the maximum concentration per sampling location were used to compute the maximum toxicity quotient at each of the sampling locations (TQ_{max}).

In addition to traditional water quality benchmarks, 17β estradiol equivalency quotients (EEQ) were determined to estimate potential for estrogenic activity of the measured chemicals. The EEQs were obtained by multiplying the measured concentrations of estrogenic compounds by their respective estradiol equivalency factor (EEF) derived from published intersex and vitellogenin induction values in fish (Vajda et al., 2008). The EEQ values were then compared to EAR values for chemicals where both were available to better understand how EARs from ToxCast compare to a more traditional approach for evaluating estrogenic potential.

2.3.3. Linking to adverse effects

Most of molecular/biochemical responses associated with the ToxCast assays do not directly translate to endpoints typically considered in ecological risk assessments (i.e., impacts on survival, growth, reproduction, etc.). However, the adverse outcome pathway framework has been developed as a systematic and transparent means to describe the plausible and empirically supported linkages between the molecular, biochemical, and/or cellular biological activities typically evaluated in ToxCast assays and adverse outcomes that are more relevant to protection goals, management decisions, and risk assessment (Ankley et al., 2010). To help interpret the significance of ToxCast endpoints with regard to potential apical hazards, efforts have been made to map ToxCast assays to corresponding key events in AOPs described in the AOP-Wiki (Fay et al., 2018; Pittman et al., 2018; Society for the Advancement of Adverse Outcome Pathways, 2018). In the present study, cross-mapping of ToxCast endpoints to key events in AOPs (Fay et al., 2018) was updated here and used to help contextualize the significance of the EAR-based analyses relative to potential ecologically-relevant hazards.

Briefly, as of February 8, 2018 there were approximately 250 user-defined AOPs in the AOP-Wiki. Key events (including molecular initiating events) in these AOPs were manually curated for relevance to response from the ToxCast assays. Key event names were considered foremost for mapping to assays, although additional text included on the event pages also were considered. Some AOP key events are not molecular in nature, but describe general or higher-level responses (e.g., mitochondrial disruption, production of reactive oxygen species, cytokine suppression), which also is the case for some ToxCast assays which measure outcomes like cell proliferation, cell death, and mitochondrial dysfunction. In these cases, a panel of ToxCast assays relevant to a generalized response was identified and corresponding AOP events were mapped to each of the assays within the panel. Applicability of ToxCast assays considered not only the intended target but also the direction of measurement (e.g., agonism or antagonism of a given receptor). Mappings were also informed by the AOP event ontology annotations in the AOP wiki (Ives et al., 2017; Table SI-5).

Once the global mapping of ToxCast assays to key events and associated AOPs was developed, the subset of AOPs relevant to ToxCast assays prioritized in the current analysis ($n = 49$) were identified. Each assay—AOP pair was further evaluated for relevance to taxa in the Great Lakes ecosystem (Table SI-6). For example, AOPs establishing links to human health outcomes/endpoints that would typically not be considered as an assessment endpoint in ecological risk contexts (e.g., fatty liver disease) were not considered. This resulted in identification of 23 ecologically relevant AOPs associated with assays that were prioritized based on the EAR analysis.

An EAR summation approach (EAR_{AOP}) that considered potential cumulative impacts along specific AOPs was developed to aid interpretation of EAR results in an ecological hazard context (Tables 1a and 1b). An important consideration in calculating this EAR summation is that there can be more than one ToxCast assay that maps to a given key event in an AOP. For example, there are multiple ToxCast assays that measure estrogen receptor activation (Browne et al., 2015) even though estrogen receptor activation is a single key event in an AOP. Likewise, since AOPs are composed of multiple key events, ToxCast assays related to multiple different biological targets may map to a single AOP (e.g., assays 1 and 2 in Fig. 1 map to different key events along the same AOP). Consequently, to control for the fact that multiple assays and thus different numbers of EAR values may map to different AOPs, EAR_{AOP} considers the maximum EAR for any chemical-assay pair relevant to that AOP.

2.3.4. Chemical mixtures

EARs and AOP networks have each been recognized as tools for evaluating the potential significance and effects of chemical mixtures (Blackwell et al., 2017; Knapen et al., 2018). Consequently, we explored how these two approaches might be combined to better understand potential hazards associated with chemical mixtures detected in Great

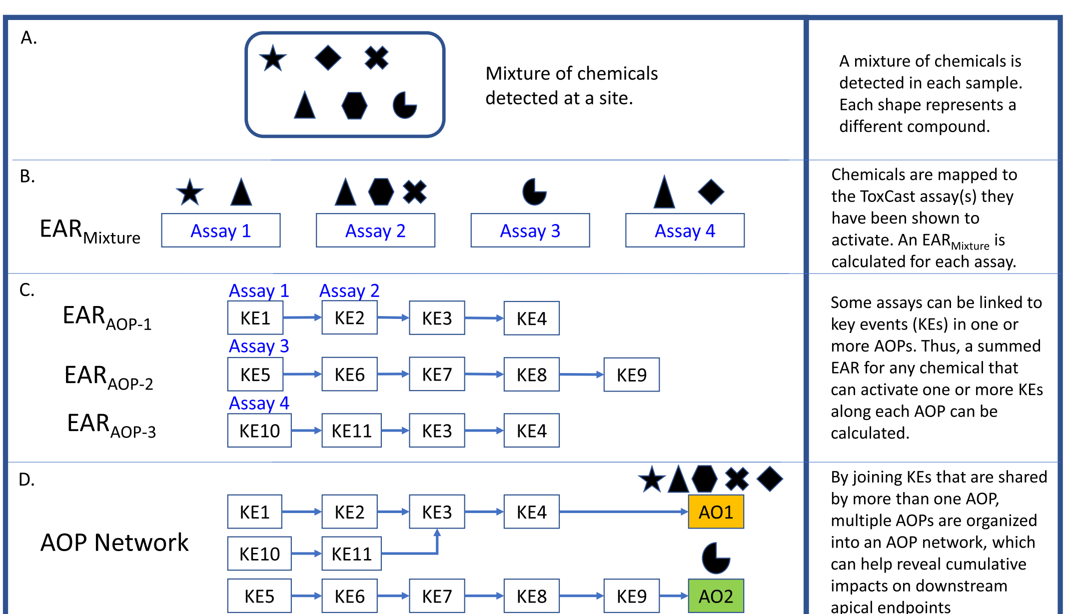

Fig. 1. Diagram of chemical mixture analysis using exposure-activity ratios for chemical-ToxCast assay pairs, adverse outcome pathways, and adverse outcome pathway networks.

Lakes tributaries. First, the number and identity of chemicals detected at each individual site was determined to establish the nature of the mixtures present (Fig. 1.A). Next, summations of EAR values for individual ToxCast assays ($EAR_{SiteMixture}$) were used to estimate potential cumulative biological activity from multiple co-occurring chemicals at a given site (Fig. 1.B). This allowed for prioritization of site-specific bioactivities, providing information on potential biological functions that may be influenced by these chemical mixtures. Third, summations of EAR values for AOPs ($EAR_{SiteAOP}$) that aligned with the biological activities detected at various sites were calculated (Fig. 1.C). After $EAR_{SiteAOP}$ values were calculated, samples that exceeded a given $EAR_{SiteAOP}$ threshold for AOPs that were designated as possibly environmentally relevant were identified. From this list of samples, chemicals that contributed at least 1% of the EAR at 5 or more sites were retained. From this list of chemicals and samples, mixtures of 2–12 co-occurring chemicals were identified.

Each AOP represents a unique sequence of biological events (key events) that link a molecular initiating event to an adverse outcome. However, AOPs employ a modular construct (Villeneuve et al., 2014). That means any given key event may be part of more than one AOP. This makes sense biologically. For example, the key event "agonism, estrogen receptor" (https://aopwiki.org/events/111) can have different biological consequences depending on the life stage exposed: altered larval development during an embryo-larval stage, impaired development of reproductive organs during sexual differentiation, or altered reproductive behaviors during adult life stages. Likewise, an apical endpoint like reproductive failure, also a key event, can have a diversity of causes. For this reason, in most real-world situations (i.e., exposure to multiple chemicals; chemicals having more than one biological activity), more than one AOP is likely to be triggered and because there are shared biological nodes among those AOPs, interactions can be expected (Knapen et al., 2018; Villeneuve et al., 2014). Based on these principles, AOP networks are defined as an assembly of two or more AOPs that share one or more key events. By linking together any shared key events, a group of individual AOPs can be assembled into an AOP network (Fig. 1.D; (Knapen et al., 2018)).

To derive an AOP network relevant to the present study, the AOP-Wiki was queried using the AOP identification number of each of the 23 ecologically-relevant AOPs of interest. Each AOP description page contains a table (relationship table) that defines the sequence of key events that make up a given AOP as a series of pairs: key event 1 leads to key event 2; key event 2 leads to key event 3; and so on. Two of the 23 AOPs did not include complete key event relationship information, and thus were not used in development of AOP networks (AOPs 52 and 53; Table SI-6). The relationship table was extracted from each of the remaining 21 AOP pages, and each pairwise key event relationship was parsed into a from-to matrix (Table SI-7). The from-to matrix was then loaded into Cytoscape (v.3.5.1; www.cytoscape.org; (Shannon et al., 2003)) to visualize the resulting network. The 21 AOPs yielded a network of seven disconnected components. The network was manually arranged for easier visualization. Three mixtures were chosen that represented different AOPs to highlight as examples, and the median $EAR_{SiteAOP}$ values for each of these three chemical mixtures was loaded as edge attributes and used to visualize the paths through the overall network that one would expect to be impacted by the mixture in question.

3. Results

Computed EAR values for individual compounds were first aggregated over the study sites for an overall assessment and examined in three primary ways: (1) by individual chemical and chemical class, (2) by monitoring location, and (3) by potential biological relevance. This sequence of analyses provided information to screen for pathway-based bioactivity and prioritize potential impacts by chemicals, watersheds, and biological functions. A goal was to enable future investigations of these tributaries to focus on a reduced set of influences at a limited number of watersheds and more efficiently identify and/or verify the potential impacts.

3.1. Prioritization of chemicals, EAR-versus TQ-based approaches

Exposure-activity ratios based on in vitro effect concentrations do not necessarily translate directly to in vivo apical responses. This is because of toxicodynamic considerations (i.e., the endpoints measured in ToxCast assays do not equate to definitive impacts on survival, development and growth, or reproduction) and toxicokinetic considerations (i.e., nominal concentrations in a test well do not necessarily translate to free chemical concentrations in plasma or environmental media). Consequently, the magnitude of EAR that is environmentally relevant for predicting adverse biological effects is not as easily defined as a TQ of concern (i.e., $TQ > 0.1$). To gain insight into the question of what EAR value would serve as a reasonable cut-off for prioritization purposes, EARs were compared with more traditional prioritization methods using water quality benchmarks and EEQ calculations (Fig. 2, B and D). Recognizing that water quality benchmarks are largely based on in vivo effects and EAR values are based on in vitro testing, this is not a perfect comparison. Nonetheless, it provided an empirical basis to start identifying what EAR levels may be of concern from a screening and prioritization standpoint.

The comparison of TQs based on water-quality benchmarks to $EAR_{SiteChem}$ levels indicated that the relation is chemical-specific. Considering $TQ_{max} = 10^{-1}$ as the benchmark for this analysis, corresponding $EAR_{SiteChem}$ levels that correspond to $TQ = 10^{-1}$ varied from approximately 10^{-4} to 10^{-1} (Fig. 2). Consequently, for the sake of screening and prioritizing the chemicals with the greatest potential for pathway-based bioactivity from a spatial distribution perspective, chemicals that were present at a minimum of 10 sites and had $EAR_{SiteChem} > 10^{-3}$ at a minimum of five sites were considered (Fig. SI-3). These chemicals included 4 nonylphenol (a detergent degradate and industrial chemical), BPA (used in plastics and epoxy resins), metolachlor and atrazine (herbicides), DEET (insect repellant), caffeine, tris(2 butoxyethyl) phosphate and tributyl phosphate (TBEP, TBP; flame retardants), benzo(a)pyrene and fluoranthene (PAHs with multiple origins), benzophenone (ultraviolet radiation protection in soaps, fragrances, plastics), and triphenyl phosphate (TPhP; plasticizer, flame retardant) (Fig. 2).

For water quality benchmarks, considering the number of sites with detections and the magnitude of the TQ_{max} for each site (detected at a minimum of 10 sites with $TQ_{max} \geq 10^{-1}$ at a minimum of five sites), the detected chemicals with the greatest potential for adverse impact were 4-nonylphenol, BPA, metolachlor, atrazine, and several PAHs (anthracene, benzo (a) pyrene, fluoranthene, phenanthrene, pyrene). Water quality benchmarks could not be identified for 23 of the detected chemicals that were present in the ToxCast database (i.e., for which EARs could be calculated); these included all chemicals within several broad usage classes (human nonprescription drugs, flame retardants, flavors and fragrances).

When endpoints for ToxCast and water quality benchmarks were both available, all chemical classes identified as a concern were consistent. There were differences when considering individual chemicals. For example, water quality benchmarks identified five PAHs as a potential concern while the ToxCast assessment identified only two, and DEET was identified as a potential concern using the EAR approach, but available water quality benchmarks for DEET did not indicate a substantial concern. While different $EAR_{SiteChem}$ levels of concern could be chosen depending on the circumstances, the choice of $EAR_{SiteChem} = 10^{-3}$ appeared to provide a reasonable level for comparison with water quality benchmarks.

Estradiol equivalency factors were available for eight compounds, four of which were not represented in the ToxCast database, and two that were not detected in samples (Fig. 2-C, D). For the compounds

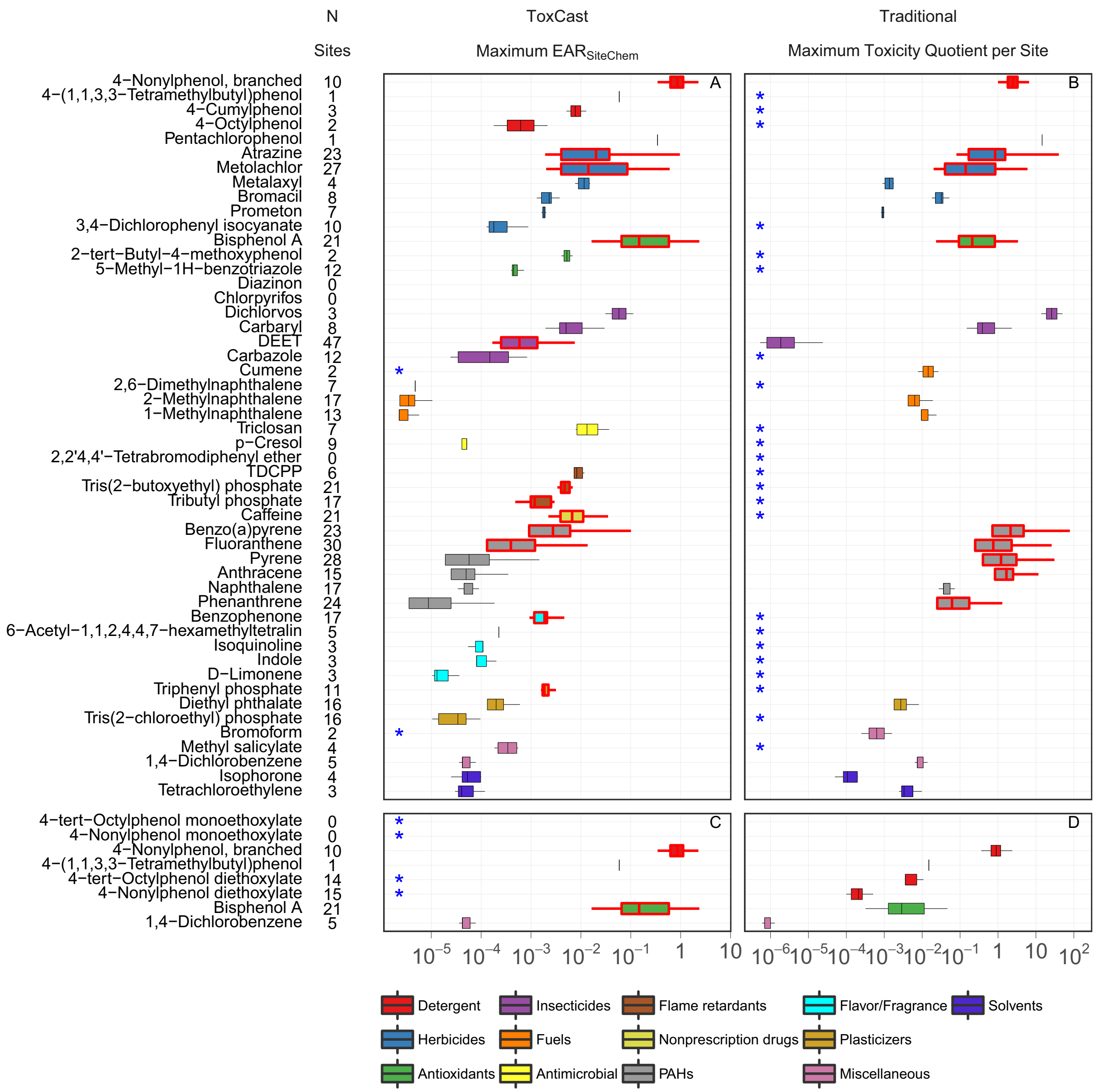

Fig. 2. Exposure activity ratios (EARs) using ToxCast endpoints for screening of potential pathway-based bioactivity from chemicals detected in water samples from 57 tributaries of the Great Lakes, 2010–2013 (A and C), toxicity quotients comparing established water quality benchmarks to sample results (B), and 17 β estradiol equivalency quotients (ng/L) for compounds with established estradiol equivalency factors (D). Compounds are grouped by chemical class and ordered by largest to smallest median EAR. Compounds where either water quality benchmarks or ToxCast information were unavailable are indicated with "*", and compounds where both were unavailable are not included in the graph. Boxplots with red borders designate chemicals that were present at 10 or more sites with EAR > 10^{-3} at 5 or more sites (panel A) or TQ > 0.1 at 5 or more sites (panel B). [N, number of sampling locations with detections of each chemical. Boxes, 25th to 75th percentiles; dark line, median; whiskers, data within 1.5× the interquartile range (IQR); circles, values outside 1.5× the IQR.]

with EEFs that were in the ToxCast database, results indicated similar priority rankings: 4 nonylphenol had the greatest potential effects and 1,4 dichlorobenzene had the least, with potential effects from 4 tert octylphenol between these two compounds. EAR values for BPA were not as comparable to EEQ results, with EAR calculations indicating greater potential effects relative to other compounds. This is because $EAR_{SiteChem}$ is computed using a summation of EAR values from all assays in which a given chemical is active. Bisphenol A is flagged not only in multiple assays related to estrogen receptors, but in several other ToxCast assays not related to estrogen signaling. Consequently, it is reasonable that the predicted impacts of a chemical that effects multiple types of biological activities, such as BPA, would deviate from a calculation considering only estrogenic activity. Nonetheless, EAR $= 10^{-3}$ appeared to be a reasonable threshold from a prioritization perspective.

3.2. Prioritization of sites, EAR-based

Sites for potential follow-up monitoring were prioritized based on the number of chemicals detected at each with an associated $EAR_{SiteChem} > 10^{-3}$ (Fig. 3, Figs. SI-4 and SI-5 A-M). Sites with at least one chemical exceeding the $EAR_{SiteChem} > 10^{-3}$ value included four of nine Lake Superior tributaries monitored, 11 of 19 Lake Michigan tributaries, five of seven Lake Huron tributaries, all 15 Lake Erie tributaries (including the Detroit River and Lake St. Claire tributaries), and three of seven Lake Ontario tributaries. Sampling locations that had >15 chemicals with $EAR_{SiteChem} > 10^{-3}$ included River Rouge, the Clinton River, and the St. Louis River. These would be a high priority for future investigation. There were an additional eight sampling locations with at least 10 chemicals and 10 more sampling locations with at least five

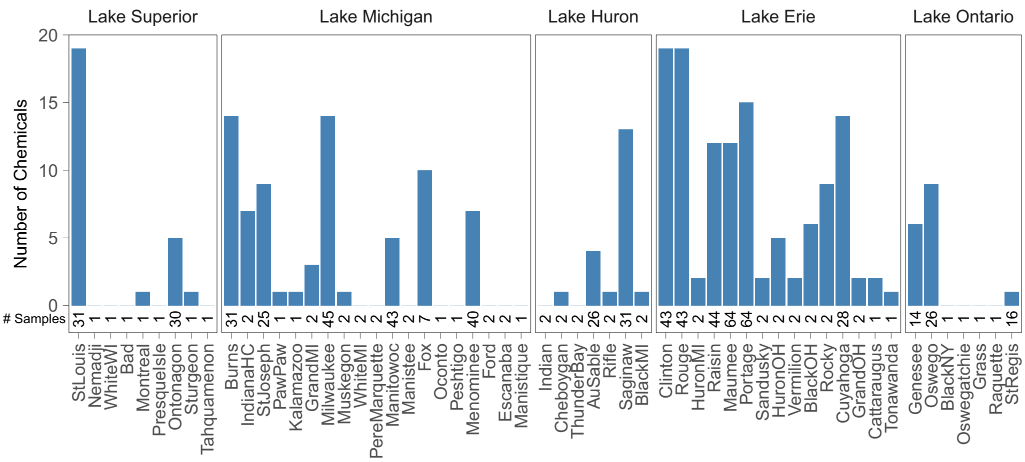

Fig. 3. Number of individual chemicals with at least one sample that resulted in a maximum exposure–activity ratio ($EAR_{SiteChem}$) > 10^{-3} for each site.

chemicals with $EAR_{SiteChem} > 10^{-3}$ (Fig. 3). Given that the number of samples collected from each site was variable (one to 64 samples) and that chemistry results varied temporally, the number of samples and timing of collection likely influenced the number of chemicals detected at each site. Nonetheless, the EARs provide an approach for differentiating sites with greater probability for biological effects from those with lesser probability.

Several sites had elevated EARs for multiple chemicals and/or chemical classes (Fig. SI-5 A-M). The EAR patterns throughout the study sites typically reflected the nature of land use within the watershed. Sites with urban influence most commonly had elevated EARs for PAHs and nonprescription drugs (Fig. SI-5 B and D). Agricultural sites were most likely to have higher EAR incidence of herbicides, primarily atrazine and metolachlor (Fig. SI-5 A). One exception to this was the St. Louis River where pentachlorophenol had an $EAR_{SiteChem} > 0.3$. The St. Louis River does not have a high percentage of either urban or agricultural land cover but does include wood and paper product industries that sometimes use pentachlorophenol in preservation processes (Karn et al., 2010). A mix of sites with differing dominant land covers had elevated $EAR_{SiteChem}$ values for insecticides, flavors/fragrances (benzophenone), plasticizers, flame retardants, antimicrobial disinfectants, and detergent degradates (Fig. SI-5 E, G, J, K, L, and M).

3.3. Prioritization by biological relevance, assay endpoints and adverse outcomes

A third approach to applying the EARs involves hypothesizing or inferring potential biological effects of concern at a given site, based on the known composition of chemical mixtures present. In this case, ToxCast assays were used to screen chemicals for biological activities (Fig. 1.A). The biological activities from those assays were aligned with key events in AOPs described in the AOP-Wiki (Fig. 1.B). Exposure activity ratios reflecting the potential effect of chemicals in a mixture on one or more key events along an AOP help to relate the potential biological activities to outcomes of concern (typically effects on survival, growth, reproduction; Fig. 1.C). In turn, recognizing that chemical-induced biological activities in vivo are occurring concurrently and can interact with one another in additive, antagonistic, and/or synergistic manners (Knapen et al., 2018), the group of AOPs potentially impacted by components of a mixture may in turn be considered in a network context (Fig. 1.D; (Villeneuve et al., 2018)).

To identify and prioritize biological activities most likely to be modulated in organisms resident to the Great Lakes tributaries surveyed, ToxCast assays with $EAR_{SiteMixture} > 10^{-3}$ at 10 or more sites were identified, resulting in a list of 46 assays (Fig. 4). For individual assays, the number of chemicals that contributed to the $EAR_{SiteMixture}$ varied from 1 to 33, further supporting the hypothesis that mixtures of chemicals are a necessary consideration for screening potential biological effects. Twenty seven of these 46 assays have been associated with AOP key events from the AOP Wiki (Fig. 4, Table SI-5; aopwiki.org). Some key events may occur in more than one AOP, and in this case the 27 assays were associated with a total of 49 AOPs (Fig. 5A and SI-6). Evaluation of the potential for effects on individual AOPs from multiple co-occurring chemicals was explored by computing summations of EAR values from all assays associated with individual AOPs ($EAR_{SiteAOP}$; Fig. 1; Tables 1a and 1b; Fig. 5A). For example, AOPs 52 and 53 are influenced by 6 of the ToxCast assays displayed in Fig. 5B. To estimate the cumulative EAR ($EAR_{SiteAOP}$) for these AOPs, the sum of EARs from all related assay-chemical combinations was computed. For any given AOP, the number of contributing assays varied from 1 to 35 (Table SI-5). Considering the ToxCast assays prioritized in Fig. 4 (i.e., those for which maximum $EAR_{SiteMixture} > 10^{-3}$ was calculated for at least 10 sites), the number of contributing assays per AOP varied from 1 to 17 (Fig. 5B).

Up to this point in the analysis, all ToxCast assays and associated AOPs potentially affected by chemicals measured in this study have been considered. However, not all ToxCast assays or associated AOPs are necessarily relevant in the context of ecological effects. Consequently, relevance of the 49 associated AOPs (Fig. 4) was evaluated. Based on this evaluation, 23 were classified as “relevant” or “potentially relevant” to resident Great Lakes tributary species (Fig. 5A, Table SI-6). Of these 23 AOPs, $EAR_{SiteAOP}$ varied from $<10^{-5}$ to 0.8.

To further understand the potential collective impacts of various mixtures detected in the Great Lakes tributaries on organisms, 21 of the 23 AOPs were assembled into an AOP network (Fig. 6). Two of the AOPs did not include sufficient information to include. The network was composed of 97 key event nodes (circles, squares, or diamonds in Fig. 6) organized into seven disconnected components (subnetworks). Overall, potential pathway-based bioactivity indicated by these AOPs includes various influences on reproduction and energy metabolism, specifically mitochondrial function (Table SI-6; Fig. 6). Detection of chemicals known to bind to and activate the estrogen receptor, produce excessive reactive oxygen species (ROS), activate peroxisome proliferator-activated receptor, inhibit histone deacetylase, or inhibit cyclooxygenase (molecular initiating events and/or “upstream” key events in Fig. 6) suggested potential for effects on reproduction and/or the reproductive system (adverse outcomes in Fig. 6). Additionally, available AOPs suggest that chemicals impacting mitochondrial function in vitro have potential to cause mortality and potential population-level impacts, including honeybee colony collapse (LaLone et al., 2017). In this case, the ToxCast assay hits did not necessarily align with the molecular initiating events of a corresponding AOP, but

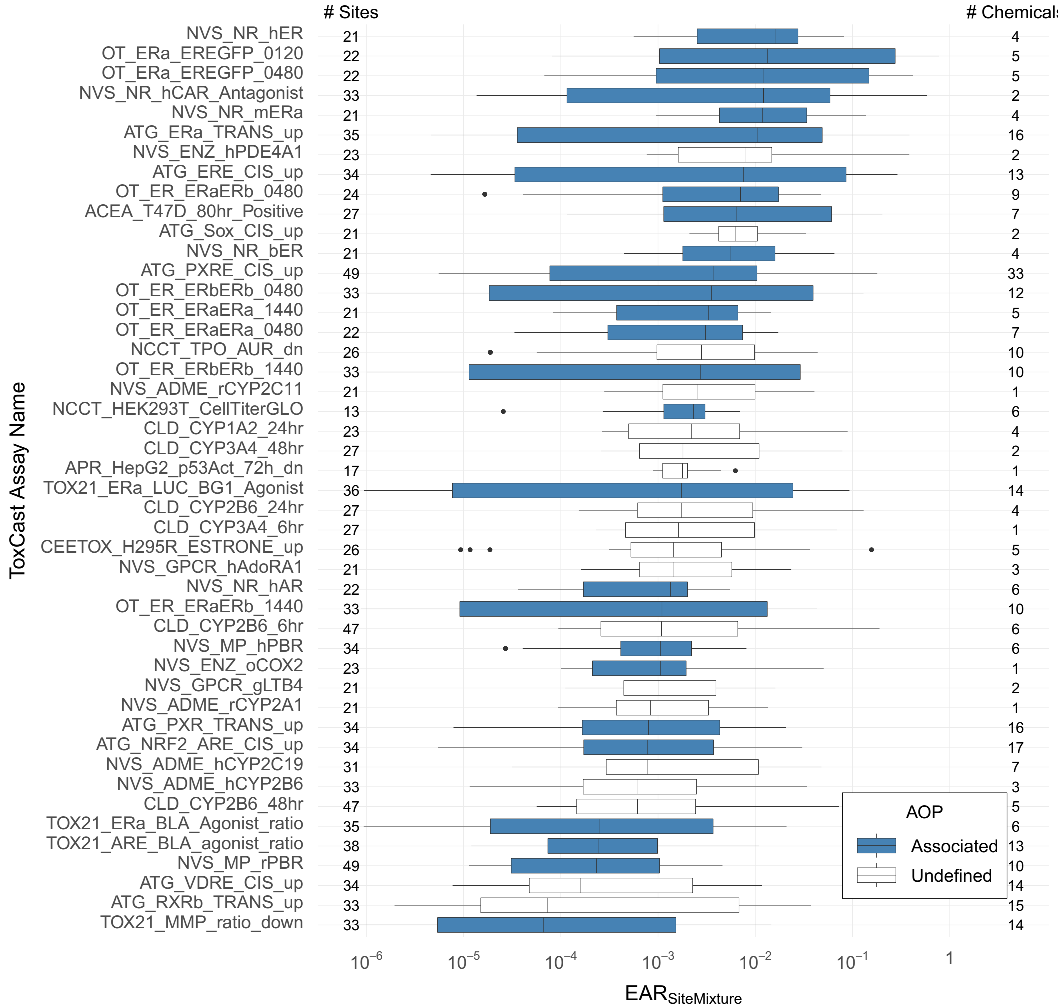

Fig. 4. Boxplots of maximum exposure-activity ratios (EARs) across sites for ToxCast assays with EARs $>10^{-3}$ from at least 10 sites. Assays with and without currently associated adverse outcome pathways (AOPs) are differentiated by color. [Boxes, 25th to 75th percentiles; dark line, median; whiskers, data within 1.5× the interquartile range (IQR); circles, values outside 1.5× the IQR. $EAR_{Mixture}$,the sum of EAR values for each individual assay endpoint across all chemicals for a given sample.]

rather the mitochondrial effects aligned with intermediate key events along an AOP (not all labeled in Fig. 6).

To illustrate how the AOP networks, along with EAR summations, can be used to hypothesize or infer potential effects of different mixtures, further consideration was given to chemical mixtures with high $EAR_{SiteAOP}$ values that occurred frequently. The list of detected chemicals was first reduced to those that contributed at least 1% of $EAR_{SiteAOP}$ in samples with $EAR_{SiteAOP} > 10^{-3}$. This identified twelve chemicals (Table SI-8). Instances when 2–12-compound mixtures occurred in samples were identified, resulting in1848 different combinations of mixtures of 2–12 compounds that occurred at between 5 and 21 sites with $EAR_{SiteAOP} > 0.001$ (Table SI-8).

Three of these mixtures were chosen as examples to illustrate how different mixture compositions (Fig. SI-7) may shift the patterns of biological responses across the network of 21 relevant AOPs (Fig. 6). To visualize this, the arrows depicting key event relationships along each AOP were weighted by their relative $EAR_{SiteAOP}$ values. Using this approach, one can see that at sites dominated by a mixture of TBP and caffeine, toxicities associated with mitochondrial function, activation of nicotinic acetylcholine receptors, activation of peroxisome proliferator activated receptor (PPAR), or inhibition of vitamin K epoxide reductase (VKOR) were predicted to be the most sensitive effects. Potential adverse outcomes linked with estrogen receptor agonism are less probable (Fig. 6A). In contrast, for mixtures containing BPA and DEET, estrogenic and anti-androgenic responses were predicted to be most likely, with mitochondrial-related toxicity potentially exacerbating some of the reproductive effects and potential impacts related with PPAR activation or VKOR inhibition a lower priority (Fig. 6B). A combination of 4 nonylphenol, atrazine, and metolachlor was predicted to have the most diverse range of potential effects on the overall network of AOPs (Fig. 6C). In particular, a series of AOPs associated with inhibition of cyclooxygenase activity, not prioritized for the other mixtures, are now identified as highly relevant, while potential estrogen receptor agonism-mediated pathways appearing less relevant for this mixture. While none of these examples are definitive, they illustrate how an EAR-based approach may be combined with an AOP network analysis (Knapen et al., 2018; Villeneuve et al., 2018) to infer potential biological hazards at different sites, and guide a hypothesis-driven approach to subsequent monitoring.

4. Discussion

A challenge for contaminant monitoring studies is to place results in the context of potential biological effects of concern to support resource management decision making. In the present study, an EAR-based application of high throughput screening data from ToxCast was explored along with AOP knowledge to provide an evidence-based approach for linking chemical occurrence with potential effects. This was done within the context of meeting the primary study objectives to prioritize chemicals and Great Lakes tributaries with the greatest likelihood for

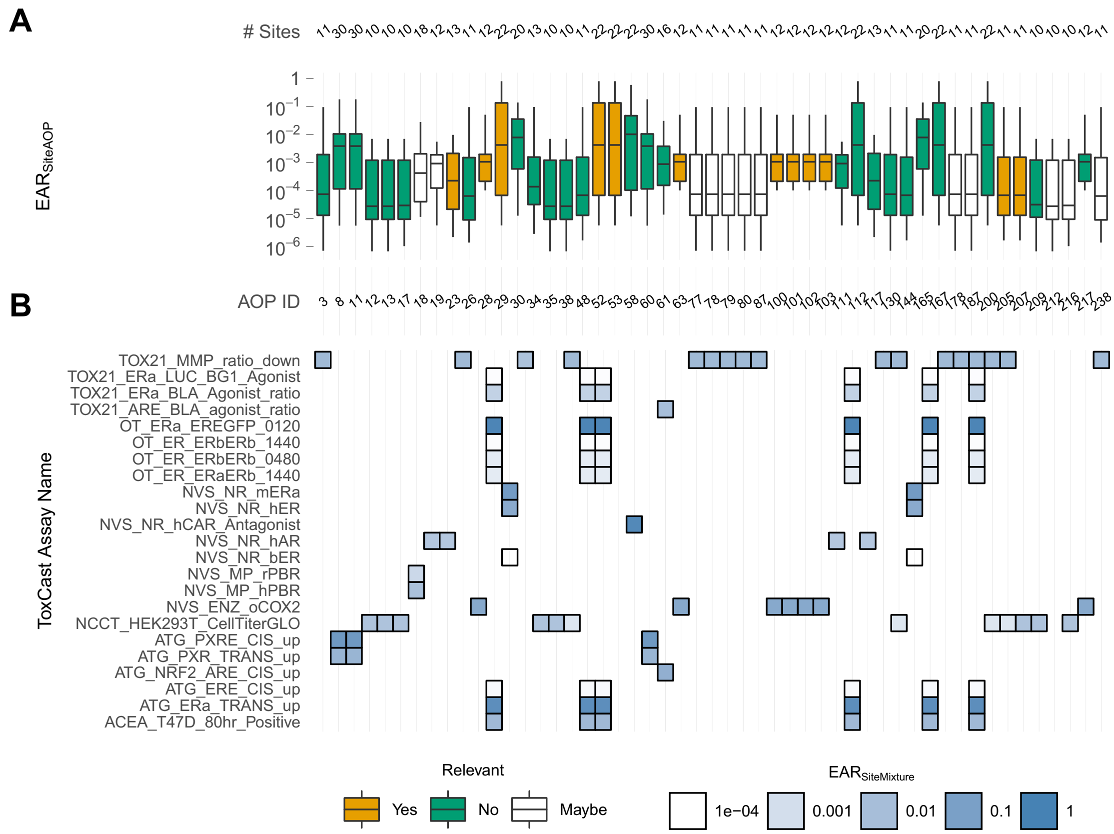

Fig. 5. Maximum exposure-activity ratios (EAR) by site for adverse- outcome pathway (AOP; A) and for ToxCast assay-AOP combinations (B) from chemicals detected in samples collected from Great Lakes tributaries, 2010–2013. Assays displayed include those that had EAR $>10^{-3}$ for at least 10 sites. [Boxes, 25th to 75th percentiles; dark line, median; whiskers, data within 1.5× the interquartile range.]

potential ecological effects. To fully understand the effectiveness of this approach, there are several questions that need to be addressed, including: (a) Can an EAR-based screening and prioritization approach provide results comparable to those obtained with traditional water quality benchmarks?; (b) What magnitude of EAR warrants concern?; (c) Are there specific chemical properties that may lead to poor representation of some chemicals using high-throughput screening results?; and (d) Are there ecologically-relevant adverse outcomes that might be anticipated using these results? It was the aim of the current analysis to examine these questions in detail.

4.1. Prioritization of chemicals

4.1.1. Individual chemicals

Among the 65 chemicals detected in the present study, traditional in vivo water quality benchmarks were available for 34 compounds, and ToxCast data were available for 54, substantially increasing the number of compounds for which comparison with biological effects data was feasible. Using a threshold of 10^{-3} as an $EAR_{SiteChem}$ level of concern, the EAR-based approach yielded a priority list of chemicals similar to that obtained using available water quality benchmarks using a TQ threshold of 10^{-1} (Fig. 2). Chemicals flagged as contaminants of potential concern by both approaches also have been identified in previous studies: 4-nonylphenol (Careghini et al., 2015), BPA (Corrales et al., 2015), metolachlor and atrazine (Busch et al., 2016; Graymore et al., 2001; Love et al., 2011; Pérez et al., 2011), and PAHs (Ankley et al., 1994; Logan, 2007; Ohe et al., 2004; Simcik and Offenberg, 2006). DEET was flagged by the EAR approach but was not identified to be of concern using TQ_{max} values from the available water quality benchmark information. The list was expanded further using the EAR approach to include several compounds for which water quality benchmarks were unavailable. These additional chemicals included caffeine, TBEP, TBP, benzophenone, and TPhP.

DEET is a commonly used insect repellant that is detected regularly in environmental waters (Weeks et al., 2012). Most traditional in vivo benchmarks for DEET (i.e., those related to mortality, growth, or reproduction) exceed 20 mg/L (US EPA, 1996; https://cfpub.epa.gov/ecotox/search.cfm).The most sensitive reported in vivo effects of DEET include a decline in hepatosomatic index and altered expression of several endocrine-related genes in fathead minnows following exposure to concentrations as low as 0.6 μg/L (Zenobio et al., 2014). Concentrations in samples for the current study exceeded 0.6 μg/L and 0.06 μg/L (10-fold safety factor) at three and 25 sites respectively. EAR analysis identified DEET as a potential concern because it was present in samples from 47 of 57 sites and 17 of those sites had samples with $EAR_{SiteChem} > 10^{-3}$. The greatest $EAR_{SiteChem}$ values for DEET corresponded to ToxCast assay activity measuring increased transcription of cytochrome P4502B6 (cyp2b6). Notably, previous studies have shown that cyp2b6 is one of the predominant cytochrome P450s involved in DEET metabolism (Usmani et al., 2002), so this chemical-biological interaction is not unexpected. Since this activity is directly linked with the metabolism of DEET, one cannot necessarily assume that induction of the protein would result in toxicity. In the case of DEET, the EAR approach may be conservative relative to traditional ecotoxicological benchmarks.

Caffeine is also detected regularly in environmental waters (Bradley et al., 2017). EAR analysis identified caffeine as a potential concern because it was present in samples from 21 sites, all of which had $EAR_{SiteChem} > 10^{-3}$. The greatest EARs for caffeine were due to its activation of Sox transcription factor (Sry-related high mobility group box) in the Attagene Cis-Factorial assay (Medvedev et al., 2018). However, given the diversity of genes regulated by this class of transcription

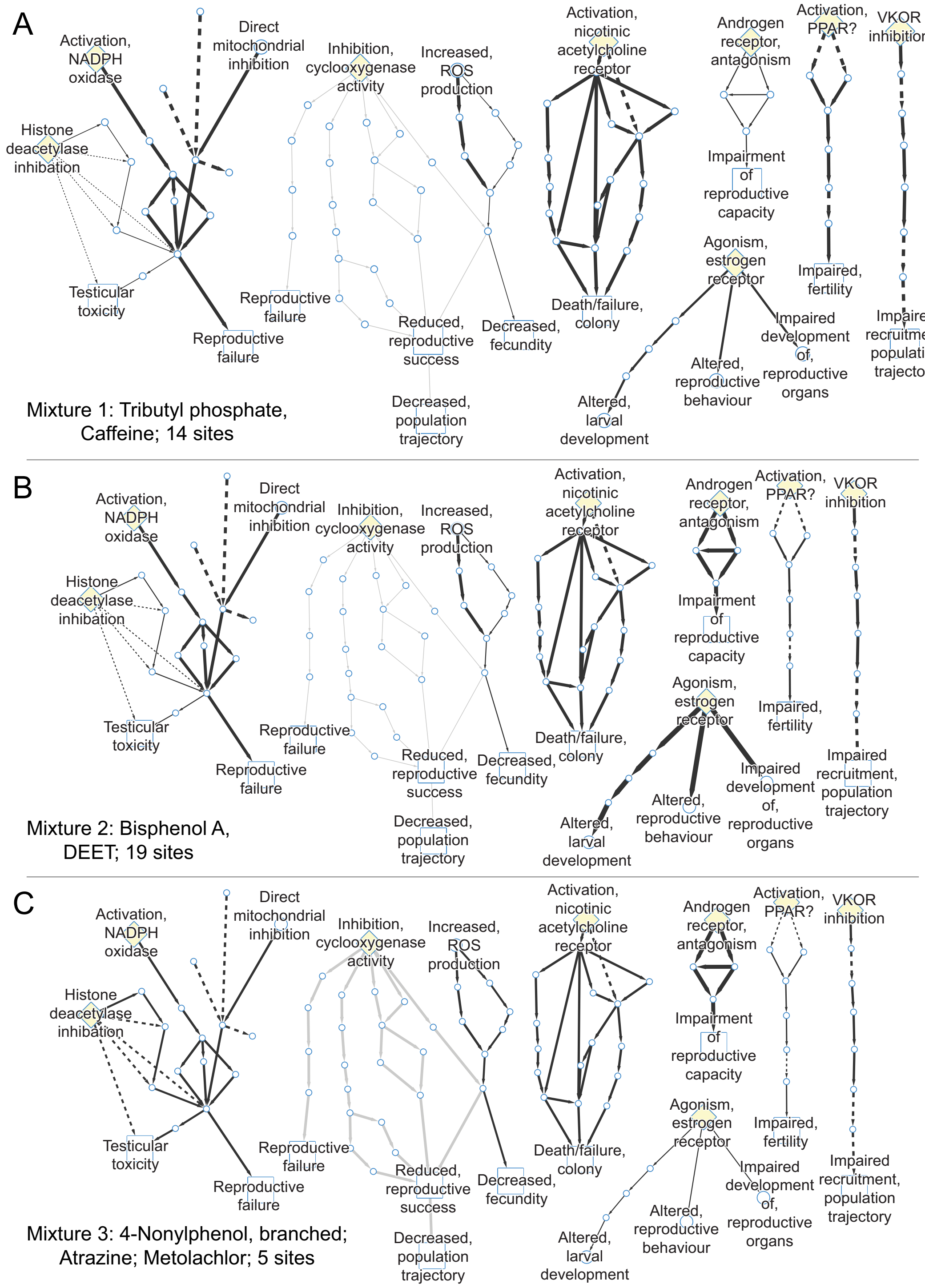

Fig. 6. Adverse outcome pathway network associated with 21 AOPs identified as relevant to the biological activities (assays) that had $EAR_{siteMixture} > 10^{-3}$. Panels represent potential relative influence for three example mixtures on the network, where arrows (key event relationships) linking the key events along a given AOP are weighted based on median $EAR_{siteAOP}$ for the mixture (thicker arrows indicate greater $EAR_{siteAOP}$ values). Biological key event nodes are represented as shapes: molecular initiating events as diamonds, adverse outcomes as squares, and intermediate key events as circles.

factors, it is difficult to infer the potential toxicological significance of the response. The second highest EARs for caffeine derive from its interaction with the human adenosine receptor a2a (assay ID NVS_GPCR_hAdoRA2a). This activity is consistent with caffeine's well-established activity as a non-selective blocker of adenosine receptors (Rivera-Oliver and Díaz-Ríos, 2014) and plays a role in many of the biological effects that caffeine is known for, including influences on sleep, cognition, heart rate, etc. However, these biological activities would not necessarily be linked to adverse effects on survival, growth, or reproduction in non-target aquatic species.

Two in vivo studies on aquatic species with TBP resulted in apical effect endpoints at 600 μg/L for regeneration of flatworms (Yoshioka et al., 1986) and between 1000 and 5000 μg/L for mortality of fathead minnows, *Daphnia magna*, and rainbow trout (Dave et al., 1979, 1981; Mayer and Ellersieck, 1986). In a study with zebrafish, TBEP caused significant early developmental toxicity by inhibiting the degradation and utilization of maternally-derived nutrients, thereby inducing apoptosis. Effects were observed at a concentration as low as 150 μg TBEP/L (Han et al., 2014). TPhP was acutely toxic to *D. magna* with a lethal concentration required to kill 50% of the population (LC_{50}) of 89 μg/L (Lin, 2009), and in zebrafish larvae, whole-body thyroid hormones and related gene expression were influenced at 40 μg TPhP/L, the lowest concentration tested (Kim et al., 2015). TBP and TPhP have been shown to display additive effects during concurrent exposures (Lin, 2009). Compared to concentrations measured in the current study, these effect concentrations for TBP and TBEP are relatively high, and substantial adverse effects might not be expected. More detailed consideration of biological responses associated with EARs $>10^{-3}$ for TBP, TPhP, and TBEP suggest relatively sensitive interactions with peripheral benzodiazepine receptor (PBR; TBP; assay IDs NVS_MP_hPBR, NVS_MP_rPBR) or the pregnane × receptor (PXR; TBP, TBEP; assay IDs ATG_PXRE_CIS_up, NVS_NR_hPXR) as well as the estrogen receptor (TBEP; assay ACEA_T47D_80hr_Positive). Activation of PXR is caused by many different types of chemicals, and generally is indicative of potential to induce xenobiotic metabolism (Kliewer et al., 2002). The significance of the interaction with PBR is less clear, but the estrogenic responses establish a clear link to one or more AOPs. While estrogenic activity would not necessarily be expected to result in lethality in either short- or long-term aquatic toxicity tests, potential effects on reproduction and/or development in vertebrate species are possible (Ankley et al., 2016).

Collectively, these examples highlight some of the differences between the application of traditional in vivo toxicity benchmarks and pathway-based activities that may flag a chemical as a potential priority. While the high throughput screening assays are detecting “real” biological activities, those activities are not necessarily directly translatable into in vivo effects in aquatic species. At the level of $EAR_{SiteChem}$ the approach serves as a practical first tier prioritization tool. A lack of EARs exceeding the prioritization threshold is a reasonable, evidence-based indicator of lower likelihood for biological effects. However, although an EAR exceeding the threshold does suggest potential for biological activity, it does not necessarily indicate that activity will result in toxicity. This points to the potential value of further alignment of ToxCast endpoints with AOP knowledge as a potential means to refine the analysis in a way that helps to better differentiate indicators of probable hazard from indicators of potentially more benign effects like induction of hepatic metabolism or of sublethal physiological effects.

Just as there are activities captured in the high throughput screening battery that would not necessarily contribute to, or drive, conventional in vivo benchmarks, there are also cases where in vivo toxicity tests may detect biological effects that would go undetected using in vitro systems. For example, even though PAHs were identified as a potential concern using both $EAR_{SiteChem}$ and TQ_{max} values, results were notably different between the two methods (Fig. 2). $EAR_{SiteChem}$ values indicated less potential for pathway-based bioactivity than those from TQ_{max} values. This difference could be attributed to the different nature of information represented in high throughput assays as compared to that used to determine water quality benchmarks. PAH water quality benchmarks used in the present study included those published by US EPA as well as the Canadian Council of Ministers of the Environment (Table SI-2). These water quality benchmarks take into consideration multiple forms of toxicity, including two that are not accounted for in ToxCast assays: metabolic- and photo-activation. Some PAHs are known to be metabolically activated to more toxic forms (Incardona et al., 2006; Stegeman and Lech, 1991), and it has been acknowledged that ToxCast in vitro assays underestimate toxicity resulting from metabolic activation (DeGroot et al., 2018; Jacobs, 2013). The more stringent Canadian water quality benchmarks for PAHs included mortality in assays from post-test ultraviolet (UV) radiation (Canadian Council of Ministers of the Environment, 2015). UV radiation exposure can induce photo-activation of PAHs, thereby decreasing concentrations of concern by several orders of magnitude over those without UV exposure (US EPA, 1996, 2003). Given that high-throughput assay results used in the present study do not account for photo-activation, this could, at least partially, explain the differences in EAR_{max} and TQ_{max} values for PAHs.

Overall, these results suggest that the EAR-based evaluation is indeed useful for hypothesis formulation to prioritize individual chemicals of concern. An EAR threshold of 10^{-3} appeared to provide conservative results tending toward inclusion of chemicals in question except for PAHs where some mechanisms to influence biological activity (e.g., metabolic- or photo-activation) are not well represented by the current assays included in ToxCast. The parameters chosen for inclusion of priority chemicals, however, are somewhat arbitrary, and may change based on study objectives. In this case, chemicals that were detected at 10 or more sites with $EAR_{SiteChem} > 10^{-3}$ were selected as a priority. Revising these parameters could result in a different list of chemicals. For example, if the threshold for the number of sites detected was reduced to five, bromacil, prometon, carbaryl, triclosan, and tris (dichloroisopropyl) phosphate (TDCPP) would have been added to the priority list using the EAR-based approach, while only carbaryl would have been added using water quality benchmarks.

4.1.2. Chemical mixtures

The first approach employed in the present study compared detected concentrations of individual chemicals in water samples to the concentrations eliciting in vitro activity across the ToxCast suite of assays (EAR_{Chem}). This type of information was useful for highlighting chemicals and sites of concern where chemical concentrations may be approaching those that elicit pathway-based biological activity (in vitro). However, another significant advantage of the EAR approach is the ability to sum the potential effects of chemicals acting on either the same biological target/pathway captured by an individual ToxCast assay (i.e., the $EAR_{Mixture}$) or chemicals acting on the set of assays that align with one or more key events along an AOP (EAR_{AOP}). In these instances, additivity of EAR values is a reasonable conservative assumption for estimation of cumulative effects. Specifically, it is plausible that each individual chemical detected in a mixture may be present at concentrations that would not, alone, exceed the EAR_{Chem} threshold of 10^{-3} selected for this study; however, collectively, multiple chemicals acting on the same target or AOP could yield an $EAR_{Mixture}$ or EAR_{AOP} $>10^{-3}$. This concept has been verified, for example, for estrogenic compounds (Thrupp et al., 2018).

In the present study, there were two additional ToxCast assays identified when $EAR_{Mixture}$ was $>10^{-3}$ at 10 or more sites as compared to those identified from individual EAR_{Chem} values. The AOPs identified when EAR_{AOP} was $>10^{-3}$ did not change from those when EAR_{Chem} was $>10^{-3}$, but EAR_{Chem} alone often underestimated the potential for impact. The mean EAR_{Chem} contribution from the individual chemical with the greatest contribution to any given $EAR_{SiteMixture}$ and EAR_{AOPMix} was 69% and 31% respectively. In addition, there were 23 chemicals that had $EAR_{Chem} > 10^{-3}$, but 29 different chemicals contributed at least 5% to EAR_{AOP} values when EAR_{AOP} was $>10^{-3}$. Thus, while some chemicals acting alone may not warrant concern, when acting in concert with other chemicals present in the mixtures, they may exacerbate the impact of one of the individually prioritized compounds.

4.2. Prioritization of potential biological effects

A primary goal of this type of analysis is to link the contaminants detected, in their relative concentrations, to apical adverse outcomes that might be of greatest concern at a given site. Recognizing that these mixtures of chemicals will interact with more than one biological pathway, and as a result, likely trigger multiple AOPs, the consideration of AOP networks is viewed as one approach to try to understand the possible cumulative effects of the chemicals (Knapen et al., 2018). Examination of an EAR-weighted network (e.g., Fig. 6) demonstrates how cumulative EARs ($EAR_{SiteAOP}$) might be used to identify what key events and outcomes may dominate at one site versus another. However, the analysis of AOP networks is in its infancy (Knapen et al., 2018; Villeneuve et al.,

2018) and it must be recognized that only a small fraction of potentially relevant pathways is currently represented in the AOP Wiki. As many as 47 sites had chemicals associated with individual assays for which the target biological activity has not yet been linked with AOPs (Fig. 4).

As a first evaluation of the adverse outcomes predicted in the current study, comparison to previous research indicates that reproductive dysfunction has been documented in multiple field studies with fish. These have included phenotypic alterations in sexual characteristics associated with exposure to substances present in wastewater discharge (Jobling et al., 1998, 2002), reduced sperm abundance and motility in resident fish from rivers receiving wastewater effluent (Blazer et al., 2012), reduced fecundity in fish downstream from or directly exposed to a wastewater effluent discharge (Cavallin et al., 2016; Prado et al., 2014), and multiple effects on male and female reproductive organs of fish exposed to wastewater effluent (Vajda et al., 2008) among others. It is recognized that neither the EAR-based identification of priority AOPs within an overall AOP network, nor previous field studies reporting effects consistent with some of these AOPs, causally implicate that concentrations of these contaminants detected in Great Lakes tributaries are having ecological effects. However, they do provide evidence to hypothesize that this may be the case when considering further monitoring and evaluation.

4.3. Limitations of the EAR approach

There are some important limitations to the EAR screening approach that need to be highlighted: (1) comparison of water chemistry results to assay results for individual chemicals does not allow for true evaluation of the integrated effect of mixtures. Assumptions (such as EAR additivity) must be made to evaluate the full sample matrix; (2) this type of evaluation is limited to those chemicals that are analyzed in samples that intersect with those chemicals that are currently represented in the ToxCast database (i.e., not all chemicals have been tested); (3) ToxCast data are limited to a specific set of assays–there are effects and some major modes of action that are not represented, including effects that are unique to non-mammalian species; (4) many of the assays lack the ability to metabolize the parent compounds being tested, which can under-and over-estimate the in vivo toxicity of some chemicals, and (5) not all ToxCast assays are currently linked to AOPs. Even with these limitations, high-throughput screening data can serve as a valuable tool for screening for potential adverse biological effects particularly where traditional in vivo toxicity data required to calculate water quality benchmarks are lacking. In addition, applying the ToxCast data in this manner could further inform and improve the ToxCast data curation and the AOP knowledgebase. For example, as hypotheses emerging from such analyses are tested, anomalous activities in the ToxCast data set can be identified and flagged, leading to more reliable pathway-based prioritization in the future. Additionally, priorities for AOP development may be identified.

4.4. Application of findings

Ultimately, the current study findings can be used to focus available resources. For example, results can help identify which of the monitored chemicals and chemical mixtures are a priority at each of the sites evaluated (Fig. SI-4, Table SI-8) and which AOPs are most likely to be relevant for the identified chemicals and mixtures at any given site (Fig. SI-6, Tables SI-8 and SI-9). Predicted bioactivities can then be confirmed through laboratory- or field-based investigation or through observations of resident organisms. However, for confirmation studies to be representative of the greatest potential hazard from chemical exposures, testing conditions must reflect the circumstances under which these chemical mixtures are most likely to be present at any given site. The contaminants present, the concentrations, and the exact mixtures vary spatially (urban vs agricultural, commercial vs residential, crops vs pasture), seasonally, and hydrologically (base flow, snowmelt, rainfall) (Baldwin et al., 2016). Well-chosen investigations that reflect the setting for which the greatest potential hazard exists based on these factors would provide substantial value and additional confidence in verifying results. After confirmation of the hypothesized bioactivities, reduction strategies for sources of these chemicals can then be investigated and implemented.

5. Conclusions

When adequate data existed for a comparative analysis, chemicals of greatest concern identified using the ToxCast database with an EAR threshold of 10^{-3} were very similar to those identified using water quality benchmarks with a TQ threshold of 10^{-1}. However, the ability to use the ToxCast database allowed for evaluation of twice as many chemicals in terms of possible biological impacts, as compared to water quality benchmarks. Several PAHs identified as a concern using water quality benchmarks were not similarly flagged using the EAR approach, perhaps due to mechanisms that can enhance their in vivo toxicity but would not be captured using in vitro systems. Several additional chemicals were identified as a potential concern through evaluation of putative bioactivity from chemical mixtures. Results from this work will enable resource managers to examine information pertinent to individual sites and identify individual chemicals and chemical mixtures of potential concern. Hypothesis-driven studies may then be designed to verify results, and if confirmed, mitigation strategies may be formulated and implemented.

Acknowledgements

The authors gratefully acknowledge the many individuals from the U.S. Geological Survey involved in sample collection, processing, and analysis. We thank Anthony L. Schroeder and Keith A. Houck for technical input and Edwin Smith and Elizabeth Murphy for program coordination. Support for this project was provided by the Great Lakes Restoration Initiative through the U.S. Environmental Protection Agency's Great Lakes National Program Office under agreement number DW-014-92453901. The views expressed in this work are those of the authors and do not necessarily reflect the views or policies of the U.S. EPA. Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2019.05.457.

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ATTACHMENT C

Impacts of Chloride and Sulfate Ions on Macroinvertebrate Communities in Ohio Streams (6/2019)

(C-1 to C-21)

water

Article

Assessing the Impacts of Chloride and Sulfate Ions on Macroinvertebrate Communities in Ohio Streams

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Abstract: Salinization of freshwaters is a growing concern, especially in urban catchments. Existing aquatic life criteria for chloride (230 mg/L; a US standard) or total dissolved solids (1500 mg/L; specific to Ohio) do not protect sensitive species, and standards for sulfate have yet to be promulgated on the national level. To help identify water quality thresholds for protection and restoration, species sensitivity distributions were compiled for chloride and sulfate based on field observations of macroinvertebrate communities co-located with water quality samples obtained from rivers and streams throughout Ohio. Additionally, attainment of biological benchmarks for macroinvertebrate communities found in headwater streams were modeled against chloride and sulfate using Bayesian logistic regression. The hazard concentration based on statewide data for chloride was 52 mg/L. The hazard concentration for sulfate based on data from the Western Allegheny Plateau ecoregion was 152 mg/L. The median effect levels from logistic regression for chloride and sulfate varied by ecoregion. Mayfly taxa were disproportionately represented in taxa comprising the lower 5th percentile of the species sensitivity distributions for chloride. However, logistic regression models of individual taxa response (as presence/absence) revealed that some taxa considered sensitive to pollution in general were highly tolerant of chloride. For 166 taxa showing directional response to chloride, 91 decreased and 75 increased. For the 97 individual taxa showing directional responses to sulfate, 81 decreased. Of the 16 taxa showing an increase, 6 are considered tolerant of pollution, 9 facultative and 1 moderately intolerant, the latter being taxa in the dipteran family Tipulidae. The hazard concentrations are useful as protective thresholds for existing high-quality waters. The logistic regression model of attainment can be used to inform management goals conditional on site-specific information.

Keywords: chloride; sulfate; salinization; macroinvertebrates; rivers; Bayesian

Citation: Miltner, R. Assessing the Impacts of Chloride and Sulfate Ions on Macroinvertebrate Communities in Ohio Streams. *Water* **2021**, *13*, 1815. https://doi.org/10.3390/w13131815

Academic Editor: Christopher Nietch

Received: 18 May 2021
Accepted: 28 June 2021
Published: 30 June 2021

1. Introduction

Dissolved ions in freshwater environments are often collectively expressed as total dissolved solids (TDS mg/L) or measured as specific conductance (SC uS/cm, specific to 25 °C). Typical ranges for relatively undisturbed freshwater streams in Ohio are 160–660 mg/L TDS, and 206–960 µS/cm. Because freshwater organisms are internally saltier than the water they live in, they must actively maintain their internal ionic concentrations and ionic compositions through osmoregulation. Most freshwater organisms are facultative with respect to external osmotic pressure as an adaptation to natural seasonal variations in external concentrations, but this requires time to acclimate. However, some macroinvertebrates, notably mayflies [1], are particularly sensitive to increases in dissolved ions, and rapid variations in concentration are more stressful compared to slow concentration changes [2].

Anthropogenic salinization also alters ionic composition [3]. The mode of ion toxicity action to aquatic macroinvertebrates is uncertain [4] but appears less related to direct disruption of osmoregulation [2,5], and more related to the actions of specific ions [6,7]. The apparent toxicity of a specific ion, however, is mediated by the presence of other ions [8–10], resulting in wide ranges in effect levels for a given ion in toxicity tests. Additionally, although disruption of osmoregulation may not be a direct cause of mortality, the energy cost needed to regulate internal ionic balance has been demonstrated to have sublethal effects at concentrations below chronic endpoints [11–13], and consequential at the assemblage level [14].

The natural background concentration and composition of dissolved ions in rivers and streams is largely governed by the geology of the catchment area. In Ohio, streams draining the Western Allegheny Plateau (WAP) tend to average 100–200 SC units lower than streams draining ecoregions in the glaciated portion of the state (Figure 1), and tend to have sulfate as the dominant anion. However, across all ecoregions, agriculture, mining, impervious cover, and wastewater loadings increase the concentrations of dissolved ions. Of these sources, loadings from impervious cover and mining are the most threatening, as those come in either high pulses [15], or steady high doses [16]. Ecoregions comprising the glaciated portion of the state are the Huron-Erie Lake Plain (HELP); the Erie-Ontario Lake Plain (EOLP); the Eastern Corn Belt Plains (ECBP); and the Interior Plateau (IP).

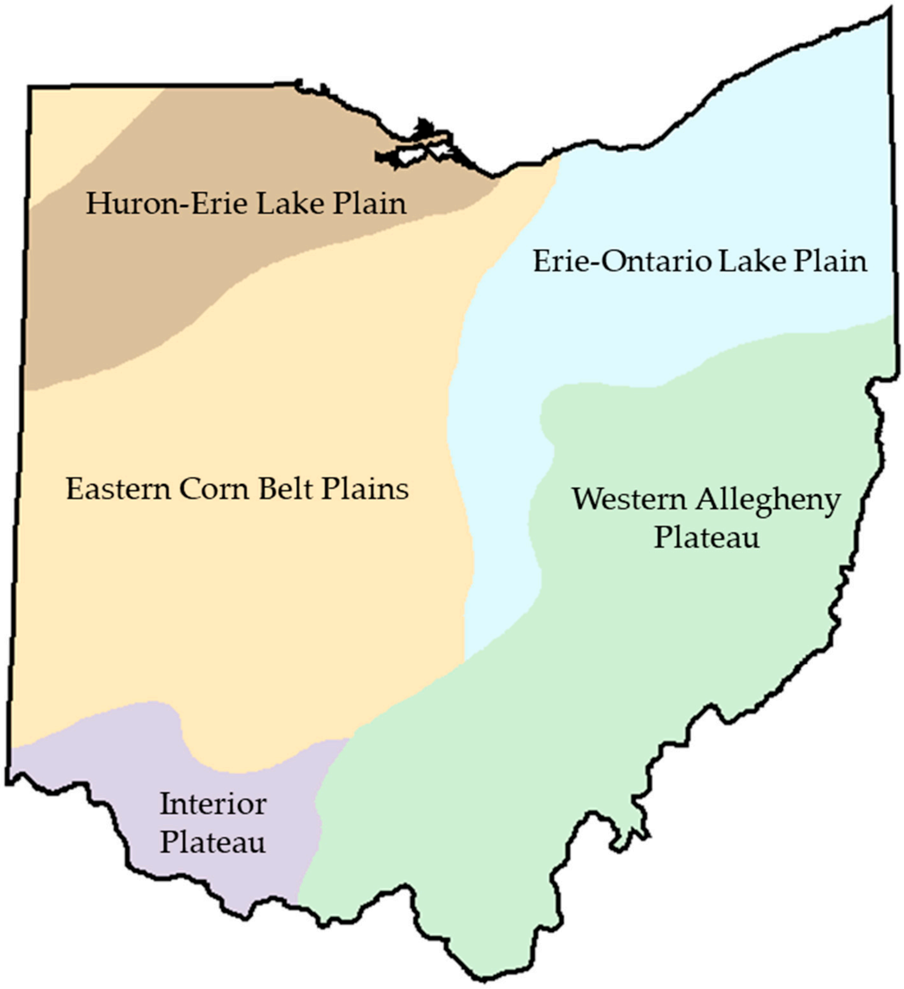

Figure 1. Level III ecoregions of Ohio.

Traditional laboratory toxicology tests focusing on lethality, or mesocosm experiments on the toxicity of TDS or specific ions (i.e., chloride or sulfate), have resulted in conditional endpoints. That is, endpoints are conditioned on various factors such as acclimation, ionic composition, synergistic effects with other toxicants (e.g., metals), and secondary effects. Additionally, many laboratory test organisms show efficient osmoregulation, as evidenced by lethal endpoints near isotonic concentrations. In the net, the results from laboratory and mesocosm studies with lethal endpoints can be interpreted as maximum thresholds not to exceed. However, mesocosm studies that account for sublethal [12] and assemblage-level effects [14] have produced protective endpoints in line with observations from field studies [17], and that are an order of magnitude less than laboratory-derived endpoints.

Species sensitivity distributions (SSDs) can be generated from field collections by observing the occurrence of species or taxa over a specific stressor gradient. For example, a field data set that has matching biological and chemical observations (of chloride, for example) can be arrayed by increasing chloride concentration for each taxon, and the corresponding 95th percentile concentration for each taxon is identified as an extirpation concentration (XC95). Then, the 5th percentile of the XC95s for all taxa is taken as the hazard effect concentration (HC5); a concentration that should be protective for most taxa. This approach can better capture the range and complexity of exposures more typical of a given environmental setting [17]. An example of this method from the data set used in this study is illustrated in Figure 2.

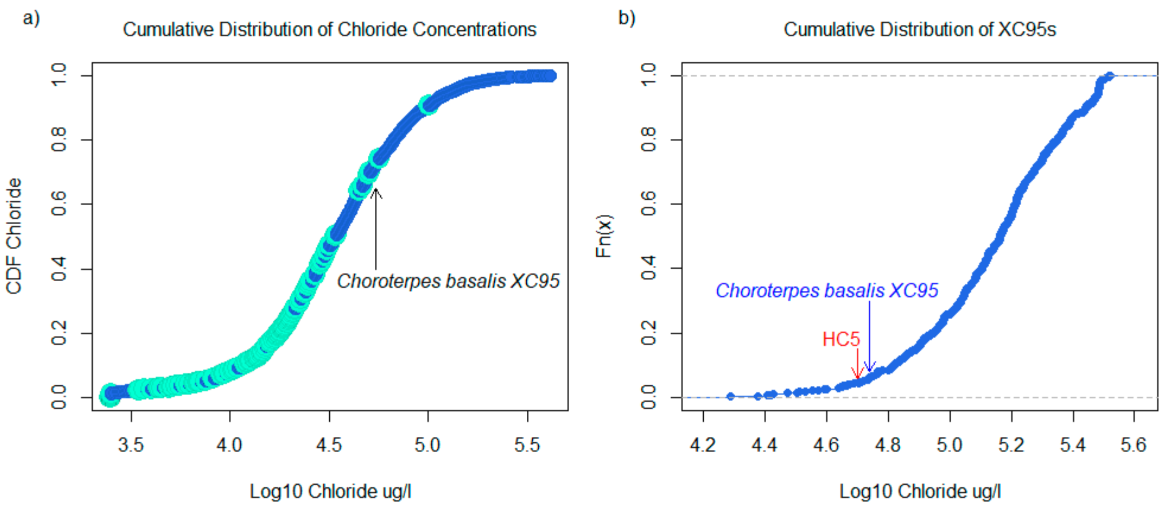

Figure 2. (**a**) The cumulative distribution of chloride concentrations from all sites. Site where the mayfly *Choroterpes basalis* occurs are highlighted in aquamarine and with a larger point size. The 95th percentile chloride concentration specific to *C. basalis* is noted as the XC95. (**b**) Cumulative distributions of XC95s for all taxa, with the XC95 for *C. basalis* noted. The hazard concentration (HC5) is taken as the 5th percentile from the distribution of XC95s.

Similarly, biological indicators or multi-metric indexes (MMI) have long been used in direct gradient analysis to identify environmental thresholds for various pollutants including nutrients [18], sediment [19], and metals [20]. MMIs have the advantage of directly expressing whether a waterbody is meeting a specified status (e.g., the goal use of the Clean Water Act), and environmental thresholds identified using MMIs thusly communicate that aspect of risk. However, the need to "decide in a field situation whether a criterion is too high or too low or just right", as suggested by US EPA [21], remains. That decision should necessarily occur in the context of the environmental setting and protection goals for a specific waterbody, a measurement of one or more communities, and a comparison of pollutant concentrations to both existing water quality criteria (typically laboratory-derived) and other empirically derived thresholds. Essentially, what this implies is that a criterion should be contextualized against the biocondition gradient (BCG).

The BCG concept serves as a useful backdrop for evaluating whether existing water quality criteria are potentially under- or over-protective, where other empirically derived thresholds lie along the gradient, and the potential for determining whether concentrations are contributing to use impairment for a given situation. The BCG describes the expected biological condition relative to a gradient of increasing environmental stress by partitioning the response into five (or more) narrative categories as: (1) natural or native condition, (2) minimal changes in structure and function, (3) evident or moderate changes in structure and minimal change in function, (4) major changes in structure and moderate changes in function, and (5) severe changes in structure and loss of function [22]. These narratives recognize that some changes to assemblage structure can occur without significant loss of ecosystem services.

The strategy used here to identify relevant thresholds for chloride and sulfate used both the SSD approach, and modeling attainment of ecoregion-specific benchmarks for macroinvertebrates using a Bayesian mixed logistic regression model. When logistic regression is applied in this context, the results are typically expressed as the probability of an outcome, in this case attainment of the benchmark, with a credible interval informed by the uncertainty from all covariates included in the model. Plotting the probability of attainment ($\pm$ the credible interval) over the full array of either chloride or sulfate concentrations, and noting where the respective HC5s and existing or proposed laboratory-based water quality criteria fall on the respective continuums, frames how those endpoints might apply to a given situation in the field.

2. Materials and Methods

2.1. Data Set

Observations of water chemistry and macroinvertebrates were obtained from 4973 unique site-year combinations from 2003 through 2019. All sites drained catchments less than 10,000 mi^2. Habitat observations were included at 4634 of those sites. Habitat observations were summarized as Qualitative Habitat Evaluation Index (QHEI) [23,24] scores. The QHEI is a qualitative visual assessment of functional aspects of stream macrohabitat (e.g., substrate quality, amount and type of cover, riparian width, siltation, channel morphology). Water quality samples were collected four to six times during a summer index period of 15 June–15 October. Water quality observations typically include field measurements of dissolved oxygen, temperature, pH and specific conductivity, plus laboratory reported values for various parameters, including metals, anions (e.g., chloride and sulfate), and nutrients (e.g., total phosphorus, nitrate and nitrite nitrogen, ammonia nitrogen, and total Kjeldahl nitrogen). All laboratory reported values are based on standard methods listed in [25,26]. To eliminate highly polluted sites, those with average pH < 6.7 or ammonia nitrogen greater than 0.1 mg/L were excluded from all further analyses. Distributions of selected water chemistry parameters and QHEI scores by ecoregion are shown in Figure 3.

Macroinvertebrate communities at each site were collected following methods described in [27,28] by sampling all available habitat types with net sweeps and by hand, and the resulting information was coded to 1 for presence and 0 for absence. Additionally, if the assemblage at a site met the ecoregion benchmark based on its MMI score [29] it was assigned a score of 1, otherwise 0. The scoring algorithm for the MMI and ecoregion benchmarks are included as Supplemental Material. The MMI has a scale of 0 to 60, and the ecoregional benchmarks reflect differences in expectations driven by prevailing land use and surficial geology. The benchmarks defining a score reflecting Warmwater Habitat (i.e., the base aquatic life use goal) are as follows: HELP—18, IP—23, EOLP and ECPB—26, WAP—34.

2.2. Individual Taxa Responses

Taxa responses over gradients of chloride or sulfate were modeled in two ways. The first using logistic regression, and the second as species sensitivity distributions. For both methods, chemistry parameters were log10 transformed and averaged by site. For the logistic regression models, only taxa with a minimum of 10 observations for either chloride or sulfate were modeled, and only models with positive or negative slopes significant at the $p < 0.01$ level were retained. Note that the p-value in this context is arbitrary but was chosen to yield a reasonable number of responses for subsequent plots. Individual taxa responses were expressed as probabilities at the 5th, 10th, 25th, median, 75th, 90th and 95th quantiles of chloride and sulfate, and plotted as distributions at those respective quantiles. All statistical models were run in R 4.0.3 [30].

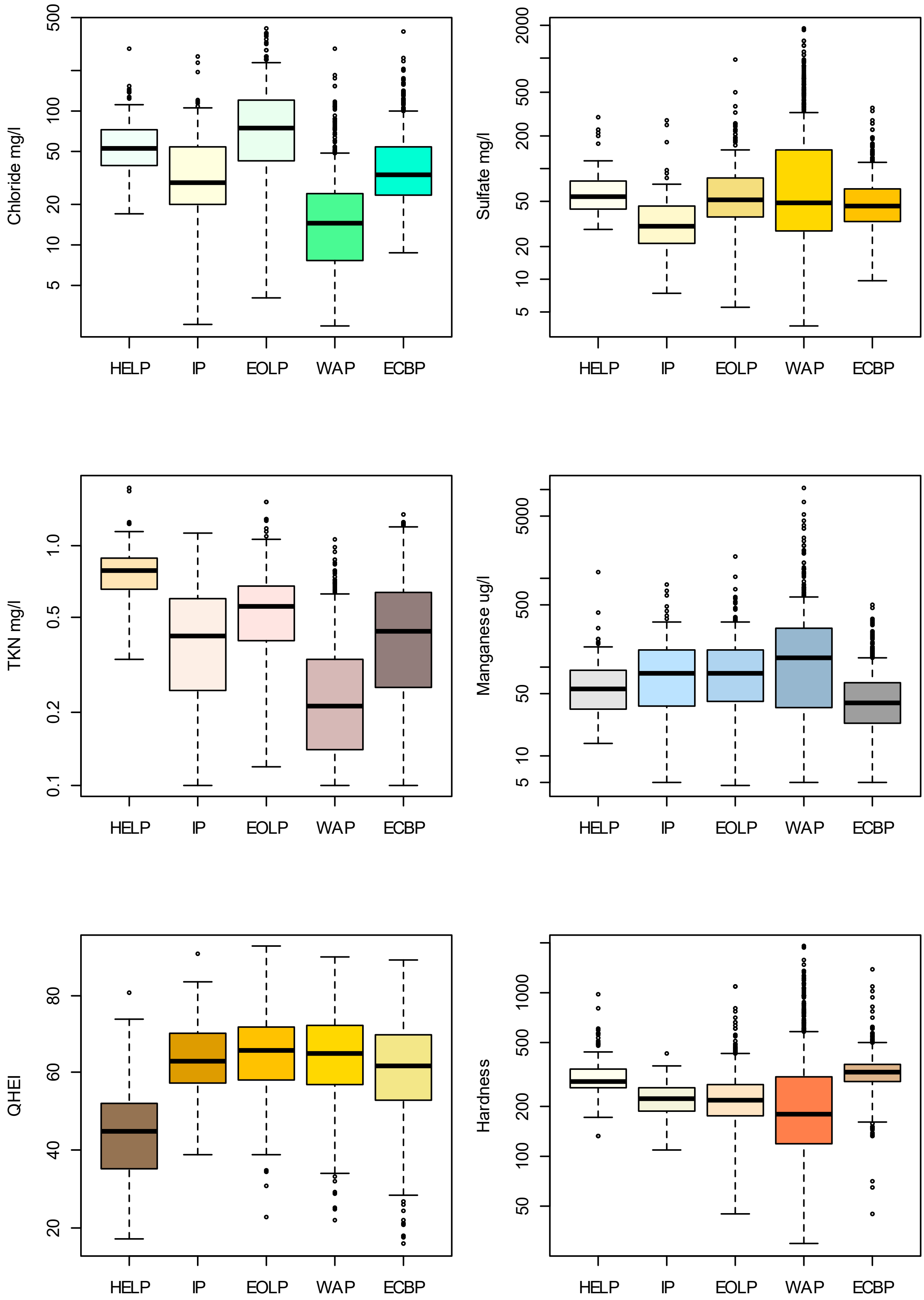

Figure 3. Distributions of water quality parameters and QHEI scores by ecoregion in Ohio. Ecoregion acronyms are as follows: HELP—Huron-Erie Lake Plain; IP—Interior Plateau; EOLP—Erie-Ontario Lake Plain; WAP—Western Allegheny Plateau; ECBP—Eastern Corn Belt Plains. Note that the y-axes for the water chemistry constituents are log10-scaled.

2.3. Species Sensitivity Distributions

SSDs were derived from field data following the methods of Cormier and Suter [31], and were based on taxa with observations for either chloride or sulfate from at least 10 different sites. From a starting pool of 981 taxa, 665 taxa had at least 10 observations, and of those, 578 had companion observations of chloride, and 556 had companion observations for sulfate. For chloride, the statewide data set was used. For sulfate, SSDs were constructed from the statewide data, and data exclusive to the WAP; the latter because sulfate is the dominant anion in the WAP. Log transformed chloride and sulfate values averaged by site were used in the calculations. The respective HC5s were obtained from the collection of XC95s resulting from the SSDs.

2.4. Bayesian Mixed Logistic Regression Model

A Bayesian mixed logistic regression model was developed for headwater streams (<20 mi^2) to assess the influence of chloride and sulfate on observing macroinvertebrate scores meeting their expected benchmark. Headwaters are modeled as they are most directly impacted by road salt and mining legacies. This model included several other variables that represent common stressors. Specifically, total Kjeldahl nitrogen (TKN) to represent organic and nutrient enrichment, manganese, and the QHEI as an indicator of habitat quality. Manganese is associated with mining, low dissolved oxygen, and streams draining soils with low redox potential. Note that because of a high degree of collinearity between chloride and TKN (r = 0.60 across ecoregions), especially in the WAP and ECBP (See Appendix B), the residuals from a regression of TKN on chloride were used in lieu of TKN, and are hereafter referred to as TKNr. This approach isolates a TKN effect divorced from chloride, but not vice versa. Chloride may facilitate enrichment through desorption of sediment bound phosphorus and the release of organic nitrogen from sediments [32], and chloride and recalcitrant organic nitrogen are discharged from wastewater plants. Thus, a singular chloride effect cannot be completely isolated, especially in the WAP and ECBP. Note that only records with complete cases for all covariates were included. In this model, stressors were considered fixed effects and ecoregion a random effect. Thus, the model is stated as:

$$Y_{ir} \sim Bernoulli(\pi_{ir}) \quad (1)$$

$$logit(\pi_{ir}) \sim x'_{ir}\beta + \gamma_r \quad (2)$$

$$\gamma \sim Normal\left(\mu,\ \sigma^2\right) \quad (3)$$

where Y_{ir} is the observation of whether a macroinvertebrate assemblage at a given site within an ecoregion is meeting its benchmark, π_{ir} is a vector of probabilities, x_{ir} is a vector of covariates, β is a vector of coefficients, and γ is one of five ecoregions denoted by the subscript r.

The model was compiled in JAGS 4.3.0 using the rjags 4.0.4 package, run using the autorun.jags function in runjags 2.2.0–2, and assessed using the coda 4.0.4 package. Three Markov chains with a burn-in of 51,000 and 155,842 samples were assessed visually for convergence in trace plots and using the Gelman-Rubin statistic (at $\leq$1.01 to indicate convergence). The Gelman-Rubin statistic essentially tests whether regression parameters estimated from the separate chains follow similar distributions. A sample length of 155,842 suggested by the Raftery and Lewis [33] diagnostic resulted in an effective sample size of at least 450 for the estimated model coefficients. The Raftery and Lewis diagnostic is used to control the error surrounding regression parameter estimates. The chains were initialized with a range of starting values suggested by standard logistic regression. The model code is supplied as Appendix A.

Inferences from the model were gathered by sampling the posterior distribution using the BayesPostEst 4.0.5 package. Predicted probabilities of meeting the macroinvertebrate benchmarks for MMI scores within an ecoregion as a function of either chloride or sulfate were obtained based on ecoregion median values for other predictors in the model. Similarly, the relative influence of each parameter in the model was assessed by compiling first differences [34]. Here, first differences estimate the change in probability of meeting the macroinvertebrate benchmark when a given predictor moves across the middle quartile of its within-ecoregion range while holding other predictors at their medians. Model fit for each ecoregion was assessed by obtaining the area under the Receiver Operating Characteristic (ROC) curve, and the Hosmer-Lemeshow [35] $\hat{C}$ statistic.

3. Results

3.1. Taxa Sensitivities

Distributions of individual taxa responses modeled by logistic regression are plotted for selected quantiles of chloride and sulfate in Figure 4. The distributions are stratified by whether taxa showed a decrease along the gradient, or an increase. Taxa sensitive to chloride appear to decrease across the range of chloride concentrations. For sulfate-sensitive taxa, a decreasing trend across the gradient is not apparent until concentrations exceed the 50th percentile. The HC5 for chloride is 52 mg/L and that corresponds to the 68th percentile concentration in the statewide data. The CCC of 230 mg/L published by US EPA [36] corresponds to the 99th percentile concentration. Similarly, the HC5 concentration for sulfate from the statewide set is 90 mg/L and corresponds to the 76th percentile. When calculated from data from the WAP, the HC5 concentration is 152 mg/L, and that corresponds to the 72nd percentile concentration for the WAP. The biggest apparent disparity between increasing and decreasing taxa appears over low chloride concentrations, and likely reflects the sensitivity of certain mayfly taxa to ions. Mayfly taxa had a higher-than-expected frequency in the lower 5th percentile of the XC95 for chloride relative to other taxa groups (χ^2 = 19.093, df = 6, *p*-value = 0.004009). Of the 29 taxa comprising the lower 5th percentile, 10 were mayfly (3.26 expected; see Appendix C for observed and expected frequencies by major taxa group). Taxa groups in the lower 5th percentile of the XC95s from the SSDs constructed for the statewide sulfate data were marginally similar (χ^2 = 11.5240, df = 6, *p*-value = 0.07348), but those for the WAP also had a higher than expected incidence of mayfly taxa in the lower 5th percentile (χ^2 = 20.6040, df = 6, *p*-value = 0.00216; 2.5 taxa expected, 7 taxa observed).

3.2. Bayesian Logistic Regression Model

Estimates of posterior means, the Gelman-Rubin convergence diagnostics and effective sample sizes are shown in Table 1. Model fits were generally good, as suggested by the Hosmer-Lameshow test (Table 2), except for the WAP ecoregion, where a subsequent investigation revealed a nonlinear relationship between macroinvertebrate index scores and sulfate. At relatively low concentrations of sulfate (i.e., for the WAP < ~50 mg/L), index scores show a slight increase over increasing sulfate concentrations. Sulfate and hardness are strongly linearly related in the WAP (r^2 = 0.74), and the tendency for increasing index scores may reflect an ameliorative effect from hardness [7,37,38] on ion toxicity in general, at least over a range of relatively low hardness concentrations (<160 mg/L). Nevertheless, the AUC score for the WAP indicated good classification accuracy. Area Under the Curve (AUC; i.e., the area under ROC curve) scores for the IP, EOLP and ECBP indicated fair accuracy, while the score for the HELP suggested poor accuracy. Because the water chemistry parameters were measured over roughly similar scales in log space, their coefficients can be compared directly to infer relative effect size. This suggests that organic and nutrient enrichment isolated from the additional effect of chloride, as represented by TKNr, is relatively more consequential in general than the other water quality measures, and comports with findings for Ohio [39]. However, first differences (Figure 5) provide a more direct comparison of effect sizes within ecoregion, and suggest that for the IP and EOLP, where TKN and chloride are not strongly correlated, the effect size for chloride is appreciable and similar to the WAP and ECBP. Sulfate and manganese have the most pronounced effect in the WAP. Note that manganese and sulfate concentrations in the WAP are not strongly correlated (r = 0.13). Because first differences are computed over the interquartile range (IQR) for a given variable while holding other variables at their medians, the magnitude of the IQR will partially dictate the size of the first difference. Plotting the first differences on scaled IQRs for a given parameter helps to place the differences into context. For example, the first differences for TKNr in the ECBP and IP were similar, but the IQR in the ECBP was narrower, suggesting a relatively larger effect per unit change in the ECBP. Similarly, the WAP should be the most sensitive of the ecoregions to a change in chloride concentration. The conspicuously muted response to chloride and organic enrichment in the HELP shown in Figure 5 reflects both the pervasively degraded habitat, and the narrow interquartile (IQR) range of TKN within the ecoregion (Figure 3). Axiomatically, a change in habitat quality in the HELP is likely to have a bigger effect than a change in any one water quality parameter.

Table 1. Parameter estimates and diagnostic statistics for the Bayesian logistic regression model.

	Posterior Means and (SD) [95% Credible Interval]	**Gelman-Rubin Point and Upper C.I.**	**Effective Size**
Intercept	0.00 (0.010) [−0.19; 0.20]	1.00–1.00	29,242
Chloride	−2.00 * (0.189) [−2.37; −1.63]	1.01–1.03	1034
QHEI	0.04 * (0.005) [0.03; 0.05]	1.00–1.00	6816
rTKN	−3.15 * (0.315) [−3.77; −2.53]	1.00–1.00	10,256
Mn	−0.85 * (0.141) [−1.13; −0.59]	1.00–1.00	1423
SO4	−1.09 * (0.159) [−1.40; −0.78]	1.00–1.01	1354
HELP	16.83 * (1.375) [14.19; 19.62]	1.01–1.01	484
IP	16.70 * (1.364) [14.09; 19.47]	1.01–1.01	465
EOLP	16.53 * (1.397) [13.84; 19.37]	1.01–1.01	451
WAP	15.45 * (1.342) [12.89; 18.18]	1.01–1.01	465
ECBP	15.44 * (1.322) [12.90; 18.13]	1.01–1.01	463

* 0 outside 95% credible interval.

Table 2. Model fit statistics.

	HL χ^2	***P***	**AUC**
HELP	5.31	0.72	61.8
IP	9.49	0.30	78.3
EOLP	3.72	0.88	76.1
WAP	18.92	0.02	83.6
ECBP	6.91	0.55	70.1
Over-all	9.89	0.27	

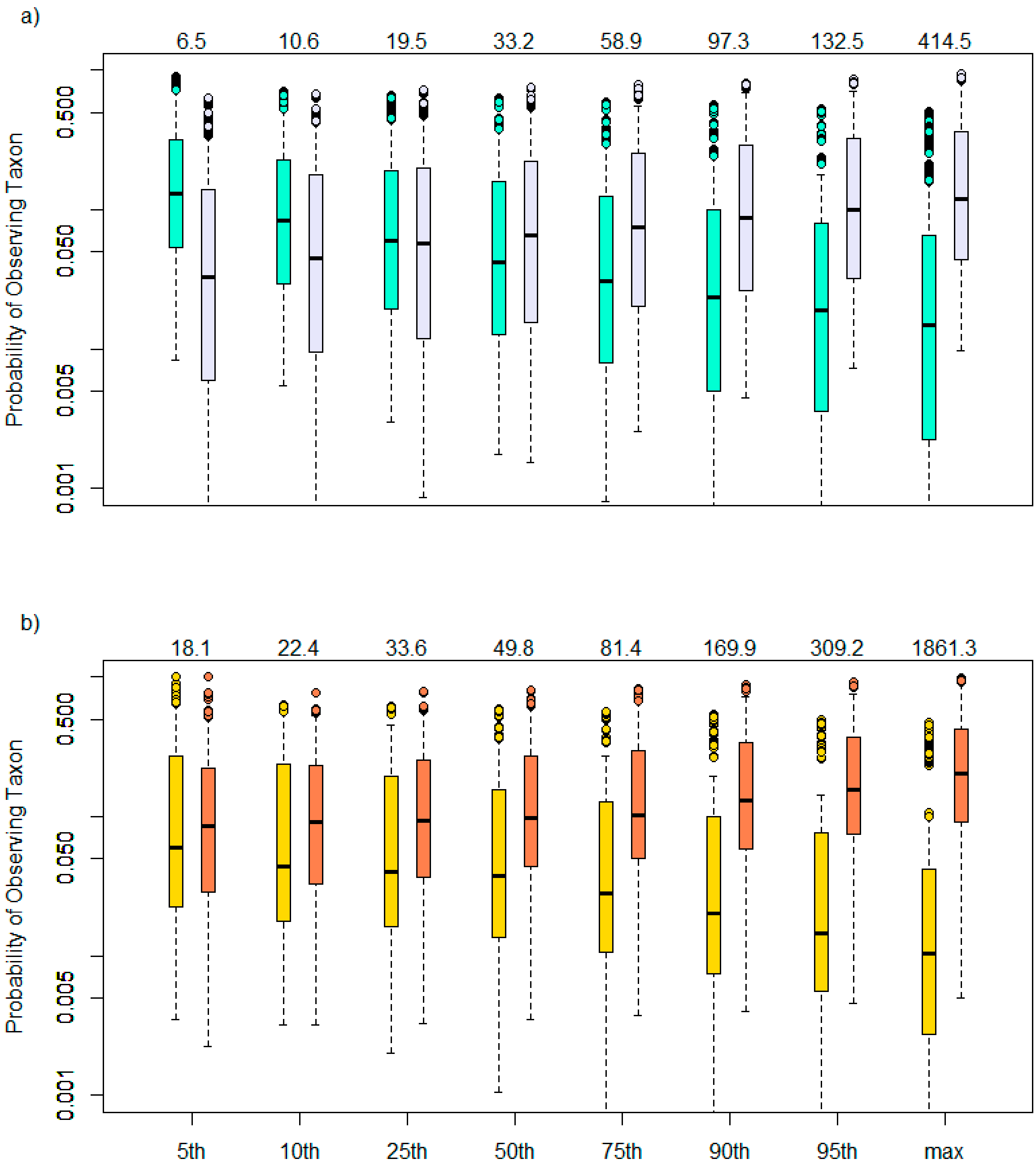

Figure 4. Distributions of taxa observation probabilities (y-axes) from logistic regression plotted by quantile intervals (e.g., <5th, 5th–10th, etc.); concentrations for the stated quantiles are arrayed across the top margins. (**a**) Probabilities for chloride where taxa having decreasing slopes are shown in light green, and those with increasing slopes in light purple. For reference, the HC5 from the species sensitivity distribution constructed from statewide data is 52 mg/L. The chronic continuous concentration (CCC) published by US EPA [36] is 230 mg/L. (**b**) Probabilities for sulfate where taxa having decreasing slopes are shown in yellow and those with increasing slopes are shown in orange. For reference, the HC5 from the species sensitivity distribution constructed from the WAP subset is 152 mg/L, and the HC5 from the statewide SSD was 94 mg/L. The Criterion Maximum Concentration (CMC) from the Illinois toxicity model [37] would default in many cases to 500 mg/L for Western Allegheny Plateau headwaters because of high levels of hardness associated with mine drainage. Note that acute to chronic ratios are typically ~10 [40].

posed by collinearity between sulfate and chloride (see Appendix B for correlation
ecoregion). Collectively, these results suggest that sulfate concentrations approaching
mg/L are consequential, and concentrations exceeding 300 mg/L are generally incom
ble with macroinvertebrate communities meeting their benchmarks.

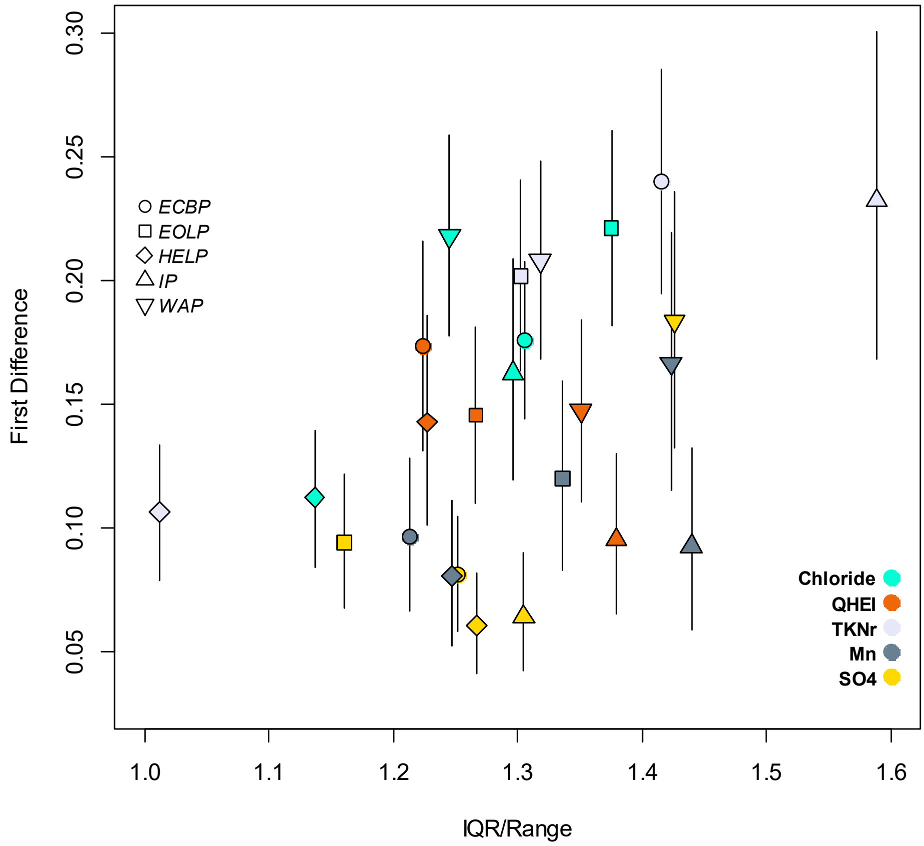

Figure 5. First differences (+/− 95% credible interval) as a function of scaled interquartile ranges. Note that absolute values for the first differences are plotted on the y-axis.

Figures 6 and 7, respectively, show the predicted probabilities of meeting ecoregion-specific macroinvertebrate MMI benchmarks as a function of chloride and sulfate concentrations when the other covariates included in the model are held at their ecoregion median values. Median effect levels (i.e., the point where the probability of meeting the ecoregion benchmark is 0.5) for all the ecoregions are less than the chloride CCC of 230 mg/L. Only in the IP does the upper credible interval suggest a better than even chance of meeting the benchmark if the CCC is exceeded, but the CI at that point encompasses a wide range. Relative to the HC5 (52 mg/L), the median effect level was lower in the HELP, WAP and ECBP, and higher in the IP and EOLP. As was evident with first differences, the lower effect level in the HELP reflects the magnitude of poor habitat quality. For the WAP and ECBP, the lower effect levels are likely due, in part, to the added contribution of TKN, and in the WAP, higher levels of sulfate. In the IP and EOLP where the effect of chloride is less entangled with TKN, the median effect levels may approximate an operational benchmark in lieu of the CCC.

For sulfate, median effect levels in the EOLP, WAP and ECBP approximate the respective HC5s, especially in light of where the CIs intersect that point on the graph. The comparatively high median effect level in the IP reflects relatively low chloride concentrations. Similarly, lower median chloride and TKN concentrations in the WAP, relative to the other ecoregions, also results in a higher median effect level. If the median value for chloride from the EOLP is substituted in the IP, then the median effect level for the IP becomes 70 mg/L, as might be expected given fixed effects and similar intercepts between the two ecoregions. The wide credible intervals for the IP likely reflect uncertainty imposed by collinearity between sulfate and chloride (see Appendix B for correlations by ecoregion). Collectively, these results suggest that sulfate concentrations approaching 100 mg/L are consequential, and concentrations exceeding 300 mg/L are generally incompatible with macroinvertebrate communities meeting their benchmarks.

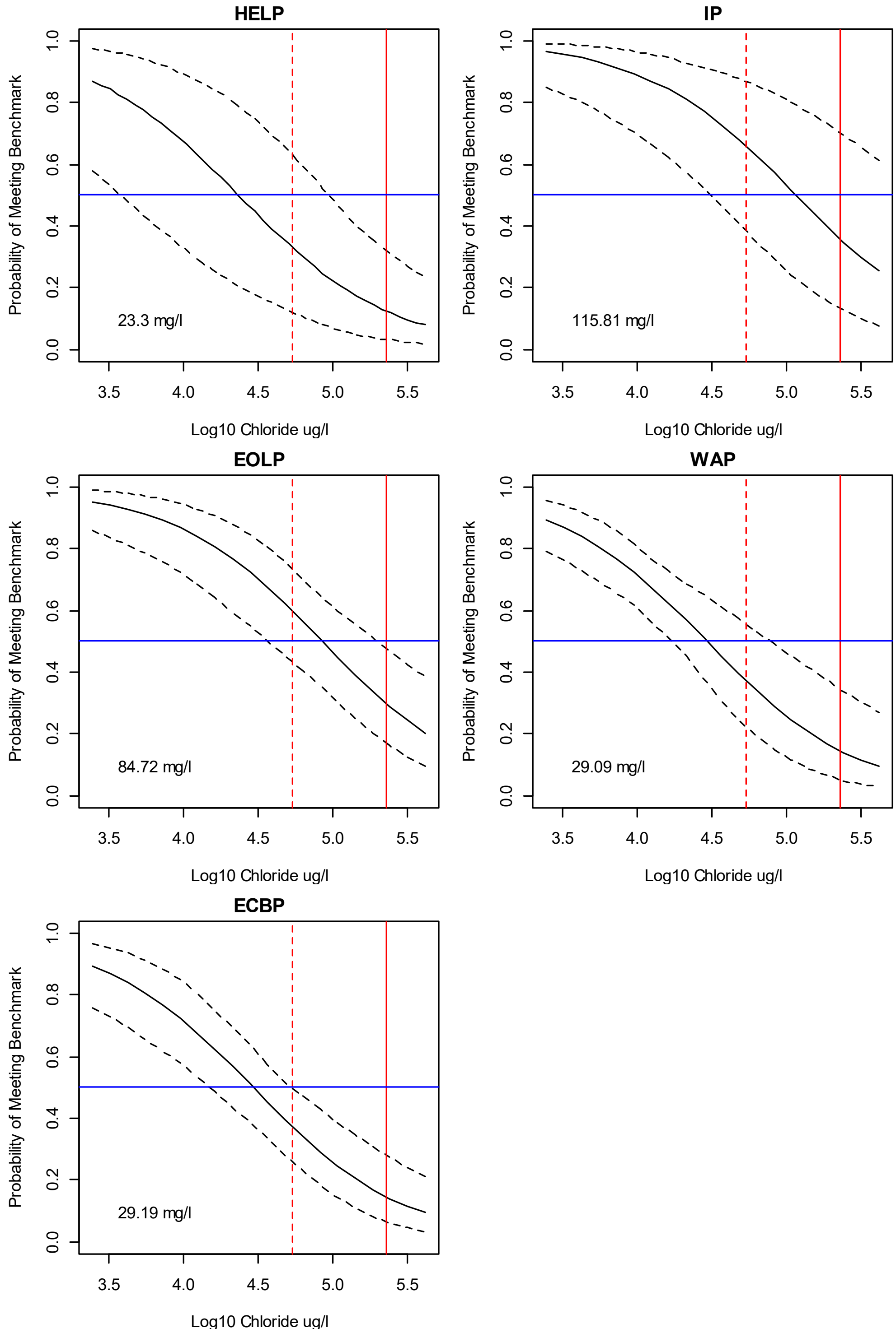

Figure 6. Predicted probabilities and 95% credible intervals of macroinvertebrate communities meeting their ecoregion benchmark as function of chloride concentrations when other model parameters are held at their ecoregion medians. The vertical dashed red line is the HC5 (52 mg/L), the solid vertical red line is the CCC (230 mg/L). The blue horizontal line is drawn at p = 0.5 for reference. Respective median effect levels in standard units are inset as text in the lower left of each plot.

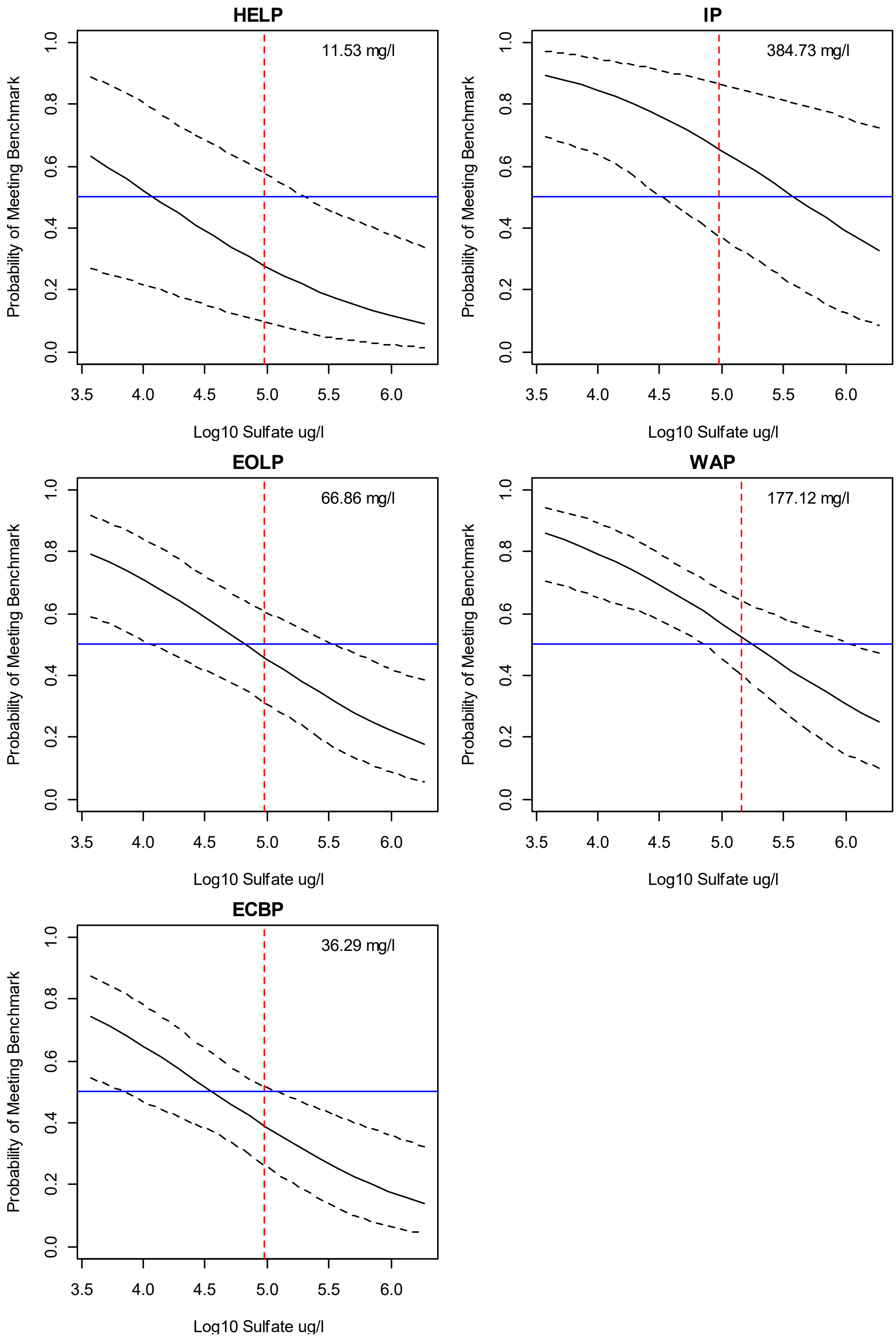

Figure 7. Predicted probabilities and 95% credible intervals of macroinvertebrate communities meeting their ecoregion benchmark as function of sulfate concentrations when other model parameters are held at their ecoregion medians. The vertical dashed red line is the HC5, equating to 90 mg/L for the four ecoregions exclusive of the WAP, and 152 mg/L for the WAP. The blue horizontal line is drawn at p = 0.5 for reference. Respective median effect levels in standard units are inset as text in the upper right of each plot.

4. Discussion

The individual taxa responses from the logistic regression models, when considered collectively and, respectively, against chloride and sulfate (Figure 4), broadly reflect expectations suggested by the present state of knowledge. Mayfly taxa have a relatively high frequency in the lower 5th percentile of the XC95s for chloride and sulfate, but some mayfly taxa are tolerant of chloride, as evidenced by increasing slopes in the logistic regression models (Figure 8a). This generally comports with findings from other studies e.g., [3,4,41,42], and the observation that some mayfly taxa can acclimate to increased salinity by reducing the number of chloride cells on tracheal gills in response to increased salinity [43]. Interestingly, of the 166 taxa showing a directional response to chloride, 75 (45%) have an increasing response, and of those, 21 taxa are considered sensitive [28], at least to other types of pollution. This suggests that turnover of taxa can maintain richness components of MMI scores consistent with attaining benchmarks. However, Drover et al. [44] cautioned that species turnover could impact functional aspects of an assemblage in a negative way that is not capture by richness metrics, implying that when evaluating biological impacts associated with chloride, functional differences associated with species turnover should be considered. Moreover, in instances where measured chloride concentrations are low, the presence of chloride tolerant taxa may signal a history of exposer [45].

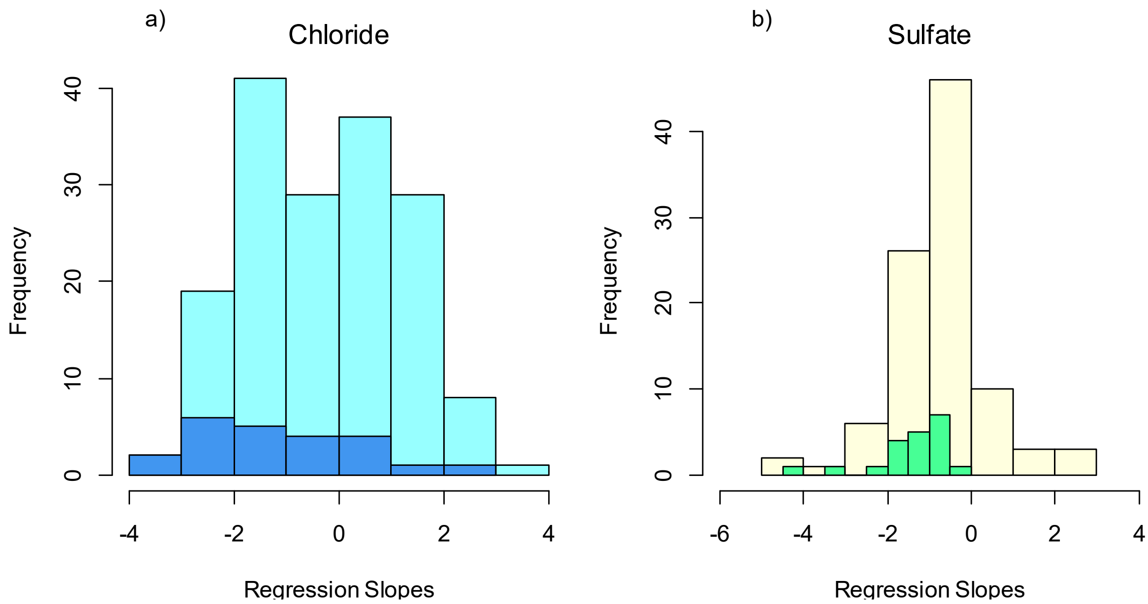

Figure 8. Histograms of slopes (x-axis) for individual taxa reported from logistic regression for (**a**) chloride and (**b**) sulfate. The subset of slopes for mayfly taxa are shown as inset overlaying histograms.

Most of the taxa showing a directional response against sulfate had decreasing responses, and of those with increasing responses, none was from mayfly taxa (Figure 8b). This suggests a lower overall ability for macroinvertebrates to acclimate to higher concentrations of sulfates, and the broader decreasing response across taxa may reflect a more direct toxic response to the associated cation. Magnesium is more toxic than either sulfate or chloride [6,7], and has a more direct effect due to the activity of the cation [46].

Application of Results to Management

The distributions shown in Figure 4 highlight potential shifts in assemblage structure that occur over each gradient. With respect to chloride, the HC5 (52 mg/L) from the statewide data coincides with a point on the gradient (i.e., the 68th percentile) where assemblage structure is materially altered, as inferred by the increasing disparity between chloride-tolerant and chloride-sensitive taxa past that point. In terms of the BCG, this might coincide with moderate changes in structure and minimal to moderate changes in function. The HC5 for sulfate from the WAP SSDs (152 mg/L) is similarly situated in terms of the percentile concentration, but a relative increase in sulfate-tolerant taxa is not as pronounced as for chloride. The probabilities plotted in Figures 6 and 7 show where changes in function are increasingly likely to have a material consequence, specifically, loss of the beneficial use when macroinvertebrates communities fail to meet their benchmarks. The CCC of 230 mg/L for chloride is clearly past the point on the BCG where communities are likely to meet their benchmarks. Based on hardness values typical for WAP headwaters affected by mine drainage, the CMC value for sulfate from the Illinois laboratory model defaults to 500 mg/L in most cases, and represents a point on the BCG where the risk of losing function is high, given the probability of meeting the WWH benchmark at that point is ~0.38 (when holding all other parameters at their respective medians). An acute to chronic ratio of 10 [40] gives a CCC of 50 mg/L, but that appears overly protective. A study by Wang et al. [47] found acute to chronic ratios of ~3.33 for three invertebrates tested against sodium sulfate. Cast in that light, a CCC of ~160 mg/L appears to match observations here.

In terms of managing condition status, the HC5s best represent either protective caps for existing high-quality waters, or restoration targets for presently impaired waters in some cases, but not as criteria to be applied independently. Coming from the other direction, and with respect to chloride, waters with average chloride concentrations that exceed the CCC of 230 mg/L (over a suitable averaging period) should be considered impaired. For waters with biological impairment linked to chloride, but having measured concentrations less than the CCC, a site or waterbody-specific restoration target should be developed and informed by the Bayesian logistic regression model.

Management decisions can be informed by the Bayesian model by examining first differences and effects over observed cases [48]. Figure 9 plots expected effects associated with four levels of chloride given values for the four other covariates within three slices of the observed data. The examples are drawn from the Eastern Corn Belt Plains (ECBP) and Erie-Ontario Lake Plains (EOLP). The levels for chloride are given by sequencing the ecoregion-specific range from the 25th to 90th percentile, and the three data groups correspond to the lower, middle and upper 25th quantiles of TKN for the respective ecoregions (n.b., the TKNr residuals associated with those strata are used in the calculation). In the ECBP, a reduction in TKN from the upper quartile to middle quartile appears to have a substantial effect, whereas in the EOLP, the effect is less dramatic. Because these effects are modeled on observed data, distributions of other covariates in the data slices will influence the outcome. In the ECBP, streams with high TKN also tend to have poor habitat (mean QHEI = 53 $\pm$ 15) compared to streams with TKN in the middle quartile (QHEI = 63 $\pm$ 11). In the EOLP, the distributions of QHEI scores are similar between the upper and middle quartile slices (64 $\pm$ 11 and 66 $\pm$ 11, respectively). Both examples illustrate the need to consider the influence of multiple stessors in restoration efforts, especially in the context of pollution from diffuse sources. In the case of the ECBP, restoration efforts that address both nutrient enrichment and habitat quality are likely to be more successful than a singular effort. Failure to observe multiple stressors has resulted in limited success in restoration efforts [49,50], and it is increasingly recognized that the biological response to habitat restoration is likely to be tempered by watershed-scale characteristics [51–54]. In the EOLP, where salinization from deicers is a significant problem, the success of best management practices (BMPs) directed at reducing chlorides, when measured by aquatic life, will depend on levels of other stressors. In other words, the need for BMPs in case of deicers is singular, but assessment of efficacy is necessarily multivariate, especially in light of the potential range of water quality and biological effects associated with chloride enrichment [32,55]. Similar plots for the HELP and IP ecoregions are provided in Appendix D.

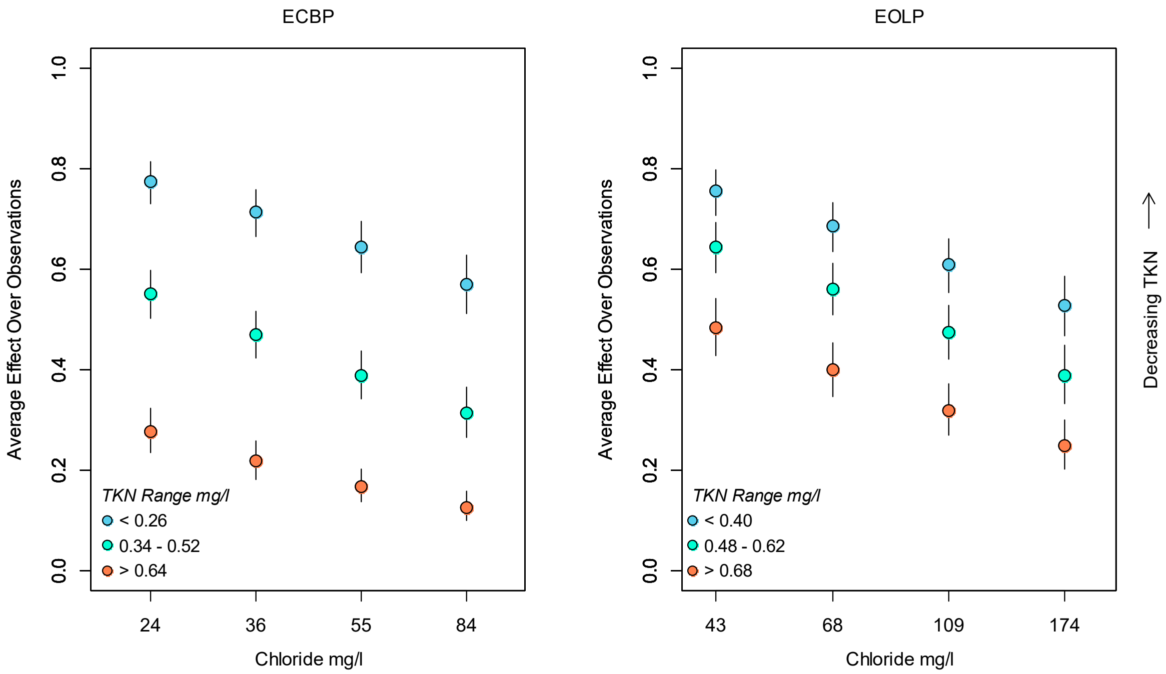

Figure 9. Probabilities of observing macroinvertebrate communities meeting their ecoregion benchmark given relevant levels of chloride and observations of covariates within three data slices defined by quantile levels of TKN (lower, middle and upper 25th quantiles). The chloride levels were obtained by sequencing the range from the 25th to 90th percentiles (in log space). Examples are for the Eastern Corn Belt Plains (ECBP) and Erie-Ontario Lake Plain (EOLP).

In the Wester Allegheny Plateau (WAP), where ions from active and legacy mining are a concern, the model suggests that management and reclamation directed toward reducing manganese concentrations are likely to have a significant effect across a wide range of sulfate levels, especially if manganese concentrations can be reduced to less than ~35 ug/L (Figure 10). Passive treatment systems for manganese are capable of high removal efficiencies and are relatively inexpensive [56–58], whereas removing sulfate is likely impractical. The results for the WAP also illustrate the potential difficulty in defining and administering parameter-specific water quality criteria when the effects of pollutants are additive. Laboratory studies that identify the mode of action and hazard concentrations are necessary to establish a basis for causation for a given parameter, but should be complemented by effect levels and models derived from field studies. Both should inform causal determinations, listing decisions, and restoration targets for impaired waters.

5. Conclusions

Water quality standards for the protection of aquatic life are intended to maintain attainment of the beneficial use. Compliance with a given standard has traditionally been judged by comparing water quality observations against a numeric endpoint derived from laboratory tests. However, the fact that changes in biological communities are detectable across entire pollution gradients, as was observed here for chloride and sulfate, calls for a more holistic framework for managing pollution. This framework should include protective endpoints derived from field and laboratory studies; the former to identify thresholds necessary to maintain existing and relatively unperturbed conditions, and the latter as backstops against overt pollution. The results here clearly demonstrate that the existing CCC for chloride (230 mg/L) is under-protective as a backstop, and that HC5s for chloride (52 mg/L) and sulfate (152 mg/L for the WAP and 90 mg/L for all other ecoregions) are needed to maintain a relatively unperturbed condition. Biological communities should be evaluated against position on the pollution gradient, and where impairment is observed, restoration strategies and targets should be informed by all relevant chemical and physical information.

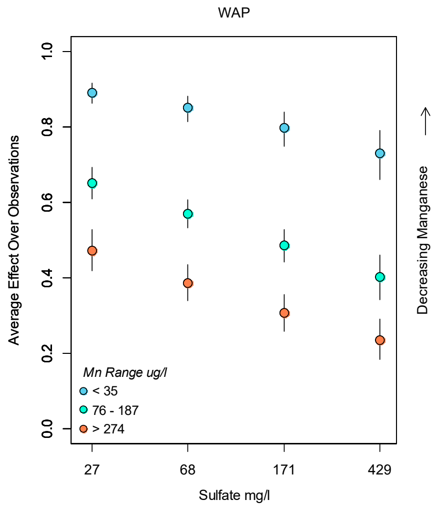

Figure 10. Probabilities of observing macroinvertebrate communities meeting the ecoregion benchmark for the Western Allegheny Plateau given four levels of sulfate and observations of covariates within three data slices defined by quantile levels of manganese (lower, middle and upper 25th quantiles). The sulfate levels were obtained by sequencing the range from the 25th to 90th percentiles (in log space).

Supplementary Materials: The following are available at https://www.mdpi.com/article/10.3390/w13131815/s1, A list of taxa and extirpation concentrations (XC95) for chloride and sulfate, and development and scoring methods for a macroinvertebrate biotic index based on presence/absence data.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to agency policy.

Acknowledgments: The author thanks the dedicated field staff at Ohio EPA and two anonymous reviewers who provided helpful comments on earlier versions of this manuscript.

Conflicts of Interest: The author declare no conflict of interest.

Appendix A. Model Code for Bayesian Logistic Regression

```
sink("lrmod.txt")
cat("model
{
for(i in 1:N){
ac[i]~dbern(p[i])
logit(p[i])<-alpha + beta1 * cl[i] + beta2 * qhei[i] + beta3 * tkn[i] + beta4 * mn[i] + beta5 *
so4[i] + u[REG[i]]
}
alpha ~ dnorm(0, 100)
sigma_a ~ dunif(0, 100)
tau_a <- 1 / (sigma_a * sigma_a)
for(j in 1:M){
u[j]~dnorm(0,tau_a)
}
beta1 ~ dnorm(0.0,0.00001)
beta2 ~ dnorm(0.0,0.00001)
beta3 ~ dnorm(0.0,0.00001)
```

```
beta4 ~ dnorm(0.0,0.00001)
beta5 ~ dnorm(0.0,0.00001)
}
", fill = TRUE)
sink()
line_init<-list(
list(alpha = 40,beta1 = −2,beta2 = 0.05,beta3 = −2.8,beta4 = −0.5,beta5 = −1.1),
list(alpha = 1,beta1 = −0.1,beta2 = 1,beta3 = −10,beta4 = −10,beta5 = −10),
list(alpha = 0,beta1 = −0.5,beta2 = 0.01,beta3 = −1,beta4 = −1,beta5 = −0.5)
)
modout<-autorun.jags(model = "lrmod.txt",monitor = c("alpha", "beta1","beta2","beta3",
"beta4","beta5","u"),
data = hwdata, n.chains = 3, inits = line_init, startsample = 51,000)
```

Appendix B. Correlations Between Water Chemistry Measures and Habitat Quality Scores Stratified by Ecoregion. Cl—Chloride; TKN—Total KJELDAHL Nitrogen; Mn—Manganese; SO4—Sulfate; Hard—Hardness

Huron-Erie Lake Plain

	Cl	TKN	Mn	SO4	Hard	QHEI
Cl	1.00	0.04	−0.09	0.23	0.21	0.10
TKN	0.04	1.00	0.28	−0.14	0.04	−0.06
Mn	−0.09	0.28	1.00	−0.05	0.21	0.10
SO4	0.23	−0.14	−0.05	1.00	0.51	0.12
Hard	0.21	0.04	0.21	0.51	1.00	0.09
QHEI	0.10	−0.06	0.10	0.12	0.09	1.00

Interior Plateau

	Cl	TKN	Mn	SO4	Hard	QHEI
Cl	1.00	0.17	−0.24	0.65	0.31	−0.16
TKN	0.17	1.00	0.39	−0.07	−0.53	−0.24
Mn	−0.24	0.39	1.00	−0.42	−0.32	−0.22
SO4	0.65	−0.07	−0.42	1.00	0.54	−0.07
Hard	0.31	−0.53	−0.32	0.54	1.00	0.12
QHEI	−0.16	−0.24	−0.22	−0.07	0.12	1.00

Erie-Ontario Lake Plain

	Cl	TKN	Mn	SO4	Hard	QHEI
Cl	1.00	0.27	−0.48	0.29	0.21	0.06
TKN	0.27	1.00	0.07	0.02	−0.13	−0.11
Mn	−0.48	0.07	1.00	−0.10	0.02	−0.23
SO4	0.29	0.02	−0.10	1.00	0.83	−0.09
Hard	0.21	−0.13	0.02	0.83	1.00	−0.08
QHEI	0.06	−0.11	−0.23	−0.09	−0.08	1.00

Western Allegheny Plateau

	Cl	TKN	Mn	SO4	Hard	QHEI
Cl	1.00	0.42	0.10	0.24	0.32	−0.05
TKN	0.42	1.00	0.46	0.14	0.23	−0.28
Mn	0.10	0.46	1.00	0.13	0.16	−0.24
SO4	0.24	0.14	0.13	1.00	0.85	0.01
Hard	0.32	0.23	0.16	0.85	1.00	0.01
QHEI	−0.05	−0.28	−0.24	0.01	0.01	1.00

Eastern Corn Belt Plains

Cl TKN Mn SO4 Hard QHEI
Cl 1.00 0.51 0.14 0.26 −0.10 −0.10
TKN 0.51 1.00 0.43 0.21 −0.26 −0.31
Mn 0.14 0.43 1.00 0.29 −0.04 −0.36
SO4 0.26 0.21 0.29 1.00 0.30 −0.25
Hard −0.10 −0.26 −0.04 0.30 1.00 0.00
QHEI −0.10 −0.31 −0.36 −0.25 0.00 1.00

Appendix C. Frequencies of Taxa Groups Occurring at Less Than or Greater Than, the Hazard Concentration (HC5) for Chloride and Sulfate. Taxa Groups Are: C—Caddisfly, D—Dipterans, M—Mayfly, N—Non-Insects, O—Other, S—Stonefly, T—Midges in the Tribe Tanytarsini. Expected Frequencies are Based on the Formula for χ-Square

	Observed		Expected	
Taxa Group	≤HC5	>HC5	≤HC5	>HC%
Chloride				
C	3	67	3.5121107	66.48789
D	7	176	9.1816609	173.8183
M	10	55	3.2612457	61.73875
N	3	108	5.5692042	105.4308
O	3	105	5.4186851	102.5813
S	2	16	0.9031142	17.09689
T	1	22	1.1539792	21.84602
Sulfate				
C	1	69	3.5251799	66.47482
D	11	163	8.7625899	165.2374
M	5	58	3.1726619	59.82734
N	9	94	5.1870504	97.81295
O	1	105	5.3381295	100.6619
S	1	16	0.8561151	16.14388
T	0	23	1.1582734	21.84173

Appendix D

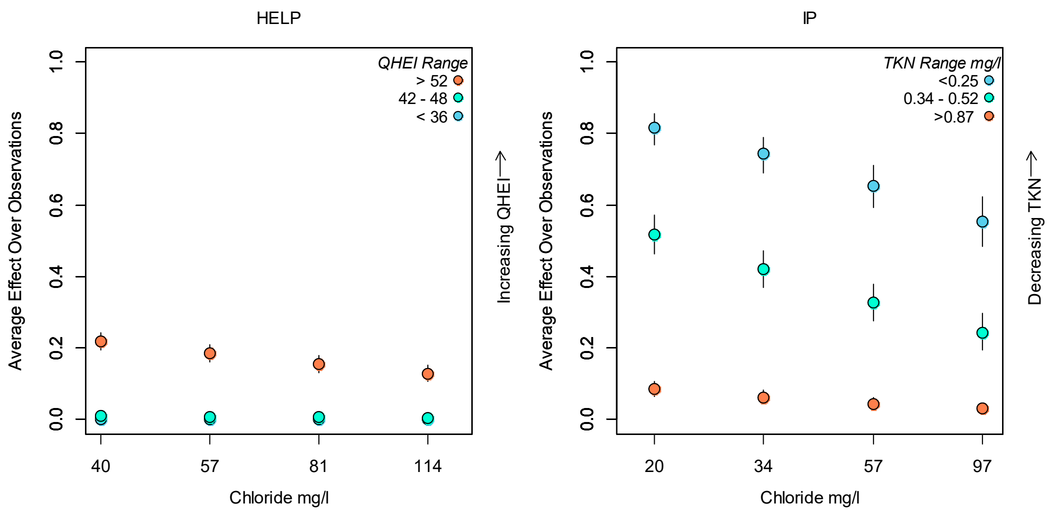

Figure A1. Probabilities of observing macroinvertebrate communities meeting the ecoregion benchmarks for the Huron-Erie Lake Plain (HELP, left panel) and the Interior Plateau (IP, right panel) given four levels of chloride and observations of covariates within three data slices defined by quantile levels of the QHEI for the HELP and TKN for the IP (lower, middle and upper 25th quantiles). The respective chloride levels were obtained by sequencing the ecoregion range from the 25th to 90th percentiles (in log space). The results for the HELP illustrate the dramatic influence of poor habitat quality. The results for the IP suggest that ecoregion is particularly susceptible to negative effects from organic and nutrient enrichment.

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ATTACHMENT D

<u>Plastic Pollution Campaign</u>

- **Plastic Pollution Initiative Flyer (10/2021)** *(D-1)*
- **Quad Cities Plastic Pollution Initiative: Results Summary** *(D-2)*
- **Quad Cities Science Report: Mississippi River Plastic Pollution Initiative (12/13/2021)**
  [Link to report is available here:
  https://www.unep.org/resources/report/quad-cities-science-report-mississippi-river-plastic-pollution-initiative
- **The Mississippi River Plastic Pollution Initiative Web Page**
  [Link to web page is available here:
  https://www.unep.org/regions/north-america/regional-initiatives/mississippi-river-plastic-pollution-initiative

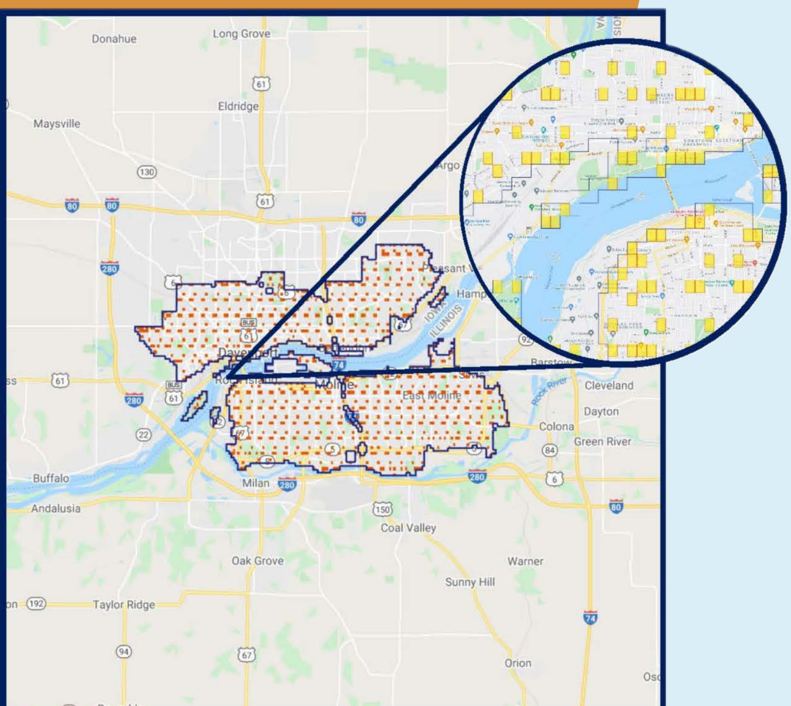
Do you have what it takes to be a marine debris detective?

Join the Mississippi River Plastic Pollution Initiative in the Quad Cities!

IMPORTANT DATES:

9/29/2021 4pm	K-12 Educator specific Zoom meeting with National Geographic Society – **Register HERE**
9/30/2021 6:30pm	Public Debris Tracker App training – **Register HERE**
10/01/2021	Data collection begins
10/15/2021	Plastic Pollution Initiative "Launch" Press and Public Event (location TBD)
10/31/2021	Final data collected to be added to the report

Find out how to join a cleanup event at **www.xstreamcleanup.org/upcoming-events**



Click to access the map for Quad Cities

1. Download the **Marine Debris Tracker App** on your device.
2. **Select 'Mississippi River Plastic Pollution/MRCTI'** from the organization list.
3. **Use this link to pick a data collection area on the map.** Google Maps will open, showing yellow squares so you can easily select a location.
4. **Find your data collection site.** Find a 3ft (1m) wide pathway where litter accumulates, like a roadside or sidewalk.
5. **Take a walk, collect data!** Follow the 3ft-wide path and record all items within it for at least 30 minutes. Do your best to track the debris you find within the marked square or just adjacent to it. We'll use this randomly sampled data for scientific analysis.

For detailed instructions, view the Debris Tracker Citizen Science Field Guide.

*Up to **80% of marine plastic originates on land**, and rivers are a major route for litter to travel to the ocean. **You can be part of the solution** to combat plastic pollution along the Mississippi River by helping to collect data on marine litter here in the Quad Cities!*

Want to find out more about the Mississippi River Plastic Pollution Initiative? Click here!

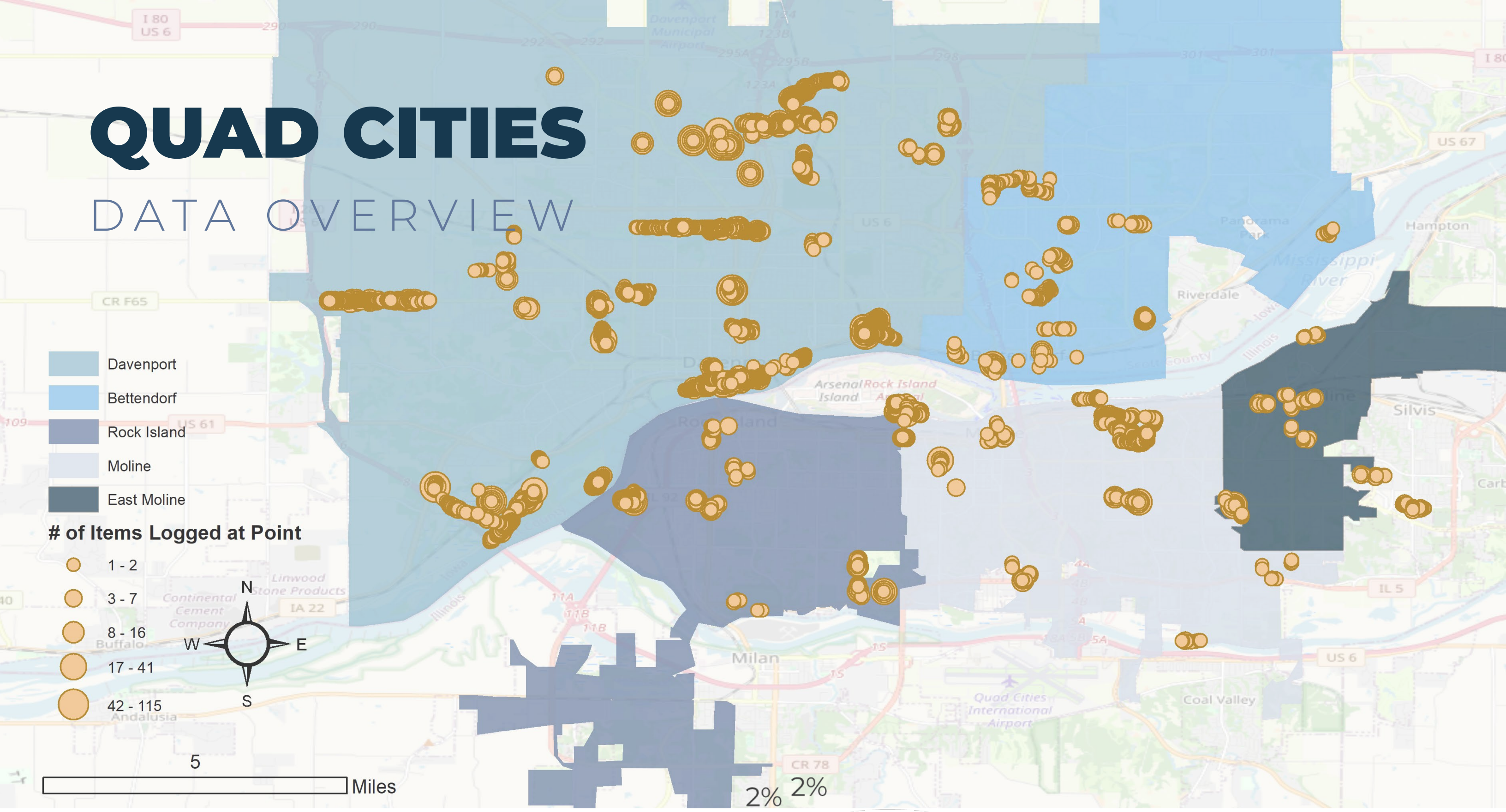
MRCTI

University of Georgia

UN environment programme

QUAD CITIES

DATA OVERVIEW



THE RUNDOWN

24,943 items logged

Average density = 0.53 items/m²

73 square kilometers analyzed

Equivalent to >10,000 football fields

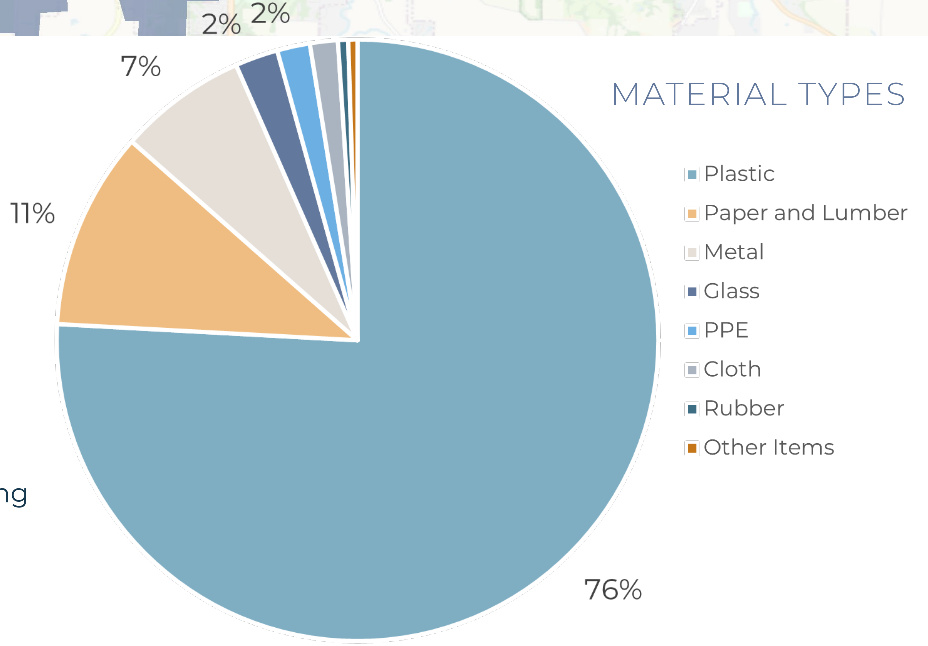
More than 45,000 m² of transects

6,476 volunteer minutes tracking

Equivalent to 4.5 days non-stop tracking

266 volunteers logged data

MATERIAL TYPES



TOP LITTER ITEMS