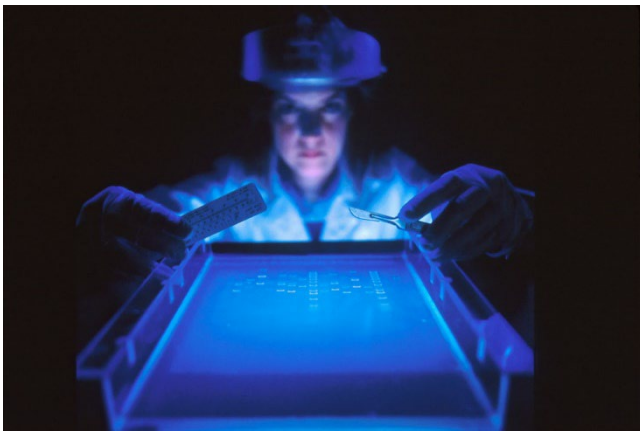
Great Lakes Microbial Water Quality Study




A report submitted to
the International Joint Commission
by the Health Professionals Advisory Board
July 2023

Prepared by:
LimnoTech, Ann Arbor, Michigan

Foreword

Towards a Great Lakes Microbial Source Water Quality Assessment

The International Joint Commission's Health Professionals Advisory Board adopted the following contractor report to complete its project: "Great Lakes Microbial Source Water Quality Assessment."

The International Joint Commission (IJC) has a long history of working to help the Canadian and United States governments clean up the Great Lakes and protect them for the benefit of today's citizens and future generations. In 1913, the IJC conducted a large study of microbial water quality across the Great Lakes (over 19,000 water samples) to assess fecal pollution in the transboundary waters, outbreaks of typhoid, and the links between disease and sewage pollution. The results from this study identified the prevalence of significant sewage pollution in some areas of the Great Lakes, and helped guide communities to improve sewage treatment and better safeguard their drinking water supplies.

Today, over 100 years later, the IJC's Health Professionals Advisory Board (HPAB) has drawn attention to microbial water quality concerns that are continuing, as well as other newer microbial water quality concerns that are emerging. The HPAB was established in 1995 to provide advice to the IJC and its Boards about current and emergent clinical and public health issues in the area of transboundary environmental health. HPAB has drawn attention to continuing concerns such as large untreated sewage releases, beach closures, waterborne disease and outbreaks in recreational waters, and boil water advisories, as well as newer emerging concerns such as harmful algal blooms, taste and odor problems, and the spread of antimicrobial resistance. Many of these concerns are increasingly compounded by climate change and extreme weather events, as well as by aging wastewater and drinking water infrastructure. Across the United States, swimming, paddling, boating and fishing are now estimated to account for more than 90 million cases of gastrointestinal, respiratory, ear, eye, and skin-related illnesses per year, with an annual cost of $2.9 billion. These types of concerns are expected to continue to adversely impact human health and local economies around the Great Lakes into the future.

HPAB recently sought to examine health concerns about microbial water quality in the Great Lakes over the last 100 years since the 1913 IJC study. Despite recent concerns about viral, bacterial, cyanobacterial, and protozoan microbes posing risks to human health, many assessments of microbial source water quality for drinking and recreation continue to be made using microscopes or other decades-old methods such as culturing for fecal indicator

bacteria like E. coli. These older methods are inadequate for comprehensive source water quality assessments; advances in newer methods are critically needed. HPAB has identified the potential of molecular and genomics technologies to modernize the assessment of microbial water quality. With leadership from the IJC's HPAB, the new vision is to conduct a large-scale study that demonstrates the effectiveness and utility of modern molecular and genomics technologies to strategically assess microbial source water quality across the Great Lakes and support decision-making. This study would enable a new era of analysis and mapping of human health risks, and better targeting of future risk management needs to protect the Great Lakes and transboundary waters. The report that follows represents Phase 1 of this effort, with key findings and recommendations summarized below.

Phase 1 – Great Lakes Microbial Water Quality Study Project

The Project was initiated by HPAB in 2020 with an initial workshop and scoping report that focused on comparing microbial water quality in the Great Lakes between 1913 and today, and investigating opportunities for advances in microbial source tracking to improve microbial water quality assessment. The Project continued through 2022 recognizing there were opportunities for modernizing microbial water quality assessment using advanced molecular and genomics technologies in three areas: 1) microbial source tracking of fecal pollution; 2) harmful algal bloom (HABs) assessment; and 3) metagenomic analyses of microbial communities. A series of workshops with water scientific and policy experts and other stakeholders was held in 2022 focusing on each of the three areas, followed by a synthesis workshop, and development of a project report for a Great Lakes Microbial Water Quality Study, provided here. Based on the general consensus from the workshops, the report finds:

- That advanced molecular and genomic tools (e.g. PCR techniques and metagenomics) are sufficiently developed and available for the three areas of fecal pollution source tracking, HABs and ecosystem/human health assessment.

- In addition, expertise and laboratory capacity exists within the basin to support a large-scale Great Lakes Microbial Source Water Quality Assessment, especially within the realm of the Centers of Excellence approach advocated by workshop participants and articulated in the draft IJC Great Lakes Science Strategy.

- A large-scale microbial water quality study could build upon growing public familiarity with PCR technology from the COVID pandemic and recent lab investments in PCR technology around the Great Lakes in Canada and the United States for tracking the COVID SARSCoV2 virus in municipal wastewater.

- The proposed large-scale study would also align well with water surveillance under the Cooperative Science and Monitoring Initiative (CSMI) framework, and broader IJC objectives of advancing the Great Lakes Science Strategy based on: increasing science capacity of the region; development of water research and monitoring infrastructure; and creation of distributed centers of excellence.

This final Phase 1 Project report for a Great Lakes Microbial Water Quality Study summarized the 2022 workshops and in particular the synthesis workshop led by HPAB reviewed the three reports from the previous workshops and further developed goals and recommendations for moving forward. Workshop participants initiated preliminary efforts to inventory the existing labs and expertise in the basin.

The report recommends:

- To build a laboratory network and Community of Practice for harmonizing use of these molecular methods across the Great Lakes basin, and

- Addressing a preliminary validation study of molecular methods between labs, and a pilot study to demonstrate the molecular methods in a defined area like the Lake St. Clair-to-Lake Erie corridor, prior to rolling out a larger basin-wide study.

- Recognizing the need for oversight and coordination of such a study, that the IJC could serve study needs by convening committees and work groups, managing communications and report-outs, facilitating any bi-national agreements needed, and contributing financial support for initial phases of implementation.

- Further discussion and engagement with the Parties (e.g. ECCC, US EPA) and national research funding programs (e.g., United States Geological Survey, Natural Sciences and Engineering Research Council of Canada, US National Science Foundation, US National Oceanic and Atmospheric Administration), to discover strategies for primary sources of foundational support for the pilot and large-scale rollout of a Great Lakes Microbial Water Quality Assessment, including enhanced funding of academic laboratories for applied and basic research.

The report estimated that very rough costs for this study over 10 years could be as follows (in US$ and not considering in-kind contributions): $50-100K inter-lab round robin study; $500K-1.5M for pilot study; $5-10M for a large-scale study roll out in annual phases throughout the Great Lakes basin. While this large-scale study would be focused on the Great Lakes, the tools, techniques and approaches could have direct applicability to other water bodies and coastal areas across the two nations and lead global efforts on managing water quality in transboundary water basins. Federal leadership could be augmented by

State and Provincial agencies, large municipal governments, private philanthropies, and professional organizations.

This work has provided the foundation for continued work on future activities to support implementing such a basin wide assessment. The HPAB provides a summary of priority areas for future activity in Phase 2 below.

Phase 2 – Implementing the Great Lakes Microbial Source Water Quality Assessment (GL-MSWQA)

Going forward, HPAB recommends establishing in 2023:

- a Steering Committee for setting objectives, strategy, and securing funding to implement the Great Lakes Microbial Source Water Quality Assessment; and
- a Technical Working Group for detailed scientific planning and coordination of interlaboratory round robin validation studies, a pilot demonstration study, and eventual rollout of the large basin-wide study.

The IJC approved a 3-year work plan in April 2023, commencing in the fall of 2023, to support continued planning for a Great Lakes Microbial Source Water Quality Assessment. A multi-nation Steering Committee (SC) could be co-led by USEPA Region 5 and the Ontario Regional Office of Environment and Climate Change Canada, and include key members (leaders) from Tribes, First Nations and Métis, state and provincial health and environment departments, municipalities, and academics. The IJC-HPAB could have oversight for the Technical Working Group (TWG), which would include key scientific expert members from US and Canada. The GL-MSWQA key objectives are to:

Year 1, 2023-2024

1.1 Establish the Steering Committee and Technical Work Group to plan for an Inter-lab Round-Robin Study, Pilot Study, and eventual rollout of the GL Microbial Source Water Quality Assessment.

1.2 Identify participating laboratories and centers of excellence in the basin, and develop a GL Community of Practice (CoP) for Use of Advanced Molecular Tools.

Year 2, 2024-2025

2.1. Conduct a series of on-line meetings to develop the plan for the Round Robin Lab Study, and plans for data management going forward.

2.2 Convene SC to review proposed CoP and plan for the Round Robin Lab Study.

Year 3, 2025-26

3.1 Conduct on-line meetings and hold one in-person workshop and site visit to develop the plan for implementing the Pilot Study in the Lake St Clair to Lake Erie corridor area.

3.2 Convene SC to review proposed plan for the Pilot Study, and discuss the roll out of the large-scale and basin-wide GL-MSWQA.

In the final part of Phase 2, the multi-national SC would review plans for the inter-lab method validation study and the pilot study to begin developing sampling plans for a large-scale GLMSWA across all five lakes, and the communication of results and recommendations about fecal pollution sources, harmful algal blooms, and health risk maps for all five Great Lakes.
These actions will help guide future risk management actions to support the Great Lakes Water Quality Agreement and protect GL microbial water quality and human health for another 100 years.

Thomas Edge
Canadian Co-chair
Health Professionals Advisory Board

Joan Rose
US Co-Chair
Health Professionals Advisory Board

Table of Contents

List of Tables

List of Figures

Executive Summary

The International Joint Commission (IJC) is responsible for regular reporting on the status of the Great Lakes and other boundary waters and investigating the risk to ecosystems that may result from current or future stressors. The Great Lakes constitute the largest freshwater ecosystem in the world and are a dominant part of the physical and cultural heritage of North America.

In 1913, the IJC conducted a detailed microbial water quality study (over 19,000 samples) of the fecal-related pollution of the boundary waters of the Great Lakes, and the potential link between disease and sewage pollution (IJC 1918). Today, over 100 years later, despite the advances in drinking water treatment technology and source control measures, microbial water quality concerns remain and range from beach closures, harmful algal blooms, other microbes impairing sources of drinking water, taste and odor issues in finished drinking water, and the spread of antimicrobial resistance. Despite rapidly growing concerns regarding viral, bacterial, cyanobacterial, and protozoal agents of human health concern, decisions about microbial source water quality for drinking and recreation continue to be made based on decades-old methods for culturing bacteria like *E. coli*. Advances to our current approaches for source water quality assessment are critically needed.

This Great Lakes (GL) Large Basin Microbial Water Quality (LBMWQ) Study Plan project is a significant step in the IJC Health Professionals Advisory Board (HPAB) efforts to develop a major plan to advance the applications of molecular and genomics tools to modernize and strategically assess microbial source water quality in the Great Lakes Basin. The study will advance assessment in three areas: 1) microbial source tracking (MST); 2) harmful algal blooms (HABs); and 3) ecosystem/human health assessment (metagenomics) and will set the stage for another 100 years of action to support water quality in the Great Lakes. The goals of this planning project were established at the outset by the HPAB:

- Advance molecular and genomics technologies for source water quality assessment throughout the Great Lakes, including MST, HABs, and metagenomics.
- Characterize current lab capacity around the Great Lakes for each microbial area, including consideration that capacity for the Great Lakes LBMWQ study could build upon recent US and Canadian government investments in modern molecular tools to track SARS-CoV-2, the virus that causes COVID, in wastewater.
- Establish a laboratory network for harmonizing molecular methods across the basin through a round robin interlaboratory comparison.
- Develop a framework for a Great Lakes LBMWQ study to demonstrate methods and map human health risks, including whether to implement the study first through a pilot application and follow with a larger rollout across the basin.

The project was initiated by HPAB in 2020 and a initial workshop and scoping report that focused on microbial source tracking was completed first (IJC 2021a). The project continued

through a series of workshops in 2022 focused on each technical area, followed by a synthesis workshop and the development of this project summary report.

In parallel with the visioning for this project, the IJC Science Advisory Board (SAB), which provides advice on research and scientific matters to the Commission related to its responsibilities under the Great Lakes Water Quality Agreement (GLWQA), convened an effort to develop a comprehensive decadal binational science plan for Great Lakes research, which was recently published in summary form (IJC 2022). The overall goal of the Science Strategy is to establish a road map for placing the Great Lakes region on a sound scientific footing for ensuring effective management and permanent sustainability of the system. The objectives and recommendations of the Science Strategy dovetail with the goals of the Great Lakes LBMWQ Plan project (Table ES-1).

Table ES-1. Alignment of Great Lakes Large Basin Microbial Water Quality Study Goals with IJC Science Strategy Goals.

Science Strategy Goal	Alignment with Great Lakes LBMWQ Goals
Increasing science capacity of the region	Launch the GL laboratory network using inventories developed from this study to establish best methods and protocols and conduct a round-robin GL validation and intercomparison of the new technologies in multiple labs.
Development of the research and monitoring infrastructure	Establish a working group to design and implement a pilot program in Lake St. Clair-Western Lake Erie that can be scaled up to the large basin study.
Creation of distributed centers of excellence	Craft the plans for an advanced genomics technologies consortium that focuses tools on public use cases to advance healthy beaches, enhance prediction and control of HABS, and improve drinking water quality.

The value, from a human health risk perspective, of including each type of microbial technology in a new Great Lakes LBMWQ study was articulated in the workshops.

- Including MST in the LBMWQ study is primarily intended to protect the health of recreational users, though it has been used for impaired waters assessment generally and can also assist with protection of source waters for drinking. These advanced tools can be used to identify fecal pollution sources and inform more efficient mitigation measures than traditional *E. coli* methods currently in use.
- Including HABs in the LBMWQ study is designed to protect the health of the members of the public whose drinking water source is the Great Lakes, as well as recreational users of the lakes. Data derived from employing advanced microbial tools can address both species and toxins associated with HABs and serve as an early warning to drinking water utilities of impending or potentially toxic a blooms. HABs were ranked as the highest priority element by workshop participants.
- Including metagenomics in the LBMWQ study presents an opportunity to characterize the diversity of the microbial community, including providing early detection of

ecosystem disruptions that can result in conditions that pose a threat to human and ecological health. Emerging toxins and pathogens are now being found in the Great Lakes, likely from the effects of climate change, and metagenomic techniques could help to identify new toxins/hazards before their levels impact human or ecosystem health.

The general consensus from the workshops is that molecular and genomic tools are sufficiently developed for use in a Great Lakes LBMWQ study that includes MST, HABs, and metagenomics. The most advanced methodologies that are ready to be immediately implemented include the use of qPCR and dPCR applied for MST and are also used for HABs. Metagenomics research and technologies are less established and require larger teams with more complex slill sets than the other methods, however there is great promise for providing comprehensive information about the state of the lakes and there was substantial support for including its use. In addition, laboratory capacity in all topical focus areas exists to support the Great Lakes LBMWQ study, especially within the realm of the Centers of Excellence approach advocated by workshop participants and articulated in the IJC Great Lakes Science Strategy (2022). The laboratory network has the potential to be expansive if an effort is made to leverage the recently enhanced capabilities of laboratories currently doing SARS-CoV-2 (COVID) testing of wastewater with qPCR to support this Great Lakes study using qPCR methods.

One important objective of the Great Lakes LBMWQ study is to incorporate molecular methods as part of the future tool box for standard microbial analysis. A laboratory intercomparison round robin is a key study component for achieving the goal of consistent data across lakes, years, and laboratories. The round robin effort will be used to establish the appropriate markers, methods, protocols, and QA/QC requirements for use by all of the participating laboratories. This is a critical need for the study to be successful and will be the first technical effort undertaken in the study implementation.

A pilot project offers the potential to be a quick win to build agency support for the large basin study by showing the benefits of a lab network and molecular tools and demonstrating how to incorporate large datasets into future efforts. It also provides an opportunity to both demonstrate and establish protocols for molecular methods (e.g., metagenomics) for the larger basin study. The concept of conducting a pilot project was widely endorsed in the workshops. Lake St. Clair and western Lake Erie were combined (with the Detroit River connecting channel) into a single area and selected as the recommendation for a good candidate pilot project study area.

Implementation of the pilot project would require establishing “sentinel” stations, sampled at regular intervals for the purpose of tracking changes in conditions, and “baseline” stations, sampled more strategically, such as in response to an environmental trigger (e.g., storm event for MST, observed bloom for HABs) and more broadly (e.g., more locations), for the purpose of establishing a current condition for comparison to the next Centennial microbial study 100 years hence. The consensus among workshop participants was that more frequent sampling targeting different conditions is needed for the new Centennial study than was done for the 1913 study.

The vision for how the study could be executed has three organizational levels, each serving a specific and critical need(s) for the overall project, which are described below in general terms:

1. **IJC**: The role of the IJC can be simply described as communication and coordination. The IJC is well-positioned to serve the study needs with respect to convening committees and work groups, managing communications and report-outs, facilitating any bi-national agreements needed, and contributing financial support for initial phases of implementation.
2. **Steering Committee**: The role of the Steering Committee can be simply described as setting strategy and securing funding from agencies in the U.S. and Canada. An initial proposed size range would be 10-12 individuals, including HPAB members, academic subject matter experts, and funding agency study champions.
3. **Technical Work Group(s)**: A Technical Work Group could be established for a relatively short period of time (e.g., 1-3 years) for each major technical component of the project to conduct detailed planning for the associated task (e.g., laboratory round robin). The composition has been envisioned as 3-4 people as core members, who would have overall responsibility for the work group activities and outcomes. The group could be expanded at core member discretion, on a temporary or as-needed basis, to have the necessary technical expertise represented in the planning and execution of the tasks.

The organizational structure described above is consistent with and advances three distinct implementation strategies that were articulated to facilitate implementation of the laboratory round robin, pilot project, and large study, including the important notion of building interest and enthusiasm among potential partners and potential funding sources for implementing the study.

Implementation Strategy #1: Break the Great Lakes LBMWQ study into multiple phases with a Work Group for each phase that is focused on the technical planning and execution of that phase. Set up a Steering Committee to focus on setting strategy and securing funding. The work group in the initial phases would address the laboratory round robin (phase 1), the design and implementation of the pilot project (phase 2), and lead local stakeholder outreach. This work group is envisioned as a small committee (e.g., 3-4 participants) focused on an approximately three-year period, nominally 2023-2025, to complete these tasks. Specifically, this work group would focus on the laboratory round robin in 2023-2024 and the pilot project design in 2024-2025 in conjunction with the Lake Erie CSMI, with stakeholder outreach throughout.

A second, larger, multi-jurisdictional work group would be established to focus on subsequent phases or the post-pilot portion of the planning process, specifically advancing the larger study strategy, centered around linking the goals of the larger basin study to the IJC Great Lakes Science Strategy (2022). This group would tackle some of the more challenging needs of the larger basin study, including, for example, data management, coordination across countries, interagency needs, and funding mechanisms, as well as address unresolved aspects of the study. For example, one suggested approach for this work group to consider is whether to work within the Cooperative Science and Monitoring Initiative (CSMI) framework to expand sampling locations to include offshore water locations and work with beach sampling programs to sample nearshore locations (e.g., beaches, docks, marinas). Leveraging both existing programs could be an effective way to reduce the cost of the Great Lakes LBMWQ study but would need to be

balanced by additional investment in training and establishing sampling protocols, as well as considering whether this approach satisfies the data needs of the microbial study.

Implementation Strategy #2: Establish Centers of Excellence, particularly with metagenomics. Method standardization, appropriate training, and coordination across laboratories were identified as critical needs, particularly for metagenomics because there are no widespread "standard" methods[1]. The concept of Centers of Excellence was developed as a potential solution. Lead laboratories, including a minimum of one in Canada and one in the United States, would be established to train and work with a consortium of experts who would address the key methodological questions in implementing a large-scale microbial water quality study of Great Lakes water quality. The project leads recognize the value of applying this approach at a pilot scale for MST, HABs, and related analytes (e.g., nutrients, *E. coli*) as well as for metagenomics. The concept was broadened to consider a single laboratory or organization that would serve as the central entity in coordinating the sample collection, sample processing, and the distribution of samples to the participating laboratories. This model of managing the field and laboratory elements was successfully used for the HABs Grab studies (Chaffin et al. 2021).

Implementation Strategy #3: Conduct the pilot project after the round robin interlaboratory comparison and before the large basin study. A number of details remain to be sorted out before the large basin study can be executed. Conducting the interlaboratory comparison round robin before the pilot project provides several benefits, including an opportunity to engage with participating laboratories to develop or refine standard operating procedures (SOPs), validate MST methods with samples from across the Great Lakes basin, and assess the interlab stability of a HAB PCR method. The pilot project offers an opportunity to build protocols, create a system of replicability, compile necessary meta-data, test data management strategies on a smaller scale, engage with local stakeholders to identify health priorities that will inform sample collection, and use the data to inform the appropriate temporal and spatial scales to be applied in the larger basin study.

In addition to implementing the LBMWQ study, a more comprehensive model has also emerged as a concept to consider. This would take the form of the creation of a distributed and binational Great Lakes Microbial Observatory consisting of coordinated sampling locations, analytical laboratories and methods, data management, and sample archiving. In addition, this observatory would facilitate a community of practice including communications, education, training, technology transfer, and planning. A common requirement of both the pilot version of the LBMWQ study and a larger microbial observatory effort would be identifying two lead investigators and institutions – one in Canada and one in the US – to anchor the efforts. The Great Lakes LBMWQ study is not just an applied science exercise. The metagenomics element, in particular, would also materially contribute towards the development of strong reconnaissance and basic research components.

[1] USEPA has two approved analytical methods for MST analysis: 1696.1 (HF183/BacR287 TaqMan) and 1697.1 (HumM2 TaqMan). USEPA has also approved several qPCR-based microbial analytical methods, including 1642, 1643, 1609.1, 1611.1, and Method B (note that these are not source specific).

After reviewing the findings and recommendations from the Synthesis workshop, the IJC project leads have developed a set of next steps for the LBMWQ study **over the next 3-4 years, as follows:**

- In 2023-2024, develop the round-robin exercise with the established laboratory network identified in the workshops and determine whether the pilot project will be executed as part of the 2024 CSMI, develop a detailed Project Work Plan, including round-robin exercise elements and pilot study; obtain funding commitments; develop community of practice, outreach, and data management plans.
- In 2024-2025, conduct the laboratory round robin study interlaboratory comparison for MST and HABs, summarize the results, and establish standard analytical protocols for use in the pilot and larger studies.
- In 2025-2026, execute the pilot project, or alternatively, the round-robin laboratory exercise; if funding for the pilot has not yet been obtained, continue to pursue this. Outline the approach to a full basin study (synchronous or phased [e.g., tied to CSMI]) and/or a microbial observatory).

Longer term steps include:

- In 2026-2031, summarize results of the pilot project; begin implementation of the microbial study elements into existing monitoring and research programs (e.g., *R/V Lake Guardian* and *Limnos* annual lake surveys, CSMI, and beach monitoring programs) to identify potential sampling locations and test approaches for the design of the Great Lakes Microbial Observatory and defining the microbial observatory; continue to pursue funding if necessary; refine community of practice approaches.
- Year 11 (>2032): Establish the Great Lakes Microbial Observatory.

Very rough estimates for the labor costs and expenses in U.S. dollars (USD) for the components of the LBMWQ study, not considering any in-kind contributions, would include:

- $50,000 – $100,000 USD for the round-robin study
- $500,000 – $1,500,000 USD for the pilot project
- $5,000,000 – $10,000,000 USD for a comprehensive basin-scale study, possibly in annual sub-phases to achieve the necessary time series of data
- $75,000 USD per year for coordinating a community of practice and sharing results and challenges
- $200,000 – $400,000 USD per year for maintaining a basic microbial observatory

To say that the IJC mission and the government approaches to environmental health have evolved substantially since the original 1913 study is an understatement. The original study took place decades before the establishment of ECCC, USEPA, and signing of the GLWQA (1972) and passage of the US Clean Water Act (1972) and the Canada Water Act (1970). At this point in time it would be appropriate and necessary for the Parties, via their federal environmental

agencies (ECCC, USEPA, NOAA, etc.) and national research funding programs (e.g., United States Geological Survey (USGS), Natural Sciences and Engineering Research Council of Canada, US National Science Foundation) to serve as the primary sources of foundational support for the pilot and full-scale phases of the LBMWQ study, including enhanced funding of academic laboratories for applied and basic research. While the applicability of this study is squarely focused on the Great Lakes and freshwater system, the applicability of tools, techniques and approaches/methods will have direct applicability to other challenges in waterways and coastal areas across the two nations. Federal leadership could be augmented by states and provinces, large municipal governments, private philanthropies, and professional organizations. Non-financial contributions could include serving in a coordinating capacity for the study or providing staff and expertise to facilitate a new environmental microbiology collaborative.

Execution of the LBMWQ study, creation of a microbial observatory, and related activities in any form align with the objectives of existing policies, regulations, and voluntary activities and programs. The GLWQA consideration of microbiology aligns closely with Annexes 2 (LAMPs), 4 (nutrients), 9 (climate change), and 10 (science). The LAMPs prioritize management concerns and associated science priorities on a lake-by-lake basis. Many of the elements described above that would constitute the LBMWQ study or observatory are well-aligned with the priorities and proposed structural elements of the IJC Great Lakes Science Strategy (2022).

1.0 Introduction

1.1 Project background

The International Joint Commission (IJC) is responsible for regular reporting on the status of the Great Lakes and other boundary waters and investigating the risk to ecosystems that may result from current or future stressors. The Great Lakes constitute the largest freshwater ecosystem in the world and are a dominant part of the physical and cultural heritage of North America. Shared by two countries and spanning a thousand miles across Canada and the United States, the shoreline is longer than the US East and Gulf coasts combined. The lakes also hold monumental environmental, cultural, and economic value for both the region and our nations. The basin is home to 3,500 species of plants and animals, and over 170 species of fish (Michigan Sea Grant 2020). These flora and fauna contribute to the environmental integrity, resilience, and character of the region and support impressive Great Lakes tourism and recreation industries.

In 1913, the IJC conducted a detailed microbial water quality study of the fecal-related pollution of the boundary waters of the Great Lakes, and the potential link between disease and sewage pollution (IJC 1918). At that time, cholera and typhoid were the primary waterborne diseases affecting human populations in the Great Lakes. The field of microbiology was in its infancy, with germ theory slowly replacing the miasma or "bad air" theory as the prevailing model for disease formation and spread. The 1913 study applied the microbiology method of the day (a measure of total coliforms) to over 19,000 samples to relate untreated sewage discharges in the nearshore to cholera and typhoid illnesses. A key recommendation from this binational study was to implement treatment of sewage before discharge into the Great Lakes (boundary waters).

Today, over 100 years later, the lakes are more widely used for drinking water and recreation, increasing the potential to expose users to unsafe bacteria levels and waterborne pathogens, despite the advances in drinking water treatment technology and source control measures. Microbial water quality concerns today are diverse, ranging from beach closures, harmful algal blooms, other microbes impairing sources of drinking water, taste and odor issues in finished drinking water, and the spread of antimicrobial resistance. These and other challenges are anticipated to grow because water recreational demands are increasing, there are more immuno-compromised people vulnerable to waterborne pathogens, wastewater infrastructure is aging, agricultural cropping and livestock husbandry practices are changing, sewage releases are common, and extreme rain events and other manifestations of climate change are increasing (Graydon et al. 2022).

The question of whether nearshore fecal bacterial/microbial water quality is getting better or worse is fundamental to maintaining the Great Lakes for recreational use and as a source of drinking water under the general objectives of the Great Lakes Water Quality Agreement (GLWQA). Beach closing data obtained from the Swim Guide [2]over two recent three-year periods (2012-2014 vs. 2019-2021) at a subset of beaches in each Great Lake show mixed results

[2] theswimguide.org/

in water quality improvement (Figure 1-1).[3] Three of the lakes (Ontario, Erie and Superior) appear to be improving overall, two lakes (Huron and Michigan) appear to be worsening overall, and Lake St. Clair has the same percentage of beaches improving and worsening (though a limited number of beaches (6) were assessed).

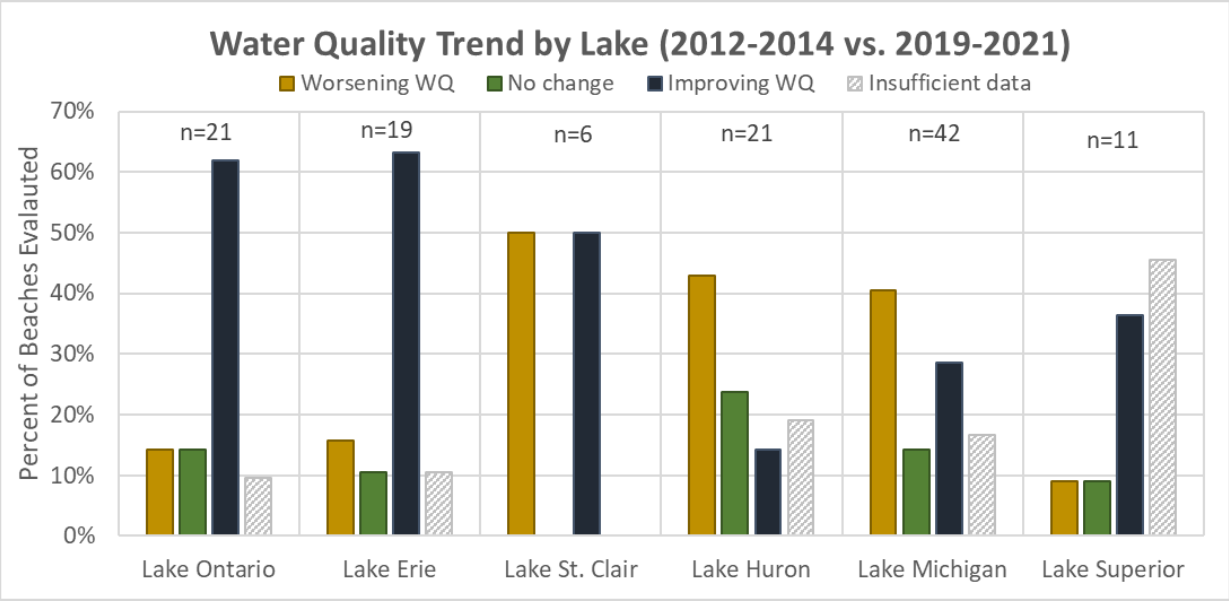


Figure 1-1. Water Quality via Beach Closing Trends by Great Lake (and Lake St. Clair)

A Water Quality Centennial Study conducted by the IJC (2021) provided the Health Professionals Advisory Board (HPAB) with an assessment of the state of knowledge on fecal contamination in the Great Lakes through the objectives of comparing the 1913 study data to contemporary fecal bacteria data to assess whether nearshore water quality is getting better or worse, the sources of pollution, and the public health risks associated with changing near shore water quality. Despite rapidly growing concerns regarding viral, cyanobacterial, and protozoal agents of human health concern, decisions about microbial source water quality for drinking and recreation continue to be made based on decades-old methods for culturing bacteria like *Escherichia coliform (E. coli)*. Advances to our current approaches for source water quality assessment are critically needed.

To set the stage for another 100 years of action to support water quality in the Great Lakes, the HPAB recommended in the Centennial Study report that the IJC oversee a binational multiphase project addressing water quality across the Great Lakes basin over a five-year timeframe. The first phase of this project would be to establish a committee of federal, Tribal, First Nations and

[3] "Worsening WQ" indicates the percentage of beaches that had more periods of unsafe pathogen conditions in 2019-2021 than in 2012-2014. "No change" indicates a similar frequency of unsafe beach conditions in each three-year period. "Improving WQ" indicates the percentage of beaches with fewer periods of unsafe beach conditions in 2019-2021 than in 2012-2014. "Insufficient data" indicates that one or both periods at one or more beaches did not have enough data to assess differences.

the Métis Nation of Ontario, provincial, state and municipal agencies to oversee and coordinate a multiyear study of fecal pollution and its sources. The goals of this study would be to:

- Demonstrate molecular and genomics technology advances over the last ~100 years to better assess and map microbial water quality threats like human sewage, livestock wastes, and harmful algal blooms (HABs).
- Modernize and coordinate microbial water quality assessments across the Great Lakes to advance source tracking to manage fecal pollution -- i.e., protect swimmers, prevent and predict HABs, and use advanced metagenomics to improve ecosystem and human health.
- Conduct a Large Basin Microbial Water Quality Study to protect beaches and sources of drinking water, address water quality restoration of coastal ecosystems, identify science and management gaps, and advance water quality restoration.

This project report describes the results of the efforts by the HPAB to advance a new Large Basin Microbial Water Quality Study Plan to accomplish the goals described above.

1.2 Project overview

This Great Lakes (GL) Large Basin Microbial Water Quality (LBMWQ) Study project is a significant step in the HPAB's efforts to develop a major plan to advance the applications of molecular and genomics tools to modernize and strategically assess microbial source water quality in the Great Lakes Basin. The study will advance assessment in three areas: 1) microbial source tracking (MST); 2) harmful algal blooms (HABs); and 3) ecosystem/human health assessment (metagenomics). Three workshops were held, one for each evaluation area. A final workshop was held to synthesize the results of the first three workshops and develop implementation strategies (Figure 1-2). Project leads and contractor team members then met to conduct further synthesis and planning.

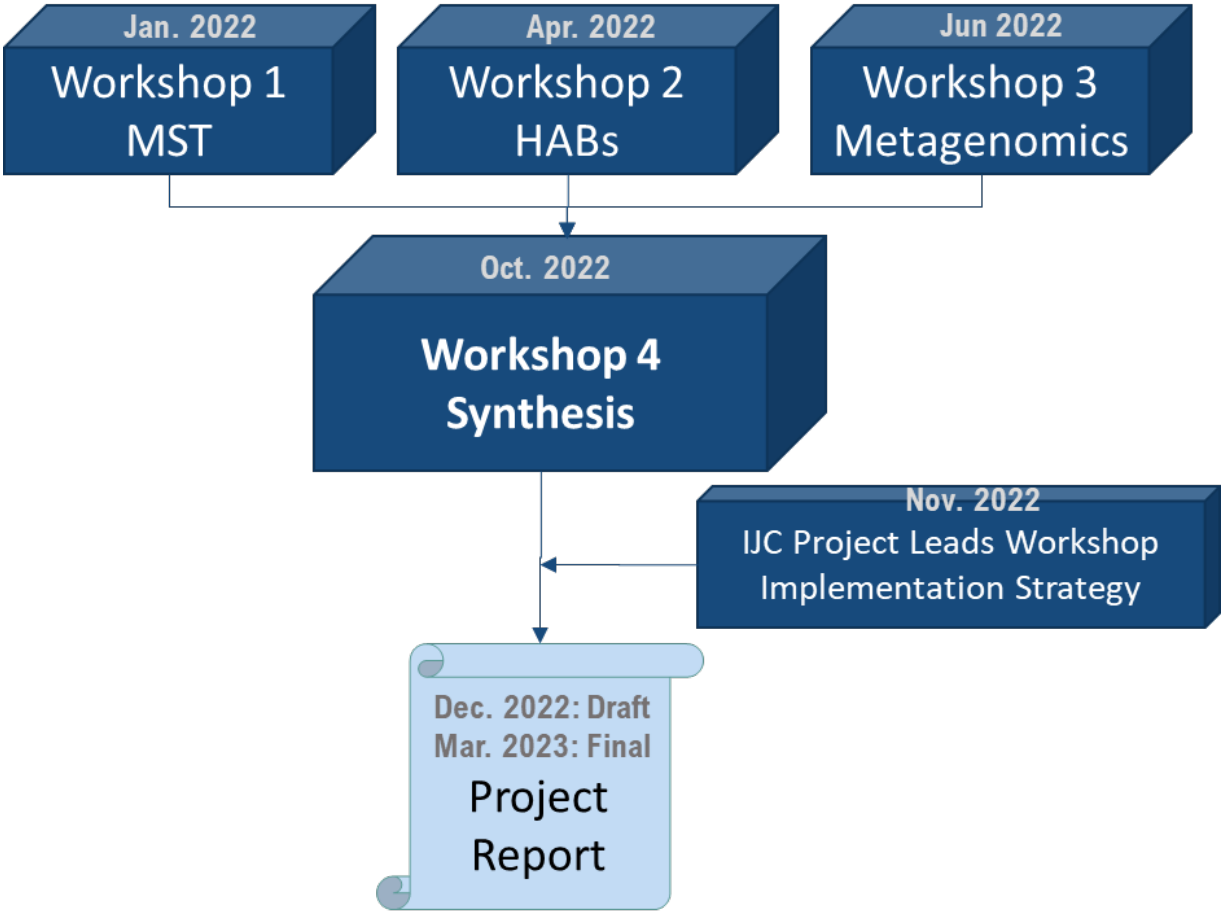


Figure 1-2. Great Lakes Large Basin Microbial Water Quality Study Project Overview.

The first topical workshop in the series was held virtually on January 20-21, 2022 and focused on Microbial Source Tracking (MST). The second topical workshop in the series was held virtually on April 13-14, 2022 and focused on Harmful Algal Blooms (HABs). The third topical workshop in the series was held virtually on June 27-28, 2022 and focused on ecosystem/human health assessment (metagenomics). The final synthesis workshop was held virtually on October 6-7, 2022 and focused on synthesizing the recommendations from the first three workshops and building out an implementation strategy. Following the workshops, a small in-person meeting with IJC project and workshop leads was held on November 3, 2022 to further the implementation strategy through development of a pilot project application in the Lake St. Clair-Detroit River-Western Lake Erie area.

This report provides a summary of the project, goals and objectives, key outcomes and associated recommendations from the workshops, and study implementation considerations, including socializing the project with federal agencies, potential sampling and analysis partners, timing, and next steps.

1.3 Project goals

The project goals were established at the outset of the project by the HPAB:

- Advance molecular and genomics technologies for source water quality assessment throughout the Great Lakes, including MST, HABs, and metagenomics.
- Characterize current lab capacity around the Great Lakes for each microbial area, including consideration that capacity for the Great Lakes LBMWQ study could build upon recent US and Canadian government investments in modern molecular tools to track SARS-CoV-2, the virus that causes COVID, in wastewater.
- Establish a laboratory network for harmonizing molecular methods across the basin through a round robin interlaboratory comparison.
- Develop a framework for a Great Lakes LBMWQ study to demonstrate methods and map human health risks, including whether to implement the study first through a pilot application and follow with a larger rollout across the basin.

In parallel with the visioning for this project, the IJC Science Advisory Board (SAB), which provides advice on research and scientific matters to the Commission related to its responsibilities under the Great Lakes Water Quality Agreement (GLWQA), convened an effort to develop a comprehensive decadal binational science strategy for Great Lakes research, which was recently published (November 2022) in draft summary form (2022). The overall goal of the Science Strategy is to establish a road map for placing the Great Lakes region on a sound scientific footing for ensuring effective management and permanent sustainability of the system. The objectives and recommendations of the Science Strategy dovetail with the goals of the Great Lakes LBMWQ Plan project. Figure 1-3 illustrates the key components of the draft Science Strategy that the Great Lakes LBMWQ supports.

Figure 1-3. IJC Science Strategy Goals Related to Protecting Human Health.

Table 1-1 provides a crosswalk between the goals of the Science Strategy and the Great Lakes LBMWQ study.

Table 1-1. Alignment of Great Lakes Large Basin Microbial Water Quality Study Goals with IJC Science Strategy Goals.

Science Strategy Goal	Alignment with Great Lakes LBMWQ Goals
Increasing science capacity of the region	Launch the GL laboratory network using inventories developed from this study to establish best methods and protocols and conduct a round-robin GL validation and intercomparison of the new technologies in multiple labs.
Development of the research and monitoring infrastructure	Establish a working group to design and implement a pilot program in Lake St. Clair that can be scaled up to the large basin study.
Creation of distributed centers of excellence	Craft the plans for advanced genomics technologies consortium that focuses tools on public use cases to advance healthy beaches, enhance prediction and control of HABS, and improve drinking water quality.

1.4 Geographic extent

One key consideration in developing a study plan for the Great Lakes LBMWQ study is to define the geographical extent of the plan. Figure 1-4 shows the watershed for each of the five Great Lakes. The portion of the St. Lawrence River that serves as an international boundary extends to the area of Cornwall, ON near the Moses-Saunders Dam. These areas and lake-influenced tributaries will be used to further develop the LBMWQ study plan. However, it should be noted that the HPAB anticipates that a Great Lakes LBMWQ plan can be adapted to other geographic areas (e.g., lower St. Lawrence River), and including, to a limited extent, inland rivers within the Great Lakes basin.

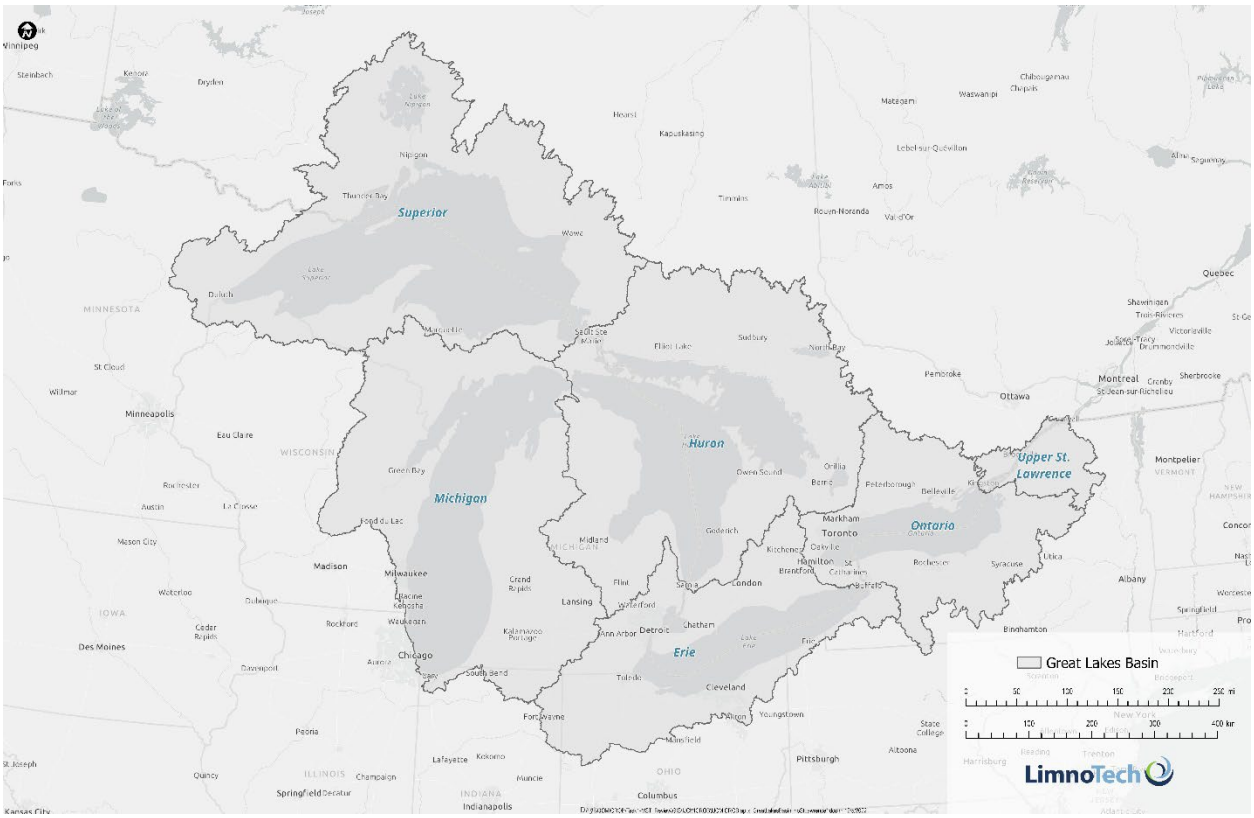


Figure 1-4. Geographic Extent of Great Lakes Large Basin Microbial Water Quality Study.

1.5 Role of IJC and its boards

Noted at the outset of the project by the IJC, the HPAB advises and supports IJC, as well as facilitating the planning of the study and socializing the plan with U.S. and Canadian agencies. While the IJC may be able to continue to provide coordination (with the HPAB and study work groups, and among the participating entities), scientific expertise, and convene workshops, additional funds and support from outside the IJC would be needed to implement the Study. This is discussed in more detail in Section 4 of the report.

Data management, which was identified as a vital component of planning and executing this study, and the potential role the IJC could have in this area, was also discussed during the workshops and follow-up meetings. Although the IJC, by virtue of its binational purview, would be an ideal entity to host the data from this study, it was determined that this task is not consistent with the IJC mission and is not an appropriate role for the organization. The nature of microbial and genomics data are such that IJC possesses neither the expertise nor the resources to manage such data within the organization. Potential collaboration with partners who could offer support for this need is discussed in more detail in Section 3 of the report.

2.0 Review of Molecular and Genomics Tools

A review of the state of current and emerging microbial assessment tools was conducted via three technical workshops conducted in 2022 (Figure 1-2). Each workshop focused on a specific area of microbial assessments, including microbial source tracking; harmful algal blooms; and metagenomics[4]. Each workshop allowed input from dozens of scientists, engineers, policy experts, and interested individuals, representing a cross section of United States and Canadian agencies, academic researchers, and managers (including beach and water utilities). Participants in each workshop are provided in Appendix A.

Desired outcomes of these technical workshops included:

1. Identifying potential laboratory network participants
2. Developing a pilot project to develop a health risk map
3. Advancing the design of a round-robin interlaboratory comparison

Each of the technical workshops included three separate breakout groups where each breakout group focused on a particular aspect of the scope of the large basin study, including: laboratory methods, sampling logistics, and management considerations. Background and findings from each of these three technical workshops are described in this section. The purpose of this section is to recap the findings from each workshop. Findings and recommendations are presented here in the interest of accurately capturing the workshop discussion, however, this documentation does not constitute an endorsement from the IJC or HPAB project leads. Key findings and recommendations described in this section were further refined and adjusted as part of the synthesis workshop and subsequent HPAB project team meetings, as described in Sections 3 and 4.

2.1 Microbial source tracking tools

2.1.1 Background

Microbial source tracking (MST) methods offer an advancement over traditional fecal coliform culture-based and probabilistic methods. Fecal indicator bacteria (FIB), such as *Escherichia coli* (*E. coli*) and *enterococcus* sp., have been used as potential indicators of harmful pathogens in waterways for over 50 years (USEPA 1986). However, FIB can survive and reproduce in natural environments, such as sands, sediments, soils and aquatic vegetation (Ishii et al. 2007; Badgley et al. 2011; Staley et al. 2015), which may contribute to poor correlations between FIB and pathogens that have been reported in various studies (Ahmed et al 2019). Most importantly, FIB provide no information on the sources of fecal contamination, which makes it difficult to develop

[4] "Metagenomics" is used in this report as an umbrella term for multiple types of genomic analyses, including metabarcoding, proteomics, metabolomics, phenomics, transcriptomics, and metagenomics.

and implement cost-effective mitigation strategies. MST methods address this last shortcoming in the use of FIB, by providing tools to identify sources of contamination, such as humans, birds, pets, livestock species (cows, pigs, chickens), and wildlife (e.g., opossum, deer, raccoons, etc.). Linking these sources to likely transport pathways, such as wastewater discharges, stormwater, agricultural runoff, or direct deposition, can then result in more cost-effective strategies to reduce contamination and associated health risks to water users.

MST may include a combination of microbiological, genotypic, phenotypic, and/or chemical based methods (Scott et al. 2002; Mayer et al. 2018). Early MST studies were primarily dominated by library-dependent methods, which relied on biochemical or antibiotic resistance-based typing of cultured isolates like *E. coli*. These isolates (fingerprints) are compared to isolates from known fecal sources (Boehm et al. 2013). Examples of these included repetitive sequence-based PCR, pulse-field gel electrophoresis, and ribotyping methods (genotypic), as well as antibiotic resistance analysis (phenotypic) methods (Korajkic et al. 2016). While some of these may still be used today most research has moved to library-independent methods such as quantitative PCR (qPCR) and digital PCR (dPCR). Indeed, MST research over the last decade has primarily focused on developing qPCR assays, and more recently community analysis methods (e.g., DNA microarrays; Phylochip, and high-throughput Illumina DNA sequencing). One of the recommendations from the literature review conducted for the IJC predecessor study (IJC 2021b) is that it may be necessary to use multiple markers (both bacterial and viral) to identify any one source (Harwood et al. 2014) and any proposed marker for use in MST should be evaluated for its appropriateness and usability (Ahmed et al. 2019).

With the predecessor study as background and the expertise of workshop participants, the MST workshop addressed the potential role and methods that could be incorporated into the Great Lakes LBMWQ study.

2.1.2 Workshop findings and recommendations

The workshop summary of findings and recommendations are provided in Appendix B and summarized in this section.

Objective 1: Method Readiness and Barriers

Finding 1: qPCR and digital PCR have been widely used around the Great Lakes for MST (Table 2-1) and participants generally agreed that these technologies make sense as an anchor for MST in the Great Lakes basin, providing a reasonable balance of method specificity, sensitivity, and cost (Table 2-2). Both types of instruments allow for broad application (Table 2-2), although digital PCR offers advantages with respect to analytical sensitivity.

Table 2-1. Degree of Use of Various MST Methods in the Great Lakes.

Method Category	Instrumentation & Variations	Maturity	Degree of Use	Recent Applications in the Great Lakes
Bacterial PCR	RT qPCR, dPCR, microarrays	Mature		Staley et al. 2018a,b; Edge et al. 2021
Viral PCR	RT qPCR, dPCR, microarrays	Mature		Ahmed et al. 2018
High Throughput Sequencing	16S rRNA, metagenomic	Developing		Brown et al. 2017; Nevers et al. 2017; Roguet et al. 2020
Optical Sensing	Library dependence	Nascent		Corsi et al. 2021
Antibiotic Resistance Analysis	Library dependence	Mature		Edge et al. 2018
Ribotyping, Pulse-field gel electrophoresis, Denaturing gradient gel electrophoresis	Library dependence	Legacy		Ram et al. 2007
Carbon and Nutrient Utilization Profiles	Library dependence	Legacy		

Table 2-2. Comparison of MST Method Categories for Sensitivity, Specificity, and Cost.

Method Category	Methodological Variables	Marker Coverage	Marker Specificity	Cost
Bacterial PCR	Molecular preparation, PCR assays			
Viral PCR	Molecular preparation, PCR assays			
High Throughput Sequencing	Molecular preparation, bioinformatics			
Optical Sensing	Optical properties, regression models		Unknown	
Antibiotic Resistance Analysis	Library depth, molecular preparation	Non-specific	Non-specific	
Ribotyping, Pulse-field gel electrophoresis, Denaturing gradient gel electrophoresis	Library depth, molecular preparation	Non-specific	Non-specific	
Carbon and Nutrient Utilization Profiles	Library depth, molecular preparation	Non-specific	Non-specific	

Recommendation: Use either the qPCR or digital PCR platform for the large-scale survey, but compare these in the round robin evaluation of methods.

Finding 2: To allow evaluation of a range of watershed types (urban, rural, and mixed use), priority fecal sources to identify are human, ruminant, waterfowl, and dog.

Recommendation: The round-robin interlaboratory comparison should focus on four markers and develop an inventory for several potential markers that could be used in the large-basin survey. The markers and methods should be validated for fecal and sewage samples from across the GL basin.

Finding 3: Consisting of more than 40 laboratories, the portfolio of existing MST analysis capabilities was considerable across universities and government agencies around the Great Lakes, although there are geographic gaps that were observed in preliminary results, such as Lake Superior in Canada and Lake Ontario in the U.S.

Recommendations:

- Consider building upon growing public familiarity with PCR and widespread government investments in PCR for COVID and build linkages between wastewater COVID testing networks and MST across the Great Lakes basin to build the portfolio of laboratory capabilities.
- Make the laboratory inventory a living document that can be updated.

Finding 4: There are number of procedures but not many "standard" methods[5], thus there is a need to ultimately provide the protocols for the large basin study.

Recommendation: Identify best practices and normalization of protocols and build upon U.S. EPA standard methods and MST reference materials to address variation in method implementation between laboratories. This could be accomplished as a part of the round-robin evaluation.

Finding 5: Most long-term data which have spatial coverage are associated with standard fecal indicator bacteria data based on recreational epidemiological studies.

Recommendation: Consider how to expand risk criteria (and standards, potentially), and map health risks beyond *enterococci* and *E. coli*, to targets such as HF183 and other options.

Objective 2: Current Policy and Management Strategy Gaps

Finding 1: Sharing data with the public will facilitate understanding and provide evidence for their support of action.

[5] USEPA has two approved analytical methods for MST analysis: 1696.1 (HF183/BacR287 TaqMan) and 1697.1 (HumM2 TaqMan). USEPA has also approved several qPCR-based microbial analytical methods, including 1642, 1643, 1609.1, 1611.1, and Method B (note that these are not source specific).

Recommendations from the workshop:

- Set up a MST database dashboard for the large basin study that can be shared with stakeholders and the public at large. IJC could take the lead in setting this up.
- Look into how GIS data can be accessed and used to support MST sampling programs.
- IJC should be the keepers of the data behind the dashboard and the GIS data.
- *[Note: As described in Section 1.5, the IJC is not in a position to serve the study in this function.]*

- Develop a management system that provides triggers for going beyond closing a beach and launching MST analysis.
- Identify gaps for knowledge outside of Areas of Concern in the Great Lakes, such as gaps related to reporting on state and trends of source water for drinking around the Great Lakes (are sources getting better/worse?).

Finding 2: There is a need to understand sources of human and zoonotic *Cryptosporidium* and *Giardia* in source drinking water.

Recommendations:

- Consider including protozoa monitoring as part of the large basin study, following on the Information Collection Rule in the U.S.
- Beach management should transition from pollution response to pollution prevention. Likewise, beach posting decisions should move towards stronger health risk-based decisions.

Objective 3: Human Health Risk Map Plan

Finding 1: Data harmonization and data management will be extremely important as a large-basin study is undertaken.

Recommendation: Develop an effective exchange and management system of existing data and for the harmonization of new data across the study and laboratories.

Finding 2: Selection of surveillance study sites for a large basin study should include consideration of beaches and source waters for drinking outside Areas of Concern. Tribal and other public perspectives consider recreational waters in the Great Lakes to extend beyond beaches.

Recommendation: Leverage data from other sources and programs. Develop partnerships with agencies and groups already collecting data (e.g., ECCC and Nearshore Framework).

Finding 3: Design of the large basin surveillance study to develop human health risk maps should consider rolling out the study on a pilot scale first, before conducting the basin-wide surveillance study.

Recommendations:

- Develop a GIS-based approach to map and communicate potential exposure, including source assessment. This could lead to future assessment of health risks.
- Select potential pilot areas: Lake Huron (and connecting channel); Lake St. Clair (with Clinton River); western Lake Erie; Lake Ontario, as all have experienced challenges meeting beach closing criteria (Figure 2-1).
- Include building recommendations for surveillance beyond Areas of Concern (AOC) into Health Risk Mapping Plan.

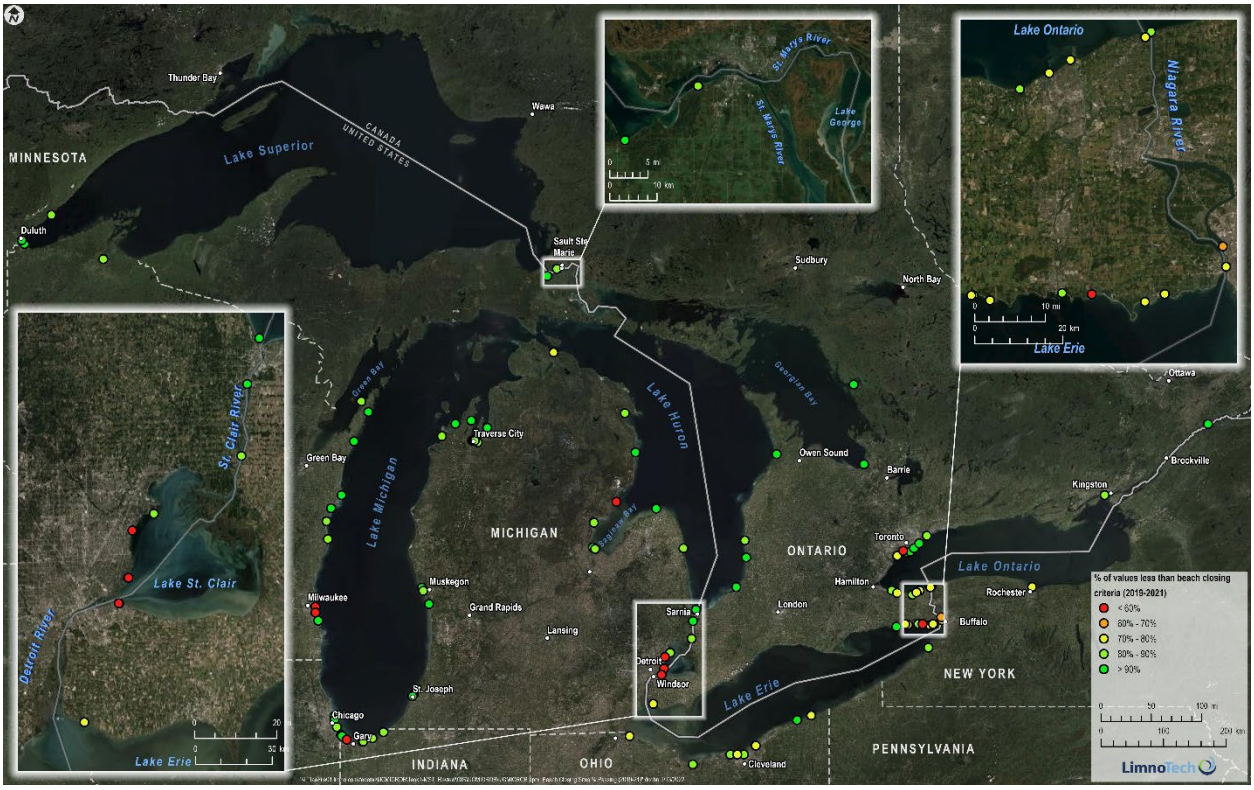


Figure 2-1. Frequency of Beach Data Below Management Criteria, 2019-2021

Objective 4: MST Laboratory Round Robin

Finding 1: To run a successful round-robin, the objectives need to be clarified along with potential sub-objectives. The workshop goal, as stated, was to identify the best MST targets for the large basin study, validate them for Great Lakes fecal pollution sources, and assess the reproducibility in using these targets across different geographies, matrices and laboratories.

A non-exhaustive marker list was included in the workshop materials (Table 2-3). Key considerations for methods and markers include specificity and sensitivity. Although there are no benchmark criteria for host specificity, the literature review conducted for the IJC Centennial study suggested methods that have a specificity >0.9 are excellent, but even methods >0.8 are useful (Ahmed et al. 2019; Boehm et al. 2013). Viral markers tend to be highly specific (e.g., HAdV, HPyV) but often lack sensitivity due to low levels.

Table 2-3. Non-Exhaustive Marker List. This list of markers is not intended to be a complete list of every marker. Methods for markers continue to be developed.

Host	Marker	Host	Marker
Human	*HF183/Bac708*	Ruminant	*Rum2Bac*
	HF183/BacR287		*CowM2*
	USEPA 1696		*CowM3*
	Hum2	Pig	*Pig2Bac*
	USEPA1697	Chicken	*LA35*
	B. theta	Bird	*GFD*
	Lachno3		*Gull4*
	crAssphage	Dog	*DG3*
	PMMoV		*DG37*
	MtDNA		
	other		

Recommendations:

- Define objectives for the round-robin exercise up front and identify nested goals.
    - Build consensus on methods, markers, etc.
    - Capture full range of sample media expected in the basin study as part of the round robin.
- Standardize protocols and implement a robust QA/QC program for comparability between labs.
    - Use reliable (e.g., NIST certified) standards (e.g., for the standard curve with qPCR), if possible. Leveraging work being done by USEPA (specifically, by Orin Shanks) to develop and promulgate methods (e.g., EPA methods 1696 and 1697 [USEPA 2019a, b]) could help achieve this recommendation.

2.2 Harmful algal bloom tools

2.2.1 Background

Harmful algal blooms (HABs) occur when key waterbody conditions (temperature, light, and nutrient levels) allow colonies of cyanobacteria or algae to proliferate out of control. Cyanobacterial HABs can produce toxins (microcystin is an example) that can cause illness or kill humans, pets, livestock, and wildlife. These toxin-producing HABs are of the most interest to this project, though it should be noted that nontoxic algae can also adversely affect the aquatic ecosystem in several ways, including causing fish kills by consuming oxygen, smothering habitat for submerged aquatic vegetation, producing taste and odor issues compounds that degrade recreational and drinking waters, and clogging the gills of fish and invertebrates.

Exposure pathways include accidental ingestion and skin contact from swimming and water recreation, drinking water with inadequate treatment to remove the toxins, inhalation of aerosols by beachgoers and lakeshore residents, and occupational exposures, such as workers in water plants, power plants, agriculture (irrigation), and commercial fishing.

Toxin-producing HABs reached mainstream consciousness with the Toledo water crisis in 2014, in which half a million people were unable to drink or use their tap water for three days because of high levels of microcystin from a HAB in western Lake Erie (used as the City's water supply). However, HABs outbreaks have been detected at multiple locations throughout the Great Lakes (Figure 2-2) and with an increased frequency because of high nutrient loads to the lakes and the effects of climate change (IJC 2017). In addition, the number of cyanobacteria species (and associated toxins) detected in the Great Lakes is also increasing as a result of climate change.

Due to concerns about the health risks posed by toxin-containing HABs, strategies to address knowledge gaps, support monitoring, set protective health criteria, and mitigate outbreaks have been developed by various stakeholders in the area, including the IJC, state government agencies (e.g., Ohio EPA), municipalities, water utilities, nongovernmental organizations, and universities (IJC 2017; State of Ohio 2017; NSTC 2017; GLC 2021).

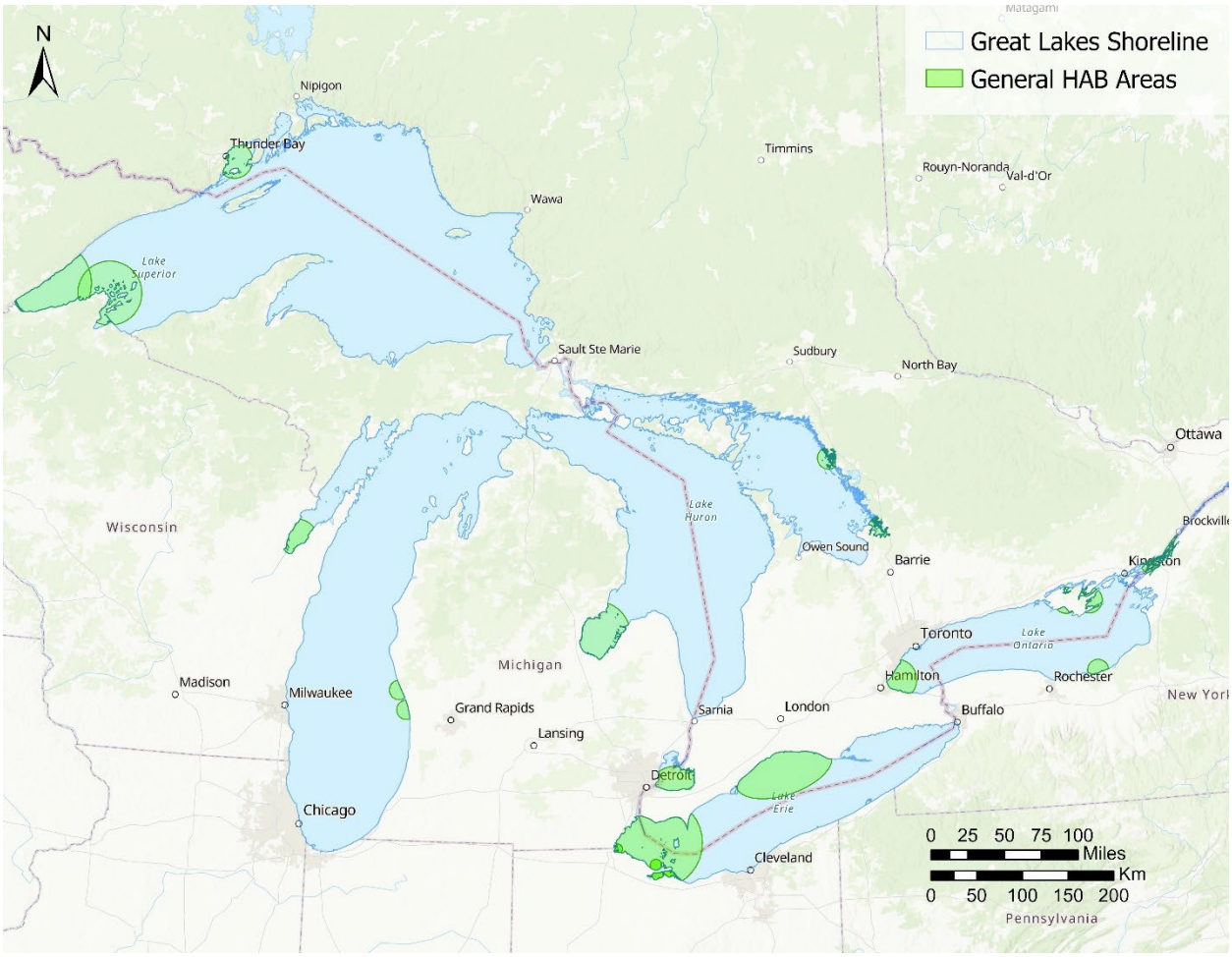


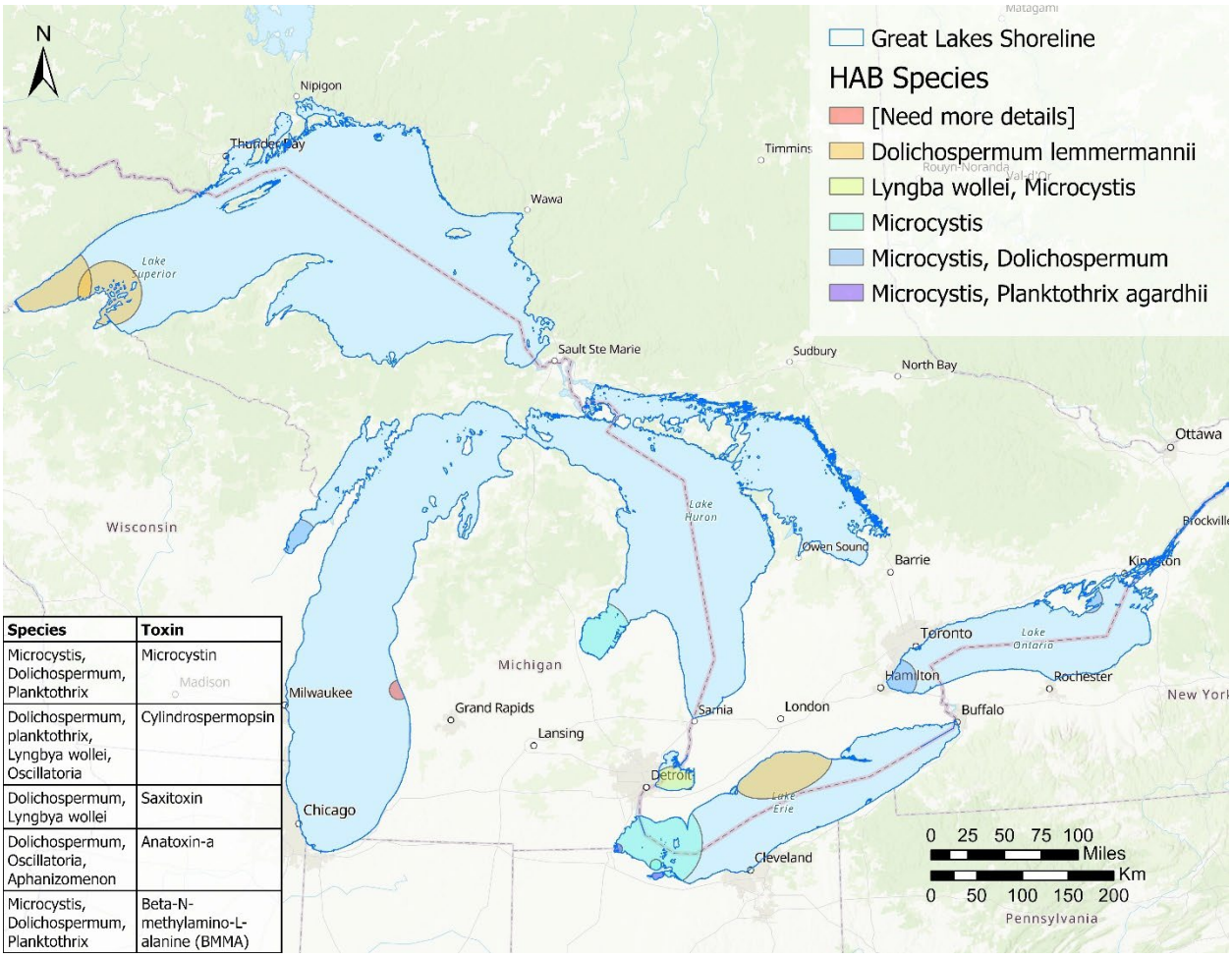
Figure 2-2. Occurrences of HABs in the Great Lakes (non-exhaustive).

The type of HAB depends on both the cyanobacterial species composition and toxins each produces. Table 2-4 provides a summary of the relationship between some HAB species and associated toxins (Graham et al. 2008, Bernard et al. 2017). It should be noted that toxins from HABs are still being discovered.

Table 2-4. Summary of HAB Species and Toxins.

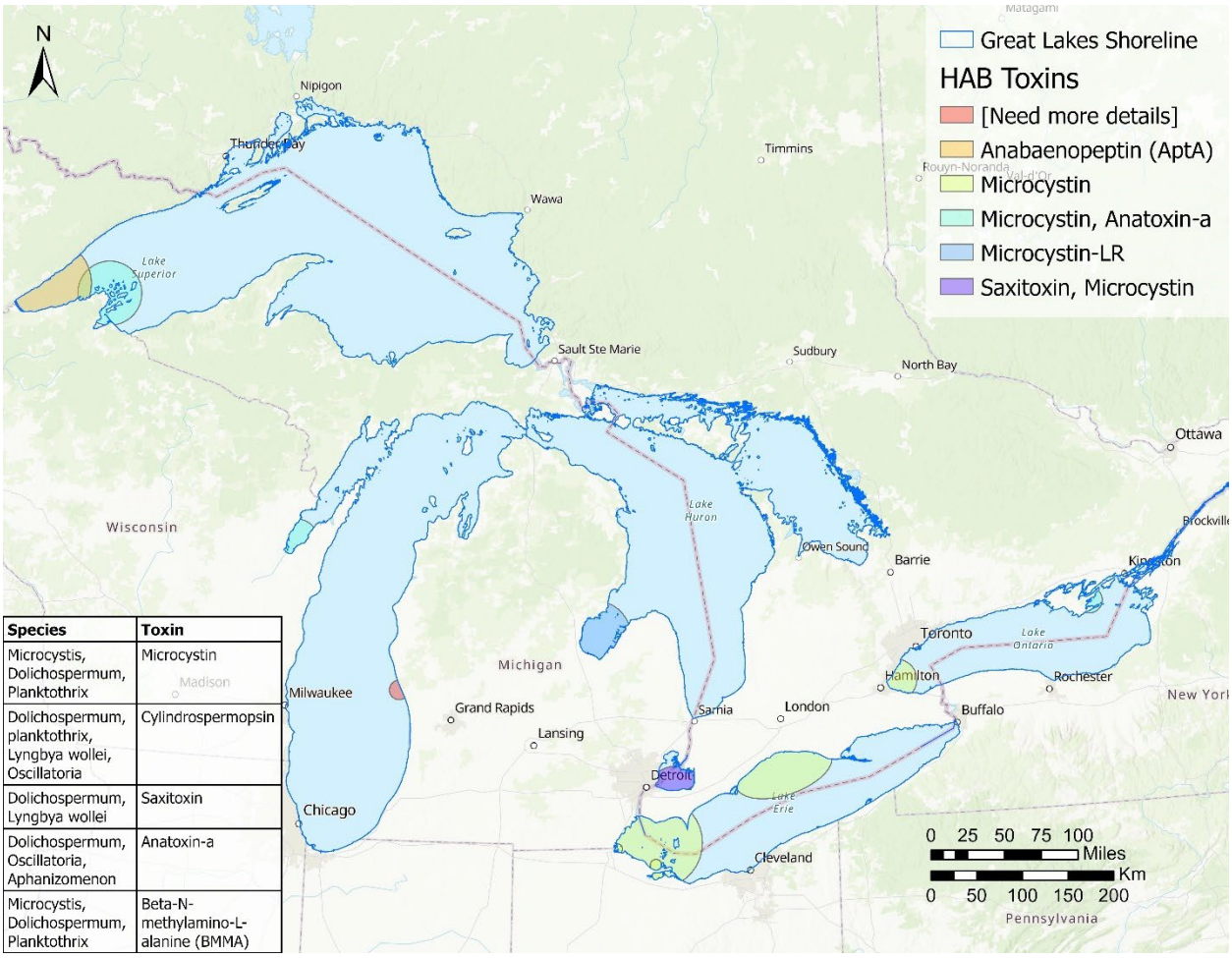
Species	Toxin				
	Microcystin	Anatoxin(s)	Cylindro-spermopsins	Saxitoxins	beta-N-methylamino-L-alanine
Microcystis	Y				Y
Pseudanabaena	Y	Y			
Planktothrix	Y	Y	Y	Y	Y
Aphanocapsa	Y				
Dolichospermum	Y	Y	Y	Y	Y
Cuspidothrix		Y		Y	
Limnothrix				Y	
Merismopedia	Y				
Aphanizomenon	Y	Y	Y	Y	
Phormidium	Y	Y		Y	
Anabaenopsis	Y				
Calothrix	Y				
Cylindrospermopsis			Y	Y	
Nostoc	Y				
Anabaena	Y	Y	Y	Y	
Cylindrospermum		Y		Y	
Synechococcus	Y				
Lyngbya wollei			Y	Y	
Oscillatoria		Y	Y		
Aphanizomenon		Y			

As Table 2-4 indicates, one cyanobacterial species can produce multiple toxins. Similarly, one toxin can be produced by more than one cyanobacteria species. Figure 2-3 and Figure 2-4 show the distribution of Great Lakes HABs by species and by toxin, respectively.



Species	Toxin
Microcystis, Dolichospermum, Planktothrix	Microcystin
Dolichospermum, planktothrix, Lyngbya wollei, Oscillatoria	Cylindrospermopsin
Dolichospermum, Lyngbya wollei	Saxitoxin
Dolichospermum, Oscillatoria, Aphanizomenon	Anatoxin-a
Microcystis, Dolichospermum, Planktothrix	Beta-N-methylamino-L-alanine (BMMA)

Figure 2-3. Great Lakes HABs by Species



Species	Toxin
Microcystis, Dolichospermum, Planktothrix	Microcystin
Dolichospermum, planktothrix, Lyngbya wollei, Oscillatoria	Cylindrospermopsin
Dolichospermum, Lyngbya wollei	Saxitoxin
Dolichospermum, Oscillatoria, Aphanizomenon	Anatoxin-a
Microcystis, Dolichospermum, Planktothrix	Beta-N-methylamino-L-alanine (BMMA)

Figure 2-4. Great Lakes HABs by Toxin

The range of cyanobacteria species, strains within a species, and possible toxins presents significant challenges for determining when a bloom presents a health risk to humans and animals. A number of strategies have been used to detect and manage HABs, including measuring the density and/or biomass of the bloom, toxicity via potential toxicity via biochemical or genetic methods, and species or strain identification and activity. Technologies and analytical methodologies that have been used to monitor HABs include:

- Satellite and aerial remote sensing
- In situ pigment sensors on buoys, mobile platforms, and fixed stations
- Microscopy
- Flow cytometry (e.g., FlowCam)
- Enzyme–linked immunosorbent assays (ELISA)
- Protein phosphatase inhibition assay (PPIA)

- Reversed-phase high performance liquid chromatographic methods (HPLC) combined with mass spectrometric (MS, MS/MS) or ultraviolet/photodiode array detectors (UV/PDA).
- Liquid chromatography/mass spectrometry (LC/MS)
- DNA-based analysis with conventional polymerase chain reaction (PCR), quantitative real–time PCR (qPCR), microarrays/DNA chips, DNA sequencing, and metagenomics

Each of these methods were explored further during this project's HABs workshop. qPCR methods were of particular interest for the Great Lakes LBMWQ study due to the overlap with the recommendations to use qPCR and dPCR for MST analysis. qPCR and dPCR methods for HABs are still developing as more primers become available but have the same advantages as when used for MST analysis, including suitability for water or sediment matrices (Mejbel et al. 2021), ability to identify and quantify species and strains, applicability to both DNA and RNA, and fast turnaround of results. Among the HABs monitoring options listed, DNA-based methods offer the best identification, sensitivity, and specificity for characterizing different HAB-forming species and toxins, as well as for distinguishing toxigenic vs. nontoxigenic strains of the same species (Feist and Lance 2021).

The workshop benefited from having many of the participants who had collaborated previously on several seminal HABs studies in Lake Erie, the "HABs Grab" (Chaffin et al. 2021). The "HABs Grab" sampling strategy was discussed as a potential model for conducting the Great Lakes LBMWQ study. With the "HABs Grab" surveys as background and the expertise of workshop participants, the HABs workshop addressed the potential role of HABs and methods that could be incorporated into the Great Lakes LBMWQ study.

2.2.2 Workshop findings and recommendations

The workshop summary of findings and recommendations are provided in Appendix B and summarized in this section.

Objective 1: Review existing lab capacity and establish a plan for developing a Great Lakes laboratory network for HABs assessment using PCR methods. Scope out the HABs laboratory intercomparison round robin effort, including implementation and costs.

Finding 1: Laboratories (over 10) were identified as resources for HABs analysis, but this was recognized as a minimum as more labs are likely.

Recommendations:

- Develop an inventory of primers and targets that people are using and incorporate them into the interlaboratory comparison.
- Bring together the large HABs community that's been working on the topic for decades (but has less application of PCR compared to other methods) with PCR researchers who are very skilled (many are early career).

Finding 2: PCR is not the most common tool used for assessing HABs, and PCR methods are less widely adopted for HABs than MST (there are probes/primers for major HAB species).

Recommendations:

- Recommended targets for basin-wide sampling: total cyanobacteria (16S), species/strains of Microcystis (eutrophic systems), Dolichospermum/Anabaena complex (oligotrophic and offshore), and Planktothrix (river mouths, Sandusky Bay, winter); McyA and McyE genes conserved across species.
- Given its increasingly common use and rapidly declining cost, advantages and efficiencies over PCR (e.g., simultaneous detection of genes for all toxins and for emerging toxins), and synergies with whole ecosystem approaches (2.3), metagenomics (i.e., whole-genome shotgun sequencing) should be considered a key tool for assessing HABs.
- For a basin-wide study, standardize sampling (vessels, drinking water intakes), sample preparation (filtering parameters, DNA extraction) and analysis.
- Consider including samples with a range of complexity (single strain, mixed known strains, lake sample) in a laboratory round robin, as well as evaluating extraction and heterogeneity issues, and deciding whether to prioritize DNA over RNA.
- The components for analysis could include bloom samples, culture samples, and extracted DNA components for analysis.

Finding 3: Practical needs (monitoring and early warning systems) are very different from research approaches (e.g., need for rapid turnaround).

Recommendations:

- The "HABs Grab" may be useful as a guide: Chaffin et al. (2021).
- Use a tiered approach (most often used), along with satellite imagery, toxins, pigments, etc., for HABs evaluation.

Objective 2: Review known HABs locations and existing qPCR and dPCR HABs data, including technologies used in workshop participants' lab, institution, or region and associated threats to human health. Outline a plan for a surveillance study to develop HABs health risk maps for each Great Lake, estimate costs, and propose one lake as an initial case study.

Finding 1: Looking at past data to better understand what triggers blooms (environmental metadata are very important) can be valuable for determining sampling strategies.

Recommendations:

- A wealth of data exists, so the first need is to fund a compilation of information on both sides of the border, with the next steps being to standardize data format and to host data in a single repository.

- There are a lot of stakeholders with different interests who can use the data; and forecasting blooms vs. reacting to them means many participants have been sampling for other parameters along with sampling blooms.

Finding 2: Adaptive sampling approach is an important sample design consideration. Sampling is often initiated in response to a visible bloom while other programs rely on routine sampling.

Recommendations:

- Use an adaptive sampling approach that includes having regular sampling stations (master/core/sentinel stations) and having reactive sampling in response to a visible bloom trigger.
- Support a pilot project to standardize sampling and analytical methods, knowing it's easier to get funding for new data collection (HABs Grab project could serve as a guide).
- Develop an inventory of primers and targets that people are using to incorporate into an interlaboratory comparison.

Finding 3: A pilot project is needed for sampling and analytical method standardization, and the study may likely get more funding if there is new data collection.

Recommendations:

- Consider including remote sensing in the study, as it is good for tracking growth and movement of large blooms and is well established in the U.S., noting the following potential hindrances to inclusion in this study:
    - Remote sensing works better in open waters than in nearshore, which may be a hindrance.
    - Additional work on remote sensing, to standardize between the U.S. and Canada, would be needed before implementing it into a Large Basin study.

Objective 3: Identify gaps in current policy strategies to address HABs water quality issues pertinent to the lakes and their management. Identify readiness and barriers to transitioning HAB-related qPCR and dPCR technologies to operational use by management agencies and give examples of successful transitions.

Finding 1: There is a need for guideline standardization across U.S.-Canada for the series of HABs toxins.

Recommendations:

- Given that current safety guideline thresholds are based on swimming exposure rather than aerosol exposure, consider adding acute and chronic exposure to HABs toxins as part of the guideline development.
- Create a binational standardized guideline for HABs toxins.

Finding 2: Data transparency is important, so public managers have the information and understand the information (need to share technical knowledge).

Recommendations:

- Support increased transparency of water quality data with respect to toxins (i.e., create a dashboard).
- Develop strategies to overcome the reality that data may be held by municipality or province/state, and so may be a challenge to obtain (Ohio might provide a good example to follow for data sharing). The jurisdiction of beaches, etc., may make it difficult to maintain lines of communication.

Finding 3: Discussion of the need to expand the role of community and citizen science in HABs management.

Recommendation:

- Develop ways to expand the role of community and citizen science in HABs management, such as with non-profits or municipal organizations.

Finding 4: qPCR is not the current gold standard adopted for assessing HABs (ELISA, LC-MS and HPLC most used for HABs assessment).

Recommendation:

- Investigate opportunities to use a variety of targets to detect HABs, including metagenomic methods.

Finding 5: Barriers to adopting new assessment approaches include lab capacity, established SOPs and technical knowledge sharing.

Recommendation:

- Develop a multi-tiered approach to create a standardized decision tree to invoke action when/if HABs are potentially detected.
- Develop capacity for education and training, i.e. workshops, short courses, etc.

2.3 Ecosystem health and metagenomics tools

2.3.1 Background

Metagenomics is a genomic-based characterization of microbial communities that can be applied to water samples for ecosystem and human health assessments. As a community-based assessment, it reflects a change in orientation towards analysis of all microbes and functions

(e.g., toxins, metabolism, etc.) in a water sample from traditional microbe-by-microbe or analyte-by-analyte testing methods that target one or a few taxa and markers of function. Metagenomics has broad applicability, including supporting both the MST and HABs portions of the Great Lakes LBMWQ study. For fecal source tracking (MST), metagenomics allows for assessments of the similarity of all water microbes to gut microbes or cells from human and diverse mammal and avian gastrointestinal systems (Staley et al. 2018b). For algal blooms (HABs), a metagenomics-based sampling strategy can and has been used to inform the taxonomic, toxin gene, and metabolite composition of blooms. An additional potential application is to soil seeding, in which the types and proportions of soil microbes in water samples are evaluated with respect to the impact of land use practices and runoff on microbial water quality. In addition, aquatic microbiomes can be considered with respect to the implications for microbial colonization and health of aquatic plants and animals.

"Metagenomics" is used in this report as an umbrella term, but there are important distinctions within this field of study in sampling and analytical approaches that are summarized below:

- Metagenomics: Study of the structure and function of entire nucleotide sequences isolated and analyzed from all the organisms (typically microbes) in a bulk sample (NIH 2022).
- Metagenome: The genetic content of any group of microorganisms (NIH 2022).
- Barcode: A short variable gene region useful for taxonomic assignment flanked by highly conserved gene regions which can be used for primer design (Hebert et al. 2003).
- Metabarcoding: The barcoding of DNA/RNA (or eDNA/eRNA) in a manner that allows for the simultaneous identification of many taxa within the same sample (Hebert et al. 2003).
  Note: 16S, 18S, and other metabarcoding are not considered metagenomics by most researchers working in this field.
- Metatranscriptomics: The metagenomic characterization of the RNA content of samples, which can be used to identify the microbial members of an ecosystem while simultaneously obtaining functional insights about a microbial community (Dash et al. 2018).
- Proteomics: Study of the proteomes, which are sets of proteins produced or modified by an organism or system (EMBL-EBI 2022).
- Metabolomics: Study of small molecules (metabolites) within cells, biofluids, tissues, or organisms; metabolites and their interactions within a biological system are known as the metabolome (EMBL-EBI 2022).
- Phenomics: Study of phenotypes (physical and biochemical traits) of organisms (Jin 2021).

A metabarcoding approach has been used with HABs testing, specifically sequencing the 16S rRNA gene, a highly specific, fast turnaround, and cost-effective way to identify the bacterial taxonomic composition (in the case of HABs, cyanobacteria in particular). Several studies utilizing the 16S sequencing methods have been conducted in the Great Lakes (MacGregor et al.

1997; Kim et al. 2015; Fujimoto et al. 2016; Denef et al. 2017; Paver et al. 2019; Butler et al. 2019; Rozmarynowycz et al, 2019; McKindles et al. 2020; Shahraki et al. 2021; Grim et al. 2021; Palermo et al. 2021; Chaudhary et al. 2021; Zepernick et al. 2022). Summaries of a select number of studies compiled for this project are included as Appendix C. Many of these studies include other sequencing methods in addition to the 16S methodology.

There are several current genomic initiatives in the Great Lakes that are relevant to consider in developing the scope of this Great Lakes LBMWQ study, including:

- Ecobiomics Project by the Canadian federal government looking at water quality and soil health: canada.ca/en/environment-climate-change/services/biodiversity/ecobiomics.html
- Great Lakes Genomics Center at the University of Wisconsin – Milwaukee School of Freshwater Sciences
- NOAA Great Lakes Environmental Research Laboratory/ Cooperative Institute of Great Lakes Research Omics Program, which includes universities and companies in the Great Lakes consortium
- Great Lakes Institute for Environmental Research Environmental Genomics Facility, which supports the Genome Canada GenFISH Program: uwindsor.ca/glier/336/environmental-genomics-facility
- Joint Genome Institute sponsored by the U.S. Department of Energy
- Open Science Earth Microbiome Project with >500 investigators earthmicrobiome.org/ (tandardsingenomics.org/content/3/3/243/)

As a relatively new technology, questions about the use of metagenomics that are pertinent to the Great Lakes LBMWQ study include identifying what can be learned from these advanced approaches, how to transition to metagenomics technologies for operational and management decisions in the near and long term (~100 years), and how a metagenomics database could support water quality preservation or enhancement. The metagenomics workshop brought together participants working within and outside of the Great Lakes to address the potential role and methods that could be incorporated into the Great Lakes LBMWQ study.

2.3.2 Workshop findings and recommendations

The workshop summary of findings and recommendations are provided in Appendix B and summarized in this section.

Objective 1: Review existing metagenomics lab capacity and establish a plan for developing a Great Lakes laboratory network for metagenomics assessment.

Finding 1: The Great Lakes basin has the necessary lab capacity to do metagenomics work in Canada and the United States.

Recommendation: Lead laboratories, including a minimum of one in Canada and one in the United States, should be established to train and work with a consortium of experts who would

address the key methodological questions in implementing a large-scale genomics study of Great Lakes water quality. Key elements needed are described below.

- The lead laboratories should cover metabarcoding, metagenomics and metatranscriptomics sequencing and analysis, with strong QA/QC, and standardized and comparable protocols.
- Standardize, as much as possible, without being too prescriptive on protocols.
- Train more personnel in metagenomics bioinformatics, data analysis and interpretation to translate the data to a public health action.
- Decide on minimum data standards and metadata requirements, consistent with the larger surveillance study.
- Attempt to standardize bioinformatics pipelines and data management, consistent with the larger surveillance study.
- The purpose and design of the surveillance study needs to inform the design of a round-robin approach (Note: this approach is different from the MST and HABs approaches, where the round robin design can be independent from the surveillance study design.)

Finding 2: There are large ongoing metagenomics studies of microbial water quality in the Great Lakes basin that can provide models for how to incorporate metagenomics into the Large Basin Microbial Water Quality Study (Lake Erie Western Basin algae research; Canadian federal government Ecobiomics Project; Genome Canada ATTRAPP Project, etc.)

Recommendation: Multiple agencies should form an alliance to formulate how data being gathered for metagenomics research could address multiple questions and projects. Emphasizing the multiple uses of the data collection can help bolster support from funders.

Finding 3: Bioinformatics, data analysis and data management need to be well-coordinated for the successful incorporation of metagenomics into the Great Lakes study.

Recommendation: Multiple agencies should form an alliance to formulate how data being gathered for metagenomics research could address multiple questions and projects. Emphasizing the multiple uses of the data collection can help bolster support from funders.

Objective 2: Review recent or current metagenomics projects and technologies used in your lab, institution, or region and associated advances and gaps related to ecological and human health. Outline a plan for a basin-wide and cross-phyla surveillance study to develop associated microbial ecological and human health risk maps for each Great Lake, estimate costs, and propose one lake as an initial case pilot? study.

Finding 1: There is a lot of great metagenomics work going on in the Great Lakes, with many technologies broadly embraced.

Recommendations:

- Link this study to the Great Lakes Decadal Science Strategy, with centers of excellence, emerging technologies, long-term monitoring/sampling stations in each lake.
- Leverage existing sequencing and bioinformatics platforms to develop the surveillance study plan.
- Develop a center for bioinformatics, as it will be critical to have bioinformatics personnel support, to integrate and train students on bioinformatics (i.e., a center of excellence for bioinformatics training).

Finding 2: Although several options were identified for risk mapping, there was broad support for using a nested approach to apply metagenomics on a subset of samples. Other options considered included coordinating sampling across the lakes all at one time (HABs grab approach), use one lake as a pilot, or establish sentinel sites on all lakes.

Recommendations:

- Use a spatial and temporal nested approach to sampling, as there is a need to acknowledge that we cannot do every analysis on every sample (but samples could be archived for later analysis).
- Focus the scope of the project to consider/include key locations, such as long-term water quality monitoring sites, drinking water intakes, recreational waters, and the land-to-water connection of major tributaries.
- Develop Western Lake Erie Basin or Lake Huron as a potential pilot location.

Objective 3: Identify what we can learn from these advanced technologies (e.g., the value that metagenomics can bring to understanding the ecological systems and effects of climate change), how utilizing these new tools can advance our knowledge, and what impacts this would have on broader policies and rules. Identify readiness of transitioning to metagenomics technologies for operational use by management agencies in the near and long term (~100 years) and give examples of successful applications or collaborations.

Finding 1: Consensus that data accessibility and management is very important.

Recommendations:

- Plan data management up front as part of study planning to address:
    - Processing raw data into manageable units for wider access
    - Archiving raw data
    - Managing associated meta-data
- Design a sample program to address both "baseline" and "background" information and consider minimum metadata requirements before sequencing.

- Make data and metadata available to the public to allow for the data to have multiple uses.

Finding 2: The value of including metagenomics in the Great Lakes study is that it provides a powerful and completely new method of tracking water quality, ecosystem health, and how ecosystems respond to climate change, emerging contaminants, invasive species and other threats. This is a huge opportunity to take a fresh look at the Great Lakes environment and gain knowledge, which is an opportunity that government agencies have supported.

Recommendations:

- Develop a strong justification for decision-makers and potential funders to support the value of metagenomics research in the Large Basin Study.
- Research existing initiatives and programs to nest the metagenomics effort within existing programs.
- Conduct a review on Great Lakes metagenomics projects and research to guide selection of study sites, and key aspects like sampling, nucleic acid extraction, and sequencing approaches to date.
- Use this review to set sentinel (e.g., background) and baseline sites, what to assess (e.g., communities of bacteria, algae, viruses, all microbes), how to assess (e.g., metabarcode, shotgun sequence, etc.), and how to interpret data (e.g., identifying changes).

Finding 3: Agreement that the data can be used beyond objectives of a Great Lakes study to understand ecosystem disruption and resiliency, and associated potential public health risks, complement restoration efforts, and support impairment and ecosystem disruptions.

Recommendations:

- Classify key environments (e.g., beaches, harbors, bays, drinking water intakes) for sampling and leverage sampling strategies for MST and HABs to include metagenomics.
- Identify key gene sequences or sentinel species that indicate resiliency and/or provide early warning of trends at a micro-level before macro-biology effects occur.

2.4 Research priorities

A survey was distributed to all workshop participants prior to the last workshop to elicit feedback on several topics, including research priorities. Twenty-four (24) responses were tallied and summarized. MST and HABs were ranked higher in research priority, in comparison to metagenomics, by the individuals who attended the workshops (Table 2-5). As the table illustrates, survey respondents were also provided an opportunity to weigh in on other important factors in developing the Great Lakes LBMWQ study plan, including the degree of established infrastructure already in place, data management, logistical coordination for sampling, and access to funding.

The survey responses likely reflect the maturity of MST and HABs research and methods relative to the field of metagenomics. The workshop attendees noted that adequate funding, training, standardization of procedures and coordination across labs were all potential concerns for implementing HABs and MST work. Metagenomics was noted as having the same concerns, with the addition of cost.

Table 2-5. Summary of Research Priorities by Survey Participants (n=24)

Topic	Priority		
	1	2	3
Element Priority	HABs	MST	Metagenomic
Established Infrastructure	MST/HABs		Metagenomic
Manageable Data	HABs/MST		Metagenomic
Logistical Coordination	MST/HABs		Metagenomic
Access to Funding	HABs	MST	Metagenomic

The workshop survey also included questions about funding sources for the Great Lakes LBMWQ study. The top potential funding sources identified by the workshop attendees were federal environmental or research agencies (Figure 2-5). Additional discussion on potential funding sources is provided in Section 4.

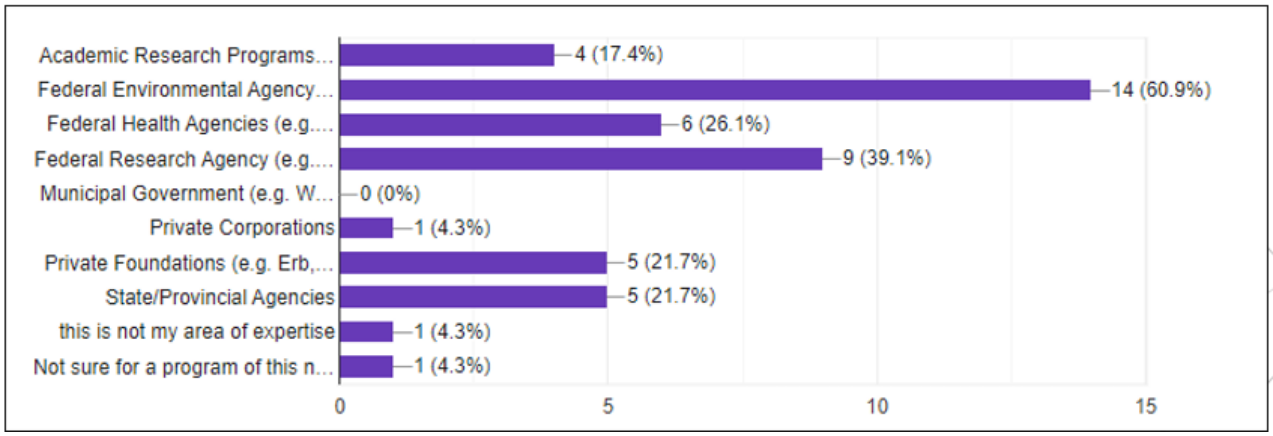


Figure 2-5. Ranking of Potential Study Funding Sources by Survey Respondents

2.5 Key recommendations

Several themes and recommendations were common to all three technical workshops, specifically:

- All three elements (MST, HABs, and metagenomics), should be used in the study, using MST and HABs structures and logistics to guide the sampling.
- Keep appropriate laboratory training, and standardization and coordination across labs as priorities in developing the framework. Limiting the number of sample processing labs may be helpful.
- Investigate funding opportunities from federal environmental agencies (U.S. Environmental Protection Agency, Environment and Climate Change Canada) and federal research agencies (U.S. National Science Foundation, Natural Sciences and Engineering Research Council of Canada). Look to expand existing sampling programs to meet the needs of this study as a way to increase the likelihood of getting funding.

3.0 Scope of Large Basin Microbial Water Quality Study Plan

A fourth workshop to synthesize the results of the three technical workshops and develop an implementation strategy was conducted in October 2022. Specific objectives were to:

- Determine the readiness of molecular and genomic tools for the Large Basin Microbial Water Quality study;
- Identify potential participating and collaborating partners; and,
- Synthesize the tabulation of potential laboratory network and capacity.

These objectives inform the scope of a large basin microbial water quality study plan. The results of the synthesis workshop are described in this section.

3.1 Readiness of molecular and genomic tools for microbial source water quality assessment

The general consensus coming out of the synthesis workshop is that molecular and genomic tools are sufficiently developed for use in a Great Lakes LBMWQ study. MST already has an established structure in place through beach monitoring programs. The Ohio EPA HABs response strategy (State of Ohio 2020) has a standardized monitoring strategy that includes qPCR-based methods

The IJC Centennial Water Quality Study report (2021) included a compilation of MST studies in the Great Lakes, which found that MST methods, including the qPCR and digital PCR methods considered in this project, have been used since the early 2000s. Several marker genes, such as *Bacteroides* HF183 (a human marker associated with sewage discharges), have become commonly applied across the basin (Alm et al. 2018; Dila et al. 2018; Edge et al. 2018; Nevers et al. 2018; Kinzelman et al. 2020; Edge et al. 2021; Shrestha et al. 2021) and HF183 has an approved EPA analysis method (Method 1696) (USEPA 2019a). These well-established and familiar methods would be the foundation of the MST portion of the Great Lakes LBMWQ study.

As discussed in Section 2.2, a wealth of data and methods for HABs are well established in the United States (Zuellig et al. 2021) and in the Great Lakes basin, spearheaded by studies in Lake Erie, particularly the western basin that affected the City of Toledo drinking water supply. Although qPCR methods for HABs are not used as often as other HABs methods, such as ELISA and LC/MS, it was agreed among workshop participants and work group leads that qPCR methods could be leveraged by MST laboratories as part of the Great Lakes LBMWQ study, along with the more traditional HABs analytical methods.

Within metagenomics, there are relatively mature methods, including sequencing of cyanobacterial genes encoding toxin biosynthesis. This sequencing is often a component of the analyses used in shotgun metagenomic studies that sequence genes from all of the organisms present in a sample (Yancey et al. 2022) (though for cost reasons, metabarcoding is often used to screen samples to determine which ones should be take through a full metagenomic analysis). Shotgun metagenomic sequencing methods serve as a starting point for a more detailed scoping of the pilot project and the larger basin-wide study discussed in Section 4.

Given that the molecular and genomic tools are sufficient for a study, the next consideration was identifying the value, from a human health risk perspective, of including each type of microbial technology in a new Great Lakes LBMWQ study.

- Including MST in the LBMWQ study is primarily to protect the health of recreational users, though it has been used for impaired waters assessment generally can can also assist with protection of source waters for drinking. These advanced tools can be used to identify fecal pollution sources and inform more efficient mitigation measures than traditional *E. coli* methods currently in use.
- Including HABs in the LBMWQ study is designed to protect the health of the members of the public whose drinking water source is the Great Lakes, as well as recreational users of the lakes. Data derived from employing advanced microbial tools can address both species and toxins associated with HABs and serve as an early warning to drinking water utilities of impending or potentially toxic blooms. HABs were ranked as the highest priority element by workshop participants.
- Including metagenomics in the LBMWQ study presents an opportunity to characterize the diversity of the microbial community, including providing early detection of ecosystem disruptions that can result in conditions that pose a threat to human and ecological health. Emerging toxins and pathogens are now being found in the Great Lakes, likely from the effects of climate change, and metagenomic techniques could help to identify new toxins/hazards before their levels impact human or ecosystem health. It can also be used as a complementary monitoring tool to assess improvement in Great Lakes Areas of Concern (AOCs) with traditional monitoring methods. These data can be used to establish linkages between limnological and biological research.

3.2 Potential participating and collaborating partners

Tables of some of the leading potential participating and collaborating partner organizations are provided below with basic information on potential roles (Table 3-1 and Table 3-2). The lists were compiled by reviewing the affiliations of workshop participants and considering other organizations that have mission responsibilities consistent with the project's goals but that did not participate in workshops. Laboratory capabilities, a critical consideration for the Great Lakes LBMWQ study, are addressed in Section 3.3. None of these lists is exhaustive and no named

entities or individuals have committed to participating in the LBMWQ study or serving in any of the roles indicated.

Table 3-1. Potential Academic Partner Organizations.

Organization	Location	Primary Lake(s)	Role	Contact
Cooperative Institute for Great Lakes Research	Ann Arbor, MI	All lakes	Investigators (multiple institutions)	Subba Rao Chaganti, Greg Dick
McMaster University	Hamilton, ON	Ontario, St. Clair, Erie	LBMWQ study co- lead	Tom Edge
Michigan State University	East Lansing, MI	Superior, Michigan, Huron, St. Clair, Erie	LBMWQ study co-lead	Joan Rose
University of Chicago	Chicago, IL	All lakes	Investigator	Maureen Coleman
University of Michigan	Ann Arbor, MI	All lakes	Investigator, data management lead	Greg Dick
University of Windsor	Windsor, ON	Huron, St. Clair, Erie	Investigator	Mike McKay

Table 3-2. Potential Agency Partner Organizations, Including Funders.

Organization	Location	Primary Lake(s)	Role	Contact
Canada Institute of Health Research	Ottawa, ON	Superior, Huron, St. Clair, Erie, Ontario	Investigator, funder	TBD
Environment and Climate Change Canada	Burlington, ON	Superior, Huron, St. Clair, Erie, Ontario	Investigator, funder	Ram Yerubandi
Erb Family Foundation	Birmingham, MI	St. Clair, Erie	Funder	Melissa Damaschke
Genome Canada	Ottawa, ON	Superior, Huron, St. Clair, Erie, Ontario	Investigator, funder	TBD
Health Canada	Ottawa, ON	Superior, Huron, St. Clair, Erie, Ontario	Funder	TBD
Michigan Dept. of Environment, Great Lakes, and Energy	Lansing, MI	Superior, Michigan, Huron, St. Clair, Erie	Investigator, funder	Shannon Briggs
National Institute of Environmental Health Sciences	Durham, NC	All	Funder	Anika Dzierlenga
National Science Foundation	Alexandria, VA	All	Funder	TBD

Organization	Location	Primary Lake(s)	Role	Contact
Natural Sciences and Engineering Research Council	Ottawa, ON	Superior, Huron, St. Clair, Erie, Ontario	Funder	TBD
Ohio Environmental Protection Agency	Columbus, OH	Erie	Investigator, funder	Amy Jo Klei
Ontario Clean Water Agency	Multiple	Superior, Huron, St. Clair, Erie, Ontario	Investigator, funder	TBD
Ontario Ministry of Environment, Conservation, and Parks	Toronto, ON	Superior, Huron, St. Clair, Erie, Ontario	Investigator, funder	Ngan Diep
Ontario Provincial Health Agency	Toronto, ON	Superior, Huron, St. Clair, Erie, Ontario	Funder	TBD
Public Health Agency of Canada	Ottawa, ON	All lakes	Funder	TBD
U.S. Environmental Protection Agency	Chicago, IL	All lakes	Investigator, funder	Beth Hinchey Malloy
U.S. Geological Survey		All lakes	Investigator, funder	

Individuals who would likely participate in the study as investigators or analyzers would consist primarily of those affiliated with laboratories housed in academic institutions or state/provincial/federal agencies in or near the Great Lakes basin, with a limited role for private sector staff and companies. County, or municipal agencies might also participate but they tend to be less active in performing these types of analyses due to a lack of the appropriate instrumentation and staff, or application of these types of capabilities to other topics (e.g., biomedical studies).

3.3 Great Lakes laboratory network and capacity

Through the workshop surveys, it is clear that laboratory capacity exists to support the Great Lakes LBMWQ study, especially within the realm of the Centers of Excellence approach advocated by workshop participants and articulated in the draft IJC Great Lakes Science Strategy (2022), which is further described in Section 4.4. The laboratory network has the potential to be large if there is a focus on leveraging the capabilities of laboratories currently doing MST and COVID testing of wastewater with qPCR to support this Great Lakes study using qPCR methods.

The top MST platforms used in both Canada and the USA are summarized in Table 3-3. qPCR and digital PCR are both the top choices for MST analysis. In general, there are more labs doing MST work in the US and Canada, as compared to HABs and metagenomics work, with metagenomics labs currently being the most limited in terms of capacity. A list of labs, and their

associated methods, is included in Appendix D. These labs are located across the Great Lakes basin and can serve as a basis for creating a laboratory network for the Great Lakes LBMWQ study.

Table 3-3. Top MST Platforms in Canada and the United States.

Platform Used	Canada (n=7)	United States (n=29)
qPCR	6	28
Digital PCR	2	19
Sequencing	5	4
Endpoint PCR	0	1
Library-MST	0	0

The project work group leads have laboratories that can serve as a base for the Great Lakes laboratory network. These leads, their associated institutions, and method capabilities are listed in Table 3-4.

Table 3-4. Analytical Capabilities of Laboratories of Project Work Group Leads.

Institution	Lab Lead at Institution	MST	HABs	Metagenomics
McMaster University	Tom Edge	x	x	x
Michigan Department of Environment, Great Lakes, and Energy	Shannon Briggs	x		
Michigan State University	Joan Rose	x		
University of Michigan	Gregory Dick	x		x
University of Windsor - GLIER	Mike McKay	x	x	x

A more detailed list of potential participating laboratories and associated expertise are provided in Appendix D. This list was developed with input from participants during each of the three technical workshops. Note that this list is not exhaustive and other laboratories may be added. Nor have the laboratories listed made any commitments to being involved in the Great Lakes LBMWQ study.

4.0 Principles and Framework for Implementation

A key goal from the synthesis workshop (and follow-up project leads meeting) was to develop a framework for implementing the Great Lakes LBMWQ study. The scope for this planning project identified four specific implementation needs:

1. Develop protocols and support a laboratory network capability assessment using a round-robin sample analysis to evaluate performance across multiple labs. The round robin is described further in Section 4.2.
2. Advance the design of a pilot-level application to standardize sample collection and pre-analysis preparation methods (filtration, extraction, preservation), as well as evaluating how to scale up to a large basin study. Recommendations regarding the pilot project are described further in Section 4.3.
3. Develop recommendations on potential funding sources for the Great Lakes LBMWQ study. As discussed in Section 1.5, this effort requires support from outside the IJC. Support can include monetary and in-kind contributions and is discussed further in Section 4.4.
4. Provide suggestions for the potential role that the Great Lakes LBMWQ study can serve with respect to human health and ecosystem priorities through U.S. and Canadian policy, regulation, or voluntary mechanisms. This is addressed in Section 4.5.

In addition to implementing the LBMWQ study, a more comprehensive model has also emerged as a possible concept to consider. This would take the form of the creation of a distributed and binational Great Lakes Microbial Water Quality Observatory consisting of coordinated sampling locations, analytical laboratories and methods, data management, and sample archiving. In addition, this observatory would facilitate a community of practice including communications, education, training, technology transfer, and planning. Examples of such microbial observatories include:

1. Lake Mendota (Wisconsin) lter.limnology.wisc.edu/taxonomy/term/3212
2. San Pedro Ocean Time series (SPOT) (Southern California) dornsife.usc.edu/labs/usc-microbial-observatory
3. Centralized site for 10 observatories serc.carleton.edu/microbelife/microbservatories/index.html
4. Boiling Springs Lake (California) bslmo.research.pdx.edu/
5. Lake Washington (Washington) depts.washington.edu/microobs/
6. Deep Mine Microbial Observatory (DeMMO) (South Dakota) frontiersin.org/articles/10.3389/feart.2019.00196/full
7. Salt Plains National Wildlife Refuge (Oklahoma) serc.carleton.edu/resources/2282.html
8. Coast Range Ophiolite (California) web.uri.edu/cromo/

9. Alpine and polar ecosystems alpinemicrobialobservatory.weebly.com/

A common requirement of both the pilot version of the LBMWQ study and a larger microbial observatory effort would be identifying two lead investigators and institutions – one in Canada and one in the US – to anchor the efforts. More details and considerations are discussed in the following sections.

4.1 Study implementation phases

After reviewing the findings and recommendations from the Synthesis workshop, the IJC project leads have developed a set of next steps for the GLMWQ study and an approximate implementation timeline.

1. Year 1-2 (2023-2024): Strategy and planning
2. Year 2-3 (2024 – 2025): Conduct Laboratory Round Robin
3. Year 3-4 (2025 – 2026): Conduct Pilot Study
4. Years 5 – 10 (2026 – 2032): Integrate microbial water quality study into existing research and monitoring programs (e.g., *R/V Lake Guardian* and *Limnos* annual lake surveys, CSMI, and beach monitoring programs) to identify potential sampling locations and test approaches for the design of the Great Lakes Microbial Water Quality Observatory
5. Year 11 (>2032): Establish the Great Lakes Microbial Water Quality Observatory.

Each of these phases is described in more detail in the following sections, followed by a discussion of recommendations regarding potential funding sources, potential mechanisms for implementation, and a recommended implementation process and organizational structure.

4.1 Laboratory round robin

A laboratory intercomparison round robin is a key study component for achieving the goal of consistent data across lakes, years, and laboratories. The round robin effort will be used to establish the appropriate markers, methods, protocols, and QA/QC requirements for use by all of the participating laboratories.

The workshop participants articulated two pathways for conducting the laboratory round robin:

1. As a stand-alone effort conducted first so that analytical protocols and quality requirements could be established prior to any sample collection either in the pilot study or in the larger study.

2. Incorporate the laboratory method development into the pilot study as a rigorous evaluation of analytical protocols with the real-world samples collected specifically for this study.

Each approach has benefits and downsides. The benefits of the first approach include having a large number of the laboratories identified in the workshops participate in the round robin and having analysis protocols established before samples are collected. The downsides are that doing the round robin first delays the pilot project, which is anticipated to be a way to build enthusiasm and interest in the larger GLMWQ study. The benefits of the second approach are that it offers some efficiency with respect to the effort for the study launch and the use of study samples for the laboratory evaluation. The downside is that it would be more challenging logistically, especially from a sample volume collection and delivery/hold time basis, to include a large number of laboratories.

As indicated in Section 4.1, the preferred approach is to conduct the laboratory round robin separately and before the pilot study. The laboratory round robin plan has been refined further to focus it on MST and HABs methods, largely in recognition of the higher number of MST and HABs laboratories identified in the workshops. The team will address metagenomics methods as part of the pilot study, which is already planned to include sampling from a variety of habitats.

One recommendation from the workshops was to use archived samples from previous studies to evaluate laboratory methods prior to launching the pilot project, though it was acknowledged that the value of doing this may merit further evaluation. A similar approach could be applied to the data management aspect of the laboratory round robin (and pilot project). Because there are many details to work out on the laboratory round robin, a critical first step is to set up a forum with the interested laboratories to better define the methods, protocols and QA/QC that should be used in the study. As part of the workshops, a list of laboratories with MST and/or HABS capabilities and experience was developed (Appendix D) and it is expected that all laboratories from the workshops will be invited to participate in informing and participating in the round robin study, possibly with an expectation of an in-kind contribution to the effort.

4.3 Lake St. Clair-Detroit River-Western Lake Erie developmental pilot project

One important objective of the Great Lakes LBMWQ study is to incorporate molecular methods as part of the future tool box for standard microbial analysis. The pilot project offers an opportunity to demonstrate protocols for molecular methods prior to initiating the larger basin study. The pilot project provides several other important benefits, such as the potential to be a quick win to build the study's profile with agencies by showing benefits of lab network, molecular tools, and integrating large datasets into future efforts.

Several pilot areas were suggested for consideration (Figure 4-1) in the synthesis workshop. Many of these suggested areas are also Areas of Concern (AOC), which are areas designated under the U.S.-Canada Great Lakes Water Quality Agreement (IJC 1972; Canada and United

States 2012) as areas that have experienced significant environmental degradation as a result of human activities. Lake St. Clair and western Lake Erie were initially considered as separate candidates for the pilot project but were ultimately combined (with the Detroit River connecting channel) into a single area and selected as the recommendation for the pilot project. Other areas considered included western Lake Ontario (Toronto/Hamilton) and Lake Huron.

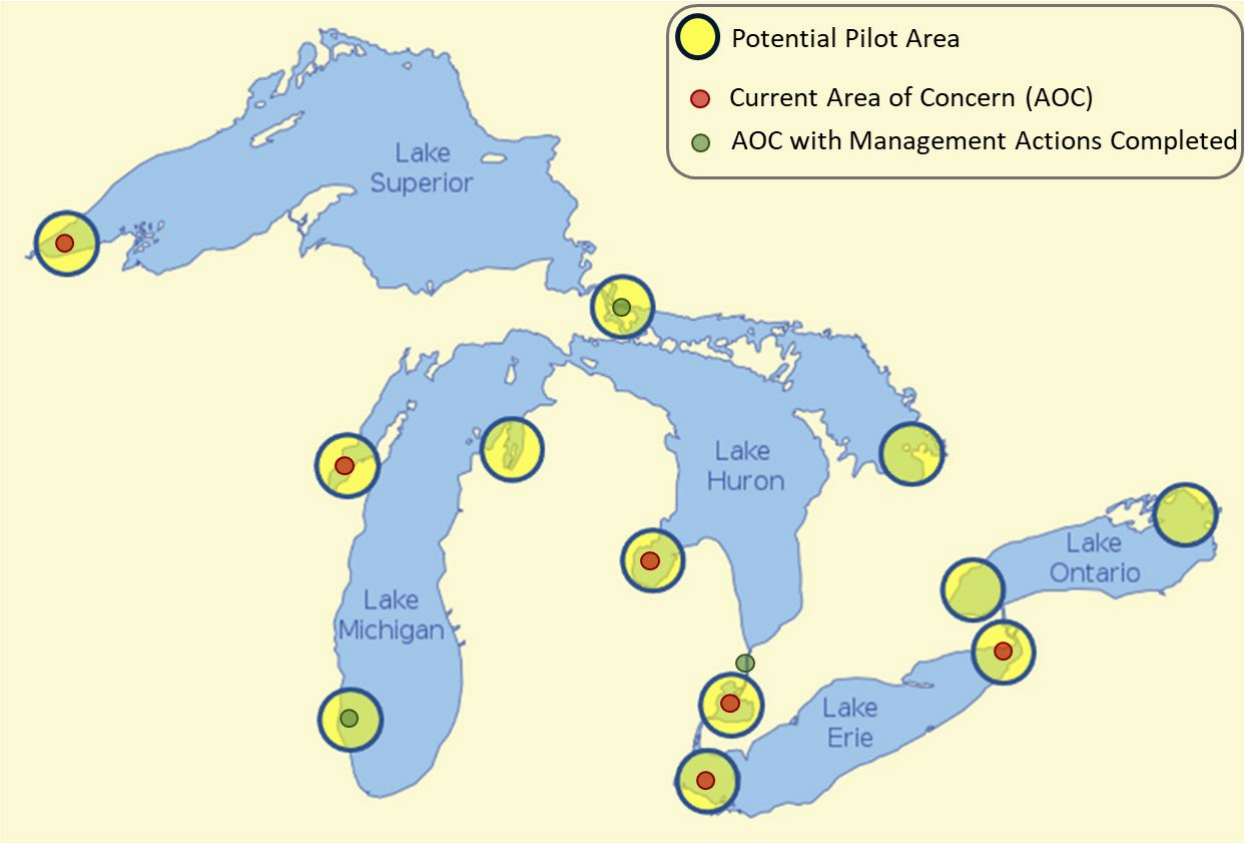


Figure 4-1. Potential Pilot Project Areas Considered for Great Lakes LBMWQ Study.

Using the Lake St. Clair-Western Lake Erie area as a pilot project provides a means to accomplish multiple objectives, including:

- Examining previous studies available to plan the sample collection and archiving procedures, laboratory methods, and data management framework. Previous work specifically mentioned included:
    - EcoBiomics Project in the Thames to Lake Erie corridor
    - Great Lakes Environmental Research Laboratory (GLERL) Beach Study
    - Western Lake Erie HABs work, including the two HABs Grab studies.
- Potentially using archived DNA samples from these previous studies as part of the round-robin interlaboratory comparison.

- Evaluating whether to connect the pilot project sampling with the Lake Erie/Lake St. Clair sampling planned in 2024 under the Cooperative Science and Monitoring Initiative (CSMI) between the United States and Canada.
- Piloting the Centers of Excellence laboratory concept (see Section 4.4) as part of the study implementation. This area has excellent laboratory capabilities nearby, including multiple university-based laboratories and commercial laboratories.
- A diverse stakeholder group for developing and testing how local stakeholder outreach can be most effective.
- Developing a health risk map for stakeholders.

In addition, the Lake St. Clair portion of the recommended pilot project is a binational waterbody with major tributaries discharging to it (the Clinton River on the U.S. side and the Thames River on the Canada side). Both Lake St. Clair and western Lake Erie have known microbial water quality issues posing potential risks to human health, including, but not limited to beach closures in both Lake St. Clair and Lake Erie from high levels of *E. coli*, increasing frequency of HABs in both lakes, and other impairments, such as heavy metals and toxic organics, being addressed through the AOC process (St. Clair Region Conservation Authority 2017; MDNR 1988; MDEQ 2011; SEMCOG 1990; MDNR 1994; Rouge River Advisory Council 2004; OEPA 2006). Note that the St. Clair-Detroit River System was sampled by USEPA in 2014 and 2015 as a pilot project using the same sample design and protocols as the National Coastal Condition Assessment, which included collection of samples at approximately 80 sites for parameters including microcystin and enterococci fecal indicator bacteria. The area has also been sampled intensively in recent years by ECCC/MECP and NOAA/CIGLR scientists with a focus on HABs.

This section of the report serves as a starting point for designing the pilot project. The final details of the pilot project sampling work plan would be undertaken by a work group dedicated to this phase of the Great Lakes LBMWQ study (see Section 4.4), initial thoughts on sampling and analysis from the synthesis workshop are described here for future consideration.

Both lakes and the Detroit River connecting channel have multiple potential exposure pathways to consider for identifying sampling locations including beaches, drinking water intakes, other recreational locations (e.g., marinas, boat launches, parks, big boat parties (such as Jobbie Nooner, Raft-Off), First Nation areas, wastewater discharge-dominated (e.g., downstream of full or partially treated effluent and untreated combined sewer overflow outfalls), stormwater outfalls, and locations tributary to areas with high septic system use. Far shore sampling, particularly for metagenomics, was also recommended to ensure the full range of habitat and hydrologic conditions are captured in the sample collection.

The suite of potential analytical parameters was also discussed as part of the pilot project design. The idea emerging from the workshops is to lead with qPCR and digital PCR methods for the microbial analyses, but pair them with other methods, including but not limited to:

- MST: *E. coli* counts, *Enterococci* sp.
- HABs: ELISA, remote sensing
- Metagenomics: potential co-factors.

Table 4-1 provides a summary of the core and companion (or co-factor) parameters suggested at the synthesis workshop. The laboratory round robin phase would focus on finalizing the and potentially additional methods for use in the pilot study. The final set of core and companion parameters will be determined by the work group focused on this phase of the Great Lakes LBMWQ study (see Section 4.6) and included in their Sampling Plan for the pilot project.

Table 4-1. Suggested Analytical Parameters for the Great Lakes LBMWQ Pilot Project.

Microbial Tool	Core Parameters	Companion Parameters
MST	Human[1] Ruminant[1] Bird[1] Dog[1]	*E. coli* and *enterococci* counts Temperature Submerged aquatic vegetation and/or Cladophora Other pathogens[3]
HABs	Taxa Toxins[1]	ELISA Phosphorus (P) – total and soluble reactive P (SRP) Nitrogen (N) Temperature Pigments (chlorophyll, phycocyanin)
Metagenomics	16S sequencing[2] Shotgun metagenomics	Nutrients (P and N) Temperature Dissolved oxygen pH

[1] Analysis using qPCR and/or digital PCR
[2] May be more appropriate for HABs
[3] e.g., giardia, cryptosporidium, norovirus, adenovirus

Two other topics discussed as part of the workshop included whether to include sediment sampling and how often samples should be collected. The final recommendation with respect to sample media was to add the collection of sediment samples to the sampling plan only if it was part of an important process that needed to be considered.

With respect to the frequency of sampling, the consensus was that more temporal sampling was needed than was done for the 1913 study. The concept of different types of sampling locations could influence the sampling frequency. One type of sampling location would serve as "sentinel" stations that are sampled on a regular interval for the purpose of tracking changes in conditions. A second type of sampling location would serve as "baseline" stations, sampled more strategically, such as in response to an environmental trigger (e.g., storm event for MST, observed bloom for HABs) and more broadly (e.g., more locations), for the purpose of establishing a current condition for comparison to the next Centennial microbial study 100 years hence.

4.4 Recommendations on potential funding sources

Because the proposed activity, based on workshop and workgroup discussions, is now being conceived as more than a single repeat survey patterned after the original 1913 study, it may be more appropriate to think of the effort as the establishment of a distributed Great Lakes Microbial Observatory, as mentioned previously. Analogous place-based entities exist in California (dornsife.usc.edu/labs/usc-microbial-observatory) and the Baltic Sea (lnu.se/en/research/research-groups/linnaeus-microbial-observatory-lmo/), among other locations. The LBMWQ study is not just an applied science exercise. The metagenomics element, in particular, would contribute strong reconnaissance and basic research components.

The IJC mission and the government approaches to environmental health have evolved substantially since the original 1913 study. The original study took place decades before the establishment of ECCC, USEPA, and signing of the GLWQA (1972) and passage of the US Clean Water Act (1972) and the Canada Water Act (1970). At this point it would appropriate for the Parties, via their federal environmental agencies (ECCC, USEPA, etc.) and research funding programs (e.g., Natural Sciences and Engineering Research Council of Canada, US National Institutes of Health, US National Science Foundation) to serve as the primary sources of support for the pilot and full-scale phases of the LBMWQ study, including funding of academic laboratories.

Federal leadership could be augmented by states and provinces, especially Michigan and Ontario. Large municipal governments (Toronto, Hamilton, Cleveland, Detroit, Chicago, Milwaukee) and private philanthropies could also play a role. Professional organizations that could contribute in a coordinating capacity would be the International Association for Great Lakes Research and the Great Lakes Beach Association. The Great Lakes Commission, which has coordinated several topical communities of practice (e.g., *Phragmites* control, invasive mussel control, HABs) could provide staff and expertise to facilitate a new environmental microbiology collaborative. If funding is continued, the Great Lakes Center for Fresh Waters and Human Health (US) could also play a role. Many of the elements described above that would constitute the LBMWQ study or observatory are well-aligned with the priorities and proposed structural elements of the draft IJC Great Lakes Science Strategy (2022).

Very rough estimates for the labor costs and expenses in U.S. dollars (USD) for the components of the LBMWQ study, not considering any in-kind contributions, would include:

- $50,000 – $100,000 USD for the round-robin study
- $500,000 – $1,500,000 USD for the pilot project (inclusive of the round-robin study)
- $5,000,000 – $10,000,000 USD for a comprehensive basin-scale study, possibly in annual sub-phases to achieve the necessary time series of data
- $75,000 USD per year for coordinating a community of practice and sharing results and challenges
- $200,000 – $400,000 USD per year for maintaining a basic microbial observatory (minimum level of support)

4.5 Recommended policy, regulation or voluntary mechanisms

Based on results of the workshops, workgroup discussion, and background research conducted as part of this project it has been confirmed that many opportunities for advancing knowledge and management of the Great Lakes microbiome and associated health risks exist. Execution of the LBMWQ study, creation of a microbial observatory of long-term monitoring locations throughout the Great Lakes basin, and related activities in any form aligns with the objectives of existing policies, regulations, and voluntary activities and programs. Opportunities for advancing these also exist.

With some exceptions, the regulatory guidance for managing swimming and drinking water quality uses basic analytical approaches at this point in both Canada and the US. Certain municipal, provincial, or state jurisdictions augment the regulatory requirements by providing additional method guidance and centralized laboratory services (e.g., Ohio EPA's requirement of routine biweekly qPCR screening for drinking water systems via state lab or commercial determination of cyanobacteria and cyanotoxin-producing genes). Mitigation of problems has also made use of advanced technologies, such as MST approaches to identifying and controlling *E. coli* sources.

The GLWQA consideration of microbiology aligns most closely with Annexes 2 (LAMPs), 4 (nutrients), 9 (climate change), and 10 (science). The LAMPs prioritize management concerns and associated science priorities on a lake-by-lake basis.

4.6 Implementation process and organizational structure

Important principles were articulated to facilitate implementation, including the important notion of building interest and enthusiasm among potential partners and potential funding sources for implementing the study. Successful implementation of the GLMWQ study and microbial observatory likely requires multiple levels of organization and planning. The vision for how the study could be executed has three levels, each serving a specific and critical need(s) for the overall project, which are described below in general terms:

1. **IJC**: The role of the IJC can be simply described as communication and coordination. The IJC is well-positioned to serve the study needs with respect to convening committees and work groups, managing communications and report-outs, facilitating any bi-national agreements needed, and contributing financial support for initial phases of implementation. Maintaining the structure of this current project, which includes the IJC Project Manager, the HPAB co-leads for the project, and representatives from related IJC Boards (Water Quality and Science Advisory Board) could serve as a starting point for the IJC management structure in the next phases of the study.
2. **Steering Committee**: The role of the Steering Committee can be simply described as setting strategy and securing funding. A Steering Committee could be established that

will exist as a standing committee under the IJC HPAB Board, including its composition, membership, duration of terms, framework, and reporting structure. The primary responsibilities of this committee are to set study strategy and secure funding from agencies in the U.S. and Canada. An initial proposed size range would be 10-12 individuals, including HPAB members, academic subject matter experts, and funding agency study champions.

3. **Technical Work Group(s)**: The role of the work groups can be simply described as working out the technical details of each phase of the project. A Technical Work Group could be established for a relatively short period of time (e.g., 1-3 years) for each major technical component of the project to conduct detailed planning for the associated task (e.g., laboratory round robin). Under this model of implementation, a separate work group dedicated for each phase (task) of the project is envisioned, though members could belong to multiple work group. The composition has been envisioned as 3-4 people as core members, who would have overall responsibility for the work group activities and outcomes. The group could be expanded at core member discretion, on a temporary or as needed basis, to have the necessary technical expertise represented in the planning and execution of the tasks.

The organizational structure described above is consistent with and advances three distinct implementation strategies that were articulated to facilitate implementation of the laboratory round robin, pilot project, and large study, including the important notion of building interest and enthusiasm among potential partners and potential funding sources for implementing the study.

Implementation Strategy #1: Break the Great Lakes LBMWQ study into multiple phases with a Work Group for each phase that is focused on the technical planning and execution of that phase. Set up a Steering Committee to focus on setting strategy and funding.

The first principle is to establish implementation phases for the study and have a dedicated leadership group for each phase. The road map described in Section 4.1 provides the implementation phases for the study. The work group in the initial phases would address the laboratory round robin (phase 1), the design and implementation of the pilot project (phase 2), and lead local stakeholder outreach.

The work group(s) would be tasked with developing a Work Plan and detailed cost estimate for their respective study phase (e.g., laboratory round robin, the pilot project, etc.). Each work group would need to work through several logistical details in their work plan development, such as:

- Laboratory round robin:
    - Participating laboratories and their roles
    - Parameters to be addressed in the round robin--e.g., should it be conducted only for MST or for HABs methods as well?
    - Specific PCR primer and probes to test for MST and HABs

    - Additional sample volume collection and distribution to laboratories participating in the round robin
    - Establish QA/QC requirements
- Pilot project:
    - Sampling locations that include a variety of aquatic habitats, exposure areas, and pollutant source discharge points
        - MST: Possible sources
        - HABs: Possible sources and lake processes
        - Metagenomics: Possible sources, lake processes, whole ecosystem processes
    - Sampling frequency and time of year (e.g., May - September for MST/HABs)
    - Sampling parameters
    - Participating laboratories and their roles
    - Sample collection responsibilities

Stakeholder outreach is a responsibility common to all of the work groups. Stakeholder outreach has multiple benefits, including building interest in and support for the pilot project and the larger basin-wide study; informing the pilot project study design with local knowledge and relevant subject matter expertise; and identifying potential partners to support sampling, analysis, and other logistical considerations of the pilot project. Specific outreach needs for the pilot project include First Nations communities (e.g., Caldwell Nation, Walpole Island, Wyandot of Anderdon Nation), local authorities (e.g., local public works commissioners), disadvantaged communities (e.g., We the People of Detroit[6]), and local experts (e.g., wastewater utilities, watershed organizations, universities). One responsibility suggested for the pilot project work group is to design and execute the stakeholder outreach effort for the pilot project so that it could serve as a model for the larger basin study.

A second, larger, multi-jurisdictional group, the Steering Committee, would be established to focus on the post-pilot portion of the planning process, specifically advancing the larger study strategy, centered around linking the goals of the larger basin study to the IJC Great Lakes Science Strategy (IJC 2022). This group would tackle some of the more challenging needs of the larger basin study, including, for example, data management, coordination across countries, interagency needs, and funding mechanisms, as well as address unresolved aspects of the study.

One example of an unresolved issue is whether to fold the Great Lakes LBMWQ study into existing programs, like the Cooperative Science and Monitoring Initiative (CSMI), or to have it as a standalone study. The CSMI is a binational effort to coordinate research and monitoring activities in one of the five Great Lakes each year. Sampling surveys focused on research areas reflecting the unique challenges and data needs within each lake and the priorities established by the Lake Partnerships of the Great Lakes Water Quality Agreement Lakewide Management Activities.

[6] wethepeopleofdetroit.com/

The CSMI is an attractive program to coordinate with because many of the same agencies that would be involved in the Great Lakes LBMWQ study are already engaged in the planning and execution of the CSMI program. Conducting the Great Lakes LBMWQ study within the CSMI program either with or without sampling augmentation provides an opportunity for systems integration between human health, ecosystem, and water quality. The downside is that each lake is sampled once every five years and there is concern that this may not align with the needs of the Great Lakes LBMWQ study.

Other questions regarding this coordination include:

- Will folding the Great Lakes LBMWQ study into CSMI take resources away from the goals of the CSMI?
- Will the Great Lakes LBMWQ study be overlooked in a CSMI-data-driven program?
- Can the CSMI sampling be expanded to include sampling other areas of interest (e.g., near shore) that are not currently part of CSMI?
- Can enough funding be garnered for a standalone Great Lakes LBMWQ study?

One suggested approach for this work group to consider is to work within the CSMI framework for sampling offshore water locations and working with beach sampling programs to sample nearshore locations (e.g., beaches, docks, marinas). Leveraging both existing programs could be an effective way to reduce the cost of the Great Lakes LBMWQ study but would need to be balanced by additional investment in training and establishing sampling protocols.

Implementation Strategy #2: Establish Centers of Excellence, particularly with metagenomics.

Several related issues with the sample processing and laboratory analysis portion of the study were raised in the technical workshops, including:

- There are number of procedures but little widespread "standard" methods, thus, there is a need to ultimately provide the protocols for the large basin study. This was first raised in the MST workshop but is a valid need for HABs and metagenomics.
- PCR is not the most common tool used for assessing HABs, and PCR methods are less widely adopted for HABs than MST (though there are probes/primers for major HAB species (Feist and Lance 2021), but methods for sequencing cyanobacteria species (16S genes) and toxin genes have become fairly well-established in the Great Lakes [see Appendix C]).
- There is a lot of exciting metagenomics work going on in the Great Lakes, with many technologies broadly embraced.

All of these findings point to the need for method standardization, appropriate training, and coordination across laboratories. This was a particular point of emphasis in the metagenomics

workshop. Ideally, there would be a sweet spot where the Great Lakes LBMWQ study work groups standardize methods, as much as possible, without being too prescriptive on protocols.

The concept of Centers of Excellence was developed as a potential solution. Lead laboratories, including a minimum of one in Canada and one in the United States, would be established to train and work with a consortium of experts who would address the key methodological questions in implementing a large-scale genomics study of Great Lakes water quality. Additional workshop discussion of this concept is provided in Section 2.3.

Originally conceived in the context of the large basin study implementation specifically for metagenomics, the project leads recognize the value of applying this approach at a pilot scale and for MST, HABs, and related analytes (e.g., nutrients, *E. coli*) as well as for metagenomics. The concept was broadened to consider a single laboratory or organization that would serve as the central entity in coordinating the sample collection, sample processing, and the distribution of samples to the participating laboratories. This model of managing the field and laboratory elements was successfully used for the HABs Grab studies (Chaffin et al. 2021).

The concept of setting up specific laboratories to serve as Centers of Excellence is consistent with the draft IJC Great Lakes Science Strategy (2022) Priority #5 recommendation to establish centers of excellence (excerpt from page 16):

> *Fostering our ongoing understanding of the Great Lakes would benefit from the creation of a small, but well supported, number of permanent Centers of Excellence, whose focus would be on long-term, basin-scale, interdisciplinary needs in key evolving areas of research, modeling, socioeconomic analysis, data acquisition, diversity and coordination. Centers of Excellence would also provide a forum to handle cross-cutting issues that may arise in interdisciplinary investigations involving various fields of natural and social science.*
>
> *Several ideas for physical or virtual Centers of Excellence, collaboratives, innovation clusters or communities of practice have been discussed by contributors to the Science Strategy. These are critical to attracting, developing and aligning talent, and the ability to address interdisciplinary questions.*

Implementation Strategy #3: Conduct the pilot project after the round robin interlaboratory comparison and before the large basin study.

A number of details remain to be sorted out before the large basin study can be executed. The pilot project offers an opportunity to build protocols, create a system of replicability, compile necessary meta-data, test data management strategies on a smaller scale, engage with local stakeholders to identify health priorities that will inform sample collection, and use the data to inform the appropriate temporal and spatial scales to be applied in the larger basin study. The two-phase approach to managing the Great Lakes LBMWQ study implementation strategy described above is predicated on the need and value of an initial pilot project prior to planning and mobilizing for the larger basin survey. Further, the pilot project can establish forward momentum for the larger study.

Conducting the interlaboratory comparison round robin before the pilot project offers several benefits, including an opportunity to engage with participating laboratories to develop or refine standard operating procedures (SOPs), validate MST methods with samples from across the Great Lakes basin, and assess the interlab stability of a HAB PCR method. Samples from the pilot project area described in Section 4.1 will span a range of hydrologic and hydraulic conditions (e.g., near shore, deeper water, river confluences, effluent-impacted, etc.), which can provide a robust test of laboratory performance.

4.6.1 Next steps

The HPAB workgroup has sketched out goals for the study planning over the next 3-4 years, as follows:

- In 2023-2024, develop the round-robin exercise with the established laboratory network identified in the workshops and determine whether the pilot project will be executed as part of the 2024 CSMI, develop a detailed Project Work Plan, including round-robin exercise elements and pilot study; obtain funding commitments; develop community of practice, outreach, and data management plans.
- In 2024-2025, conduct the laboratory round robin study interlaboratory comparison for MST and HABs, summarize the results, and establish standard analytical protocols for use in the pilot and larger studies.
- In 2025-2026, execute the Pilot Project, or alternatively, the round-robin exercise; if funding for the pilot has not yet been obtained, continue to pursue this. Outline the approach to a full basin study (synchronous or phased [e.g., tied to CSMI]) and/or a microbial observatory.
- In 2026-2032, summarize results of the Pilot Project; begin implementation of the microbial study elements into existing monitoring and research programs and defining the microbial observatory; continue to pursue funding if necessary; refine community of practice approaches.

5.0 References

Ahmed, W., Gyawali, P., Feng, S., McLellan, S.L., 2019. Host specificity and sensitivity of the established and novel sewage-associated marker genes in human and non-human fecal samples. Appl. Environ. Microbiol. 85(14) e00641-19. DOI: 10.1128/AEM.00641-19.

Ahmed, W., Payyappat, S., Cassidy, M. et al. 2019. Enhanced insights from human and animal host-associated molecular marker genes in a freshwater lake receiving wet weather overflows. Sci Rep 9, 12503. https://doi.org/10.1038/s41598-019-48682-4

Alm, E.W., Daniels-Witt, Q.R., Learman, D.R., Ryu, H., Jordan, D.W., Gehring, T.M., Domingo, J.S., 2018. Potential for gulls to transport bacteria from human waste sites to beaches. Sci. Total Environ. 615 123–130. DOI: 10.1016/j.scitotenv.2017.09.232.

Badgley, B. D., Thomas, F. I. & Harwood, V. J. 2011. Quantifying environmental reservoirs of fecal indicator bacteria associated with sediment and submerged aquatic vegetation. Environ. Microbiol. 13, 932–942.

Bernard, C., Ballot, A., Thomazeau, S., Maloufi, S., Furey, A., Mankiewicz-Boczek, J., Pawlik-Skowrońska, B., Capelli, C., Salmaso, N. 2017. Handbook of Cyanobacterial Monitoring and Cyanotoxin Analysis. Appendix 2: Cyanobacteria Associated with the Production of Cyanotoxins. John Wiley & Sons.

Boehm, A.B., Van De Werfhorst, L.C., Griffith, J.F., Holden, P.A., Jay, J.A., Shanks, O.C., Wang, D., Weisberg, S.B. 2013. Performance of forty-one microbial source tracking methods: A twenty-seven lab evaluation study. Water Res. 47(18) 6812–6828. DOI: 10.1016/j.watres.2012.12.046.

Brown, C., Staley, C., Wang, P., Dalzell, B.,Chun, C. L., Sadowsky, M. 2017. A High-Throughput DNA Sequencing Approach to Determine Sources of Fecal Bacteria in a Lake Superior Estuary. Environmental Science & Technology. 51. 10.1021/acs.est.7b01353.

Butler, T.M., Wilhelm, A-C., Dwyer, A.C., Webb, P.N., Baldwin, A.L, Techtmann, S.M. 2019. Microbial community dynamics during lake ice freezing. Nature: Scientific Reports. 9, 6231. doi.org/10.1038/s41598-019-42609-9.

Canada, US. 2012. The Great Lakes Water Quality Protocol 2012. Access online at: ijc.org/sites/default/files/2018-07/GLWQA_2012.pdf

Chaffin JD, Bratton JF, Verhamme EM, Bair HB, Beecher AA, Binding CE, Birbeck JA, Bridgeman TB, Chang X, Crossman J, Currie WJS, Davis TW, Dick GJ, Drouillard KG, Errera RM, Frenken T, MacIsaac HJ, McClure A, McKay RM, Reitz LA, Domingo JWS, Stanislawczyk K, Stumpf RP, Swan ZD, Snyder BK, Westrick JA, Xue P, Yancey CE, Zastepa A, Zhou X. The Lake Erie HABs Grab: A binational collaboration to characterize the western basin cyanobacterial harmful algal blooms at an unprecedented high-resolution spatial scale.

Harmful Algae. 2021 Aug;108:102080. doi: 10.1016/j.hal.2021.102080. Epub 2021 Jul 23. PMID: 34588116; PMCID: PMC8682807. pubmed.ncbi.nlm.nih.gov/34588116/

Chaudhary, A., Turner, S. and Poretsky, R., 2021. Bacterioplankton respond with similar transcriptional activity to allochthonous dissolved organic matter in coastal and offshore Lake Michigan. Limnology and Oceanography, 66(8), pp.3162-3175.

Corsi, S., De Cicco, L., Hansen, A., Lenaker, P., Bergamaschi, B., Pellerin, B., Dila, D., Bootsma, M., Spencer, S., Borchardt, M., and Mclellan, S. 2021. Optical Properties of Water for Prediction of Wastewater Contamination, Human-Associated Bacteria, and Fecal Indicator Bacteria in Surface Water at Three Watershed Scales. Environmental Science & Technology 55. 13770-13782. https://doi.org/10.1021/acs.est.1c02644.

Dash, H. R., Das, S. 2018. Methods in Microbiology. Chapter 4-Molecular Methods for Studying Microorganisms from Atypical Environments. Academic Press, Volume 45, pp. 89-122. https://doi.org/10.1016/bs.mim.2018.07.005.

Denef, V.J., Carrick, H.J., Cavaletto, J., Chiang, E., Johengen, T.H. and Vanderploeg, H.A., 2017. Lake bacterial assemblage composition is sensitive to biological disturbance caused by an invasive filter feeder. *Msphere*, *2*(3), pp.e00189-17.

Dila, D.K., Corsi, S.R., Lenaker, P.L. Baldwin, A.K., Bootsma, M.J., McLellan, S.L., 2018. Patterns of host-associated fecal indicators driven by hydrology, precipitation, and land use attributes in Great Lakes watersheds. Environ. Sci. Technol. 52(20) 11500-11509. DOI: 10.1021/acs.est.8b01945.

Edge, T.A., Hill, S., Crowe, A., Marsalek, J., Seto, P., Snodgrass, B., Toninger, R., Patel, M., 2018. Remediation of a beneficial use impairment at Bluffer's Park Beach in the Toronto Area of Concern. Aquat. Ecosyst. Health Manag. 21(3) 285–292. DOI: 10.1080/14634988.2018.1497401.

Edge, T.A., Boyd, R.J., Shum, P., Thomas, J.L., 2021. Microbial source tracking to identify fecal sources contaminating the Toronto Harbour and Don River watershed in wet and dry weather. Journal of Great Lakes Research, Volume 47, Issue 2, 2021, Pages 366-377. ISSN 0380-1330. https://doi.org/10.1016/j.jglr.2020.09.002.

European Molecular Biology Laboratory (EMBL) European Bioinformatics Institute (EBI). "Proteomics: An introduction to EMBL-EBI resources". https://www.ebi.ac.uk/training/online/courses/proteomics-an-introduction/what-is-proteomics/#:~:text=Proteomics%20is%20the%20large%2Dscale,for%20example%2C%20the%20liver). Accessed September 2022.

EMBL-EBI. "Metabolomics: An introduction". https://www.ebi.ac.uk/training/online/courses/proteomics-an-introduction/what-is-proteomics/#:~:text=Proteomics%20is%20the%20large%2Dscale,for%20example%2C%20the%20liver). Accessed September 2022.

Feist, S.M., Lance, R.L. 2021. Genetic detection of freshwater harmful algal blooms: A review focused on the use of environmental DNA (eDNA) in Microcystis aeruginosa and Prymnesium parvum. Harmful Algae, 110 (102124). https://doi.org/10.1016/j.hal.2021.102124

Fujimoto, M., Cavaletto, J., Liebig, J.R., McCarthy, A., Vanderploeg, H.A. and Denef, V.J., 2016. Spatiotemporal distribution of bacterioplankton functional groups along a freshwater estuary to pelagic gradient in Lake Michigan. Journal of Great Lakes Research, 42(5), pp.1036-1048.

Graham, L.E., L.W. Wilcox and J.M. Graham. 2008. Algae, Benjamin Cummings, London.

Graydon, R.C., Mezzacapo, M., Boehme, J., Foldy, S., Edge, T.A., Brubacher, J., Chan, H.M., Dellinger, M., Faustman, E.M., Rose, J.B. and Takaro, T.K., 2022. Associations between extreme precipitation, drinking water, and protozoan acute gastrointestinal illnesses in four North American Great Lakes cities (2009–2014). Journal of Water and Health, 20(5), pp.849-862 (open access). https://doi.org/10.2166/wh.2022.018

Great Lakes Commission (GLC). 2021. Great Lakes Harmful Algal Blooms: Current Knowledge Gaps. Prepared by the Great Lakes HABs Collaborative. April 21, 2021. https://www.glc.org/wp-content/uploads/HABS-Knowledge-Gaps-clean-04.21.2021.pdf.

Grim, S.L., Voorhies, A.A., Biddanda, B.A., Jain, S., Nold, S.C., Green, R. and Dick, G.J., 2021. Omics-Inferred Partitioning and Expression of Diverse Biogeochemical Functions in a Low-O2 Cyanobacterial Mat Community. Msystems, 6(6), pp. 01042-21.

Harwood, V.J., Staley, C., Badgley, B.D., Borges, K., Korajkic, A., 2014. Microbial source tracking markers for detection of fecal contamination in environmental waters: relationships between pathogens and human health outcomes. FEMS Microbiol. Rev. 38(1) 1–40. DOI: 10.1111/1574-6976.12031.

Hebert, Paul D. N.; Cywinska, Alina; Ball, Shelley L.; Dewaard, Jeremy R. 2003. "Biological identifications through DNA barcodes". Proceedings of the Royal Society of London. Series B: Biological Sciences. 270 (1512): 313–321. doi:10.1098/rspb.2002.2218. PMC 1691236. PMID 12614582.

International Joint Commission (IJC). 1918. Final report of the International Joint Commission on the pollution of boundary waters reference: University of Windsor International Joint Commission (IJC) Digital Archive. Accessed at https://scholar.uwindsor.ca/ijcarchive/34, January 22, 2021. 55 p.

IJC. 2017. “Human Health Effects of Cyanobacterial Toxins in the Great Lakes Region: A Science and Monitoring Assessment.” Report prepared and submitted by the Health Professional Advisory Board. February 27, 2017.

IJC. 2021a. “Large Basin Microbial Water Quality Study Plan, Statement of Work.” Plan prepared by the IJC, Great Lakes Regional Office, Health Professionals Advisory Board and Great Lakes Water Quality Board. July 27, 2021.

IJC. 2021b. “The Great Lakes Water Quality Centennial Study – Phase 1 Report.” Report prepared and submitted by the Health Professional Advisory Board. February 2021.

IJC. 2022. *Great Lakes Science Strategy for the Next Decade Summary Report*. Report prepared by the Great Lakes Science Advisory Board. November 2022. https://www.ijc.org/sites/default/files/SAB_Great-Lakes-Science-Strategy-summary-report_2022.pdf

Ishii, S., Hansen, D.L., Hicks, R.E., Sadowsky, M.J., 2007. Beach sand and sediments are temporal sinks and sources of Escherichia Coli in Lake Superior. Environ. Sci. Technol. 41(7) 2203–2209. DOI: 10.1021/es0623156.

Jin, Li, 2021. "Welcome to the Phenomics Journal". Phenomics. 1 (1): 1–2. doi:10.1007/s43657-020-00009-4. ISSN 2730-5848. February 1, 2021.

Kim, Y., Aw, T.G., Teal, T.K., Rose, J.B. 2015. Metagenomic investigation of viral communities in ballast water. Environmental Science & Technology. 49, 8396-8407. DOI: 10.1021/acs.est.5b01633.

Kinzelman, J., Byappanahalli, M.N., Nevers, M.B., Shively, D., Kurdas, S. Nakatsu, C., 2020. Utilization of multiple microbial tools to evaluate efficacy of restoration strategies to improve recreational water quality at a Lake Michigan Beach (Racine, WI). Journal of Microbiological Methods, Vol. 178, November 2020, 1060409. https://doi.org/10.1016/j.mimet.2020.106049

Korajkic, A., Stoeckel, D., Griffith, J.F., 2016. Validation of microbial source tracking markers and detection protocols: considerations for effective interpretation, in: Yates, M.V., Nakatsu, C.H., Miller, R.V., Pillai, S.D. (Eds.), Manual of Environmental Microbiology, Fourth Edition. American Society of Microbiology, p. 3-4. DOI: 10.1128/9781555818821.ch3.4.2.

MacGregor, B.J., Moser, D.P., Alm, E.W., Nealson, K.H. and Stahl, D.A., 1997. Crenarchaeota in Lake Michigan sediment. Applied and Environmental Microbiology, 63(3), pp.1178-1181.

Mayer, R.E., Reischer, G.H., Ixenmaier, S.K., Derx, J., Blaschke, A.P., Ebdon, J.E., Linke, R., Egle, L, Ahmed, W. Blanch, A.R., Byamukama, D., Savill, M., Mushi, D., Cristóbal, H.A., Edge, T.A., Schade, M.A., Aslan, A., Brooks, Y.M., Sommer, R., Masago, Y., Sato, M.I., Taylor, H.D., Rose, J.B., Wuertz, S., Shanks, O.C., Piringer, H., Mach, R.L., Savio, D., Zessner, M., Farnleitner, A.H., 2018. Global Distribution of Human-Associated Fecal Genetic Markers in Reference Samples from Six Continents. Environmental Science & Technology 2018 52 (9), 5076-5084. DOI: 10.1021/acs.est.7b04438.

McKindles, K.M., Manes, M.A., DeMarco, J.R., McClure, A., McKay, R.M., Davis, T.W. and Bullerjahn, G.S., 2020. Dissolved microcystin release coincident with lysis of a bloom dominated by *Microcystis* spp. in western Lake Erie attributed to a novel cyanophage. Applied and environmental microbiology, 86(22), pp. e01397-20.

Mejbel, H., Dodsworth, W., Baud, A., Gregory-Eaves, I., Pick, F. 2021. Comparing Quantitative Methods for Analyzing Sediment DNA Records of Cyanobacteria in Experimental and Reference Lakes. Frontiers in Microbiology. 12. 10.3389/fmicb.2021.669910.

Michigan Department of Environmental Quality (MDEQ). 2011. Stage 2 Remedial Action Plan Clinton River Area of Concern. Prepared by Office of the Great Lakes, Great Lakes Management Unit. October 1, 2011. https://www.epa.gov/sites/default/files/2015-08/documents/clinton-river-stage-2-rap-20111001_.pdf

Michigan Department of Natural Resources (MDNR). 1988. Remedial Action Plan for Clinton River Area of Concern. Prepared by Surface Water Quality Division. November 1988. Accessed via https://www.epa.gov/sites/default/files/2015-08/documents/1988_clinton_river_rap.pdf

Michigan Sea Grant. Great Lakes Fast Facts. 2021. https://www.michiganseagrant.org/topics/great-lakes-fast-facts/.

National Institute of Health (NIH) National Human Genome Research Institute. 2022. Genetic glossary. https://www.genome.gov/genetics-glossary/Metagenomics#:~:text=Metagenomics%20is%20the%20study%20of,or%20in%20a%20water%20sample. Accessed September 2022.

National Science and Technology Council (NSTC). 2017. "Harmful Algal Blooms and Hypoxia in the Great Lakes Research Plan and Action Strategy: An Interagency Report." August 2017.

Nevers, M.B., Byappanahalli, M.N., Shively, D., Buszka, P.M., Jackson, P.R., Phanikumar, M.S., 2018. Identifying and eliminating sources of recreational water quality degradation along an urban coast. J. Environ. Qual. 47(5) 1042. DOI: 10.2134/jeq2017.11.0461.

Ohio Environmental Protection Agency (OEPA). 2006. Maumee Area of Concern Stage 2 Watershed Restoration Plan, Volume 1. Prepared by the Maumee RAP and Duck & Otter Creeks Partnership, Inc. January 2006. Access via: https://www.epa.gov/system/files/documents/2021-08/maumee-river-rap_stagei-append.pdf

Palermo, C.N., Shea, D.W. and Short, S.M., 2021. Analysis of different size fractions provides a more complete perspective of viral diversity in a freshwater embayment. Applied and Environmental Microbiology, 87(11), pp.00197-21.

Paver, S.F., Newton, R.J. and Coleman, M.L., 2020. Microbial communities of the Laurentian Great Lakes reflect connectivity and local biogeochemistry. Environmental microbiology, 22(1), pp.433-446. https://sfamjournals.onlinelibrary.wiley.com/doi/full/10.1111/1462-2920.14862.

Rozmarynowycz, M.J., Beall, B.F.N., Bullerjan, G.S., Small, G.E., Sterner, R.W., Brovold, S.S., D'souza, N.A., Watson, S.B., and McKay, R.M.L., 2019. Transitions in microbial communities along a 1600 km freshwater trophic gradient. J of Great Lakes Res. 45, 263-276.

Ram, J.L., Thompson, B., Turner, C., Nechvatal, J.M., Sheehan, H., Bobrin, J., 2007. Identification of pets and raccoons as sources of bacterial contamination of urban storm sewers

using a sequence-based bacterial source tracking method. Water Res. 41(16) 3605–3614. DOI: 10.1016/j.watres.2007.04.013.

Roguet A, Esen ÖC, Eren AM, Newton RJ, McLellan SL. FORENSIC: an Online Platform for Fecal Source Identification. mSystems. 2020 Mar 17;5(2):e00869-19. DOI: 10.1128/mSystems.00869-19. PMID: 32184364; PMCID: PMC7380585.

Rouge River Advisory Council. 2004. 2004 Rouge River Remedial Action Plan Revision. Access via: https://www.epa.gov/sites/default/files/2015-04/documents/2004_rouge-river-rap-revision.pdf

Scott, T.M., Rose, J.B., Jenkins, T.M., Farrah, S.R., Lukasik, J. 2002. Microbial source tracking: current methodology and future directions. Appl. Environ. Microbiol. 68(12), 5796–5803. DOI: 10.1128/AEM.68.12.5796-5803.2002.

Shahraki, A.H., Chaganti, S.R., Heath, D. 2021. Spatio-temporal dynamics of bacterial communities in the shoreline of Laurentian great Lake Erie and Lake St. Clair's large freshwater ecosystems. BMC Microbiol 21, 253. https://doi.org/10.1186/s12866-021-02306-y.

Shrestha, A., Kelty, C. A., Sivaganesan, M., Shanks, O. C., & Dorevitch, S. 2020. Fecal pollution source characterization at non-point source impacted beaches under dry and wet weather conditions. Water research, 182, 116014. https://doi.org/10.1016/j.watres.2020.116014

Southeast Michigan Council of Governments (SEMCOG). 1990. Remedial Action Plan for the River Rouge Basin, Volume 1: Executive Summary. Prepared by SEMCOG and MDNR. Submitted to the Michigan Water Resources Commission. Revised September 1990. Accessed via: https://www.epa.gov/sites/default/files/2015-04/documents/1990_rouge-river-rap-update.pdf

SEMCOG. 1994. 1994 Rouge River Remedial Action Plan Update. Prepared by SEMCOG and MDNR. Accessed via: https://www.epa.gov/sites/default/files/2015-04/documents/1994_rouge-river-rap-update.pdf

St. Clair Region Conservation Authority. 2016. St. Clair River Area of Concern 2017-2022 Work Plan. St. Clair River Remedial Action Plan. Accessed via: https://friendsofstclair.ca/wp-content/uploads/2020/04/St.-Clair-River-AOC-2017-2022-Work-Plan.pdf

Staley, Z.R., Vogel, L., Robinson, C., Edge, T. 2015. Differential occurrence of Escherichia coli and human Bacteroidales at two Great Lakes beaches. Journal of Great Lakes Research. 41. 10.1016/j.jglr.2015.03.006.

Staley, Z.R., Edge, T.A. 2016. Comparative microbial source tracking methods for identification of fecal contamination sources at Sunnyside Beach in the Toronto region Area of Concern. J. Water Health. 14(5) 839–850. DOI: 10.2166/wh.2016.296.

Staley, Z.R., Boyd, R.J., Shum, P., Edge, T.A., 2018a. Microbial source tracking using quantitative and digital PCR to identify sources of fecal contamination in stormwater, river

water, and beach water in a Great Lakes Area of Concern. Appl. Environ. Microbiol. 84(20) e01634-18. DOI: 10.1128/AEM.01634-18.

Staley, Z.R., Chuong, J.D., Hill, S.J., Grabuski, J., Shokralla, S., Hajibabaei, M., Edge, T.A., 2018b. Fecal source tracking and eDNA profiling in an urban creek following an extreme rain event. Sci. Rep. 8, 14390. DOI: 10.1038/s41598-018-32680-z.

State of Ohio. 2020. "Harmful Algal Bloom Response Strategy for Recreational Waters". Prepared by the Ohio Department of Health, Ohio Environmental Protection Agency, and Ohio Department of Natural Resources. August 2020. https://epa.ohio.gov/static/Portals/35/hab/HABResponseStrategy.pdf

US Environmental Protection Agency (USEPA). 1986. Ambient water quality criteria for bacteria:1986. Office of Water Regulations and Standards, Criteria and Standards Division. Washington, DC. EPA 4405-84-002. 24 p.

US Environmental Protection Agency, 2019a. Method 1696: Characterization of human fecal pollution in water by HF183/BacR287 TaqMan quantitative polymerase chain reaction (qPCR) assay. Office of Water. Washington, DC. EPA 821-R-19-002. 56 p.

US Environmental Protection Agency, 2019b. Method 1697: Characterization of Human Fecal Pollution in Water by HumM2 TaqMan® Quantitative Polymerase Chain Reaction (qPCR) Assay®. Office of Water. Washington, DC. EPA 821-R-19-003. 56 p.

Yancey et al. (2022). Variation of the mcy operon and population diversity in a cyanobacterial bloom community reveals dynamics of novel and known Microcystis genotypes. *Applied and Environmental Microbiology* 88: e02464-21.

Zepernick, B.N., Denison, E.R., Chaffin, J.D., Bullerjahn, G.S., Pennacchio, C.P., Frenken, T., Peck, D.H., Anderson, J.T., Niles, D., Zastepa, A., McKay, R.M.L. 2022. Metatranscriptomics sequencing of winter and spring planktonic communities from Lake Erie, a Laurentian Great Lake. American Society for Microbiology. Microbiology Resource Announcements. 11 (7) 1-3. https://doi.org/10.1128/mra.00351-22.

Zuellig, R.E., Graham, J.L., Stelzer, E.A., Loftin, K.A., and Rosen, B.H., 2021, Cyanobacteria, cyanotoxin synthetase gene, and cyanotoxin occurrence among selected large river sites of the conterminous United States, 2017–18: U.S. Geological Survey Scientific Investigations Report 2021–5121, 22 p., https://doi.org/10.3133/sir20215121.

5.1 Additional resources provided during workshops

Abhilasha, S., and Dorevitch, S. 2020. Slow adoption of rapid testing: Beach monitoring and notification using qPCR has a publication with PCR startup costs for a beach monitoring program. Journal of Microbio. Methods (174): 105947. https://www.sciencedirect.com/science/article/pii/S016770122030230X

Boehm, A.B., et al. 2018. Can We Swim Yet? Systematic Review, Meta-Analysis, and Risk Assessment of Aging Sewage in Surface Waters. Enviro. Sci. Technol. 52 (17): 9634-9645. https://pubs.acs.org/doi/abs/10.1021/acs.est.8b01948

Bowling Green State University Great Lakes Center for Fresh Waters and Human Health: https://www.bgsu.edu/great-lakes-center

Burch, T.R., et al. 2021. Quantitative Microbial Risk Assessment for Contaminated Private Wells in the Fractured Dolomite Aquifer of Kewaunee County, Wisconsin. Enviro. Health Persp. 129(6): https://ehp.niehs.nih.gov/doi/full/10.1289/EHP7815

Bustin SA, Benes V, Garson JA, Hellemans J, Huggett J, Kubista M, Mueller R, Nolan T, Pfaffl MW, Shipley GL, Vandesompele J, Wittwer CT. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Clin Chem. 2009 Apr;55(4):611-22. doi: 10.1373/clinchem.2008.112797. Epub 2009 Feb 26. PMID: 19246619. https://pubmed.ncbi.nlm.nih.gov/19246619/

Chaffin, J.D., Bratton, J.F., Verhamme, E.M., et al., 2021. The Lake Erie HABs Grab: A binational collaboration to characterize the western basin cyanobacterial harmful algal blooms at an unprecedented high-resolution spatial scale. Harmful Algae, 108, p.102080. https://doi.org/10.1016/j.hal.2021.102080

Chaffin, J.D.; Mishra, S.; Kane, D.D.; Bade, D.L.; Stanislawczyk, K.; Slodysko, K.N.; Jones, K.W.; Parker, E.M.; Fox, E.L. Cyanobacterial blooms in the central basin of Lake Erie: Potentials for cyanotoxins and environmental drivers. J. Great Lakes Res. 2019, 45, 277–289. https://doi.org/10.1016/j.jglr.2018.12.006

Chik, A., et al., 2022. Protocol for evaluations of RT_qPCR Performance Characteristics: Technical Guidance. Project: Ontario MECP's Wastewater Surveillance Initiative. https://www.researchgate.net/publication/354248379_Protocol_for_Evaluations_of_RT-qPCR_Performance_Characteristics_Technical_Guidance

Corsi, S.R., et al. 2021. Optical Properties of Water for Prediction of Wastewater Contamination, Human-Associated Bacteria, and Fecal Indicator Bacteria in Surface Water at Three Watershed Scales. Environmental Science & Technology. 55 (20), 13770-13782. DOI: 10.1021/acs.est.1c02644 https://pubs.acs.org/doi/full/10.1021/acs.est.1c02644

Davis TW, Stumpf R, Bullerjahn GS, McKay RM, Chaffin JD, Bridgeman TB, Winslow C. Science meets policy: a framework for determining impairment designation criteria for large waterbodies affected by cyanobacterial harmful algal blooms. Harmful algae. 2019 Jan 1;81:59-64.

Dila, D.K., Corsi, S.R., Lenaker, P.L., Baldwin, A.K., Bootsma, M.J., McLellan, S.L. 2018. Patterns of Host-Associated Fecal Indicators Driven by Hydrology, Precipitation, and Land Use Attributes in Great Lakes Watersheds. Environmental Science & Technology, 52 (20), 11500-11509. DOI: 10.1021/acs.est.8b01945: https://pubs.acs.org/doi/10.1021/acs.est.8b01945

Dila, D.K., et al. 2018. Patterns of Host-Associated Fecal Indicators Driven by Hydrology, Precipitation, and Land Use Attributes in Great Lakes Watersheds. Environ. Sci. Technol. 52(20). 11500-11509. https://pubs.acs.org/doi/10.1021/acs.est.8b01945

Edge, T.A. et al. 2020. The Ecobiomics project: advancing metagenomics assessment of soil health and freshwater quality in Canada. Science of the Total Environment 710: 135906. https://www.sciencedirect.com/science/article/abs/pii/S0048969719359017?via%3Dihub

Hatfield, R.G., et al. 2020. The Application of Nanopore Sequencing Technology to the Study of Dinoflagellates: A Proof of Concept Study for Rapid Sequence-Based Discrimination of Potentially Harmful Algae" Front. Microbiol: https://doi.org/10.3389/fmicb.2020.00844. https://www.frontiersin.org/articles/10.3389/fmicb.2020.00844/full

Hellweger FL, Martin RM, Eigemann F, Smith DJ, Dick GJ, Wilhelm SW. Models predict planned phosphorus load reduction will make Lake Erie more toxic. Science. 2022 May 27;376(6596):1001-5.

Genomic Approaches in Earth and Environmental Sciences: ISBN: 978-1-118-70826-2: https://www.wiley.com/en- us/Genomic+Approaches+in+Earth+and+Environmental+Sciences-p-9781118708262 (email Greg Dick for PDF: gdick@umich.edu)

GEO BON – Omic BON: Standards-based body for genomic observatories: https://geobon.org/geo-bon-welcomes-a-new-thematic-bon/

Government of Canada: EOLakeWatch: Satellite earth observations for lake monitoring. https://www.canada.ca/en/environment-climate-change/services/water-overview/satellite-earth-observations-lake-monitoring.html

Government of Canada: The EcoBiomics Project: genomics research and development initiative: https://www.canada.ca/en/environment-climate-change/services/biodiversity/ecobiomics.html

Government of Canada: Guideline for Canadian Recreational Water Quality – Third Edition. Part II: Guideline Technical Documentation. 6.0 Cyanobacteria and their toxins. https://www.canada.ca/en/health-canada/services/publications/healthy-living/guidelines-canadian-recreational-water-quality-third-edition/guidelines-canadian-recreational-water-quality-third-edition-page-11.html

Government of Canada: Infectious Disease and Climate Change Fund (From Public Health Agency of Canada), to assess impact of climate change on human health, including water: https://www.canada.ca/en/public-health/services/funding-opportunities/infectious-diseases-climate-change-fund.htm

Graham, J.L., Dubrovsky, N.M., Foster, G.M., King, L.R., Loftin, K.A., Rosen, B.H., Stelzer, E.A. 2020. Cyanotoxin occurrence in large rivers of the United States, Inland Waters, 10:1, 109-117, DOI: 10.1080/20442041.2019.1700749 https://www.tandfonline.com/doi/full/10.1080/20442041.2019.1700749

Great Lakes Commission HABs collaborative: https://www.glc.org/work/habs (can join, too)

Great Lakes DataStream: An open access hub for sharing water data: https://greatlakesdatastream.ca/

Great Lakes St. Lawrence Collaborative: https://glslcities.org/initiatives/collaborative/

Health Professionals Advisory Board Report to the IJC: "The Great Lakes Water Quality Centennial Study - Phase I Report." 2021: https://www.ijc.org/sites/default/files/2021-07/HPAB_CentennialStudy_PhaseI_2021.pdf

IJC 1913 study: International Joint Commission. 1918. Final Report of the International Joint Commission on the Pollution of Boundary Waters Reference: Washington-Ottawa (p. 55). International Joint Commission; International Joint Commission (IJC) Digital Archive. https://scholar.uwindsor.ca/ijcarchive/34

International Joint Commission Report from the Health Professionals Advisory Board. February 27, 2017. Report on Human Health Effects of Cyanobacterial Toxins. https://ijc.org/sites/default/files/2019-04/HPABAlgalBloomFinal.pdf

IJC report: https://ijc.org/en/hpab/health-experts-find-heavy-storms-can-predict-risk-germs-treated-great-lakes-drinking-water

Interstate Technology Regulatory Council. Strategies for Preventing and Managing Benthic Harmful Cyanobacterial Blooms (HCB-2) https://hcb-2.itrcweb.org/introduction/

Interstate Technology Regulatory Council: 2.6 Cyanotoxin Thresholds (cyanotoxin ingestion threshold values for different creatures): https://hcb-2.itrcweb.org/cyanotoxins/#_Cyanotoxin_Thresholds

Kinzelman, J., Byappanahalli, M.N., Nevers, M.B., Shively, D., Kurdas, S. and Nakatsu, C., 2020. Utilization of multiple microbial tools to evaluate efficacy of restoration strategies to improve recreational water quality at a Lake Michigan Beach (Racine, WI). Journal of Microbiological Methods, 178, p.106049.

Lake Ontario Waterkeeper. 2006. Bluffer's Beach Report: http://www.waterkeeper.ca/blog/3173

Lake Ontario Waterkeeper. 2019. Swim Drink Fish submission for Ontario's environment plan. http://www.waterkeeper.ca/blog/ontario-environment-plan-submission

Lenaker, P.L., Corsi, S.R., Borchardt, M.A., Spencer, S.K.,Baldwin, A.K., Lutz, M.A. Hydrologic, land cover, and seasonal patterns of waterborne pathogens in Great Lakes tributaries, Water Research, Volume 113, 2017, Pages 11-21, ISSN 0043-1354, https://doi.org/10.1016/j.watres.2017.01.060. https://www.sciencedirect.com/science/article/pii/S0043135417300672https://www.sciencedirect.com/science/article/pii/S0043135417300672

Lenard, T. (2015). Winter bloom of some motile phytoplankton under ice cover in a mesotrophic lake: vertical distribution and environmental factors. Oceanol. Hydrobiol. St. 44, 164–171. https://doi.org/10.1515/ohs-2015-0016

LightDeck: Lab-quality diagnostics. https://lightdeckdx.com/

Liu, Q., Rowe, M. D., Anderson, E. J., Stow, C. A., Stumpf, R. P., & Johengen, T. H. (2020). Probabilistic forecast of microcystin toxin using satellite remote sensing, in situ observations and numerical modeling. Environmental Modelling & Software, 128, 104705 https://doi.org/10.1016/j.envsoft.2020.104705

Madani, M., Seth, R., Leon, L. F., Valipour, R., & McCrimmon, C. (2020). Three dimensional modelling to assess contributions of major tributaries to fecal microbial pollution of lake St. Clair and Sandpoint Beach. Journal of Great Lakes Research, 46(1), 159-179

Madani, M., Seth, R., Leon, L. F., Valipour, R., & McCrimmon, C. (2021). Microbial modelling of Lake St. Clair: Impact of local tributaries on the shoreline water quality. Ecological Modelling, 458, 109709.

May, N.W., Olson, N.E., Panas, M., Axson, J.L., Tirella, P.S., Kirpes, R.M., Craig, R.L., Gunsch, M.J., China, S., Laskin, A. and Ault, A.P., 2018. Aerosol emissions from Great Lakes harmful algal blooms. Environmental Science & Technology, 52(2), pp.397-405.

McLellan, S.L., et al. 2018. Sewage loading and microbial risk in urban waters of the Great Lakes. Elementa: Science of the Anthropocene 6(46). https://online.ucpress.edu/elementa/article/doi/10.1525/elementa.301/112829/Sewage-loading-and-microbial-risk-in-urban-waters

Michigan statewide TMDL: https://www.michigan.gov/egle/0,9429,7-135-3313_3681_3686_3728-376271--,00.html

Microbiome Informatics - API and service usage examples: https://github.com/EBI-Metagenomics/examples

Microbiome Support: European Microbe Effort: https://www.microbiomesupport.eu/

Millennium Ecosystem Assessment: https://www.millenniumassessment.org/

Muzzerall, P. Waterkeeper Swim Guide. 2018 Great Lakes Beach Report: https://greatlakes.guide/ideas/2018-great-lakes-beach-report

Niagara River Assessment Report, Beach Closings, BUI #10. 2021: http://ourniagarariver.ca/wp-content/uploads/2021/05/NRRAP-Beach-Closings-BUI-Status-Assessment-Report.pdf

NOAA Great Lakes Environmental Research Laboratory. Great Lakes Coastal Forecast System - Experimental: https://www.glerl.noaa.gov/res/glcfs/

Ohio EPA HAB Public Water System and Other Ohio EPA Surface Water Monitoring Portal: https://geo.epa.ohio.gov/portal/apps/opsdashboard/index.html#/e72499f345ec43579513da521d83347e

Ohio EPA: Harmful Algal Blooms (HAB) Information for Public Water Systems: https://epa.ohio.gov/divisions-and-offices/drinking-and-ground-waters/public-water-systems/harmful-algal-blooms

Ohio EPA Recreational HABs: https://epa.ohio.gov/monitor-pollution/pollution-issues/harmful-algae-blooms

Olds, H.T., et al. 2018. High levels of sewage contamination released from urban areas after storm events: A quantitative survey with sewage specific bacterial indicators. PLOS Medicine. https://journals.plos.org/plosmedicine/article?id=10.1371/journal.pmed.1002614

Ontario Ministry of the Environment, Conservation and Parks Suspected Cyanobacteria Blooms Reporting Hotline: 1-866-MOE-TIPS (663-8477)

Ontario Ministry of the Environment, Conservation and Parks. Source Protection Information Atlas. Interactive Map: https://www.lioapplications.lrc.gov.on.ca/SourceWaterProtection/index.html?viewer=SourceWaterProtection.SWPViewer&locale=en-CA

Phytoxigene: CyanoDTec: https://www.phytoxigene.com/products

Roberts VA, Vigar M, Backer L, et al. Surveillance for Harmful Algal Bloom Events and Associated Human and Animal Illnesses — One Health Harmful Algal Bloom System, United States, 2016–2018. MMWR Morb Mortal Wkly Rep 2020;69:1889–1894. DOI: http://dx.doi.org/10.15585/mmwr.mm6950a2

Salter, C., et al., 2021. Investigating the microbial dynamics of microcystin-LR degradation in Lake Erie sand. Chemosphere (272): 129873. https://doi.org/10.1016/j.chemosphere.2021.129873. https://www.sciencedirect.com/science/article/pii/S0045653521003428

Shahraki, A. H., Heath, D., & Chaganti, S. R. (2019). Recreational water monitoring: Nanofluidic qRT-PCR chip for assessing beach water safety. Environmental DNA, 1(4), 305-315.

State of the Great Lakes 2019 Report: https://binational.net/2020/06/03/sogl-edgl-2019-2/

Stokdyk, J.P., Spencer, S.K., Walsh, J.F., de Lambert, J.R., Firnstahl, A.D., Anderson, A.C., Rezania, L.W., Borchardt, M.A. 2019. Environmental Science & Technology 2019 53 (7), 3391-3398. DOI: 10.1021/acs.est.8b05446. https://pubs.acs.org/doi/abs/10.1021/acs.est.8b05446

TATAA Biocenter. TATAA Universal DNA Spike II. Commercial spike-in and primers used for quality control: https://webshop.tataa.com/products/tataa-universal-dna-spike-ii

The Great Lakes and St. Lawrence Collaborative. Action Plan to Protect the Great Lakes and St. Lawrence 2020-2030. June 2020. https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=&ved=2ahUKEwjv76zU2v77AhWzJ0QIHVEcBEMQFnoECAgQAw&url=http%3A%2F%2Fwww.glfc.org%2Fpubs%2Fpdfs%2F2020%2520Great%2520Lakes%2520and%2520St.%2520Lawrence%2520Collaborative-ENG-Web.pdf&usg=AOvVaw2xTjyH_VvgwF6U8flijGQc

The Swim Guide: Weather and Water Quality for 8,000 Beaches: www.theswimguide.org

U.S. Centers for Disease Control and Prevention. One Health Harmful Algal Blooms System: https://www.cdc.gov/habs/ohhabs.html

U.S. EPA Cyanobacteria Assessment Network Application (CyAN app): epa.gov/water-research/CyANapp

U.S. Environmental Protection Agency, Office of Wastewater Management. 2016. Report to Congress on Combined Sewer Overflows into the Great Lakes Basin (EPA 833-R-16-006; p. 104). U.S. Environmental Protection Agency; National Service Center for Environmental Publications. https://nepis.epa.gov/Exe/ZyPDF.cgi/P100OEFG.PDF?Dockey=P100OEFG.PDF

U.S. EPA: EPA Drinking Water Health Advisories for Cyanotoxins: https://www.epa.gov/cyanohabs/epa-drinking-water-health-advisories-cyanotoxins

U.S. EPA: 2019 Recreational Water Quality Criteria or Swimming Advisories for Cyanotoxins: https://www.epa.gov/wqc/recreational-water-quality-criteria-and-methods#rec3

U.S. EPA. Long Term 2 Enhanced Surface Water Treatment Rule Documents: https://www.epa.gov/dwreginfo/long-term-2-enhanced-surface-water-treatment-rule-documents

U.S. Geological Survey Drinking Water and Source Water Research: https://www.usgs.gov/mission-areas/water-resources/science/drinking-water-and-source-water-research#science

University of Montreal. Adopt a Lake Program: https://fas.umontreal.ca/adopte-un-lac/adopt-a-lake/

Utilities Kingston. Know before you go. https://utilitieskingston.com/Wastewater/SewerOverflow/Map

Van Eijsden, R.G., Stassen, C., Daenen, L., Van Mulders, S.E., Bapat, P.M., Siewers, V., Goossens, K.V., Nielsen, J., Delvaux, F.R., Van Hummelen, P. and Devreese, B., 2013. A universal fixation method based on quaternary ammonium salts (RNAlater) for omics-technologies: Saccharomyces cerevisiae as a case study. Biotechnology letters, 35(6), pp.891-900. https://doi.org/10.1007/s10529-013-1163-0

Whelan, G., et al. 2018. Capturing Microbial Sources Distributed in a Mixed-use Watershed within an Integrated Environmental Modeling Workflow. Environ Model Softw. 2018 Jan; 99:

126–146. doi: 10.1016/j.envsoft.2017.08.002.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6069999/

WHO-Geneva, CH, World Health Organization. HEALTH-BASED MONITORING OF RECREATIONAL WATERS: THE FEASIBILITY OF A NEW APPROACH (THE 'ANNAPOLIS PROTOCOL'). Protection of the Human Environment Water, Sanitation and Health Series, Annapolis, MD, November 01 - 05, 1998. WHO -Geneva, CH, World Health Organization (ed.), BULLETIN OF THE WORLD HEALTH ORGANIZATION. World Health Organization (WHO), Geneva, Switzerland, 1-50, (1999).
https://cfpub.epa.gov/si/si_public_record_report.cfm?Lab=NERL&dirEntryId=309392

Wilkinson, Grace M., Walter, Jonathan A., Buelo, Cal D., Pace, and Michael L. 2021. No evidence of widespread algal bloom intensification in hundreds of lakes. Frontiers in Ecology and the Environment, 20(1): 16-21. https://doi.org/10.1002/fee.2421

Wilkinson, M., Dumontier, M., Aalbersberg, I. et al. The FAIR Guiding Principles for scientific data management and stewardship. Sci Data 3, 160018 (2016).
https://doi.org/10.1038/sdata.2016.18
https://www.nature.com/articles/sdata201618?ref=https://githubhelp.com

6.0 Appendices

Appendix A. Workshop Participants

Appendix B. Workshop Summaries

Appendix C. Genomic Studies table

Appendix D. Detailed Laboratory network table

Appendix A. Workshop Participants Summary Table.

Name	Affiliation	MST Attendee?	HABs Attendee?	Meta-genomics Attendee?	Synthesis workshop Attendee?	# of Workshops Attended?
Abhilasha Shrestha	UIC School of Public Health	Y				1
Albert Simhon	Ontario Ministry of the Environment	Y	Y	Y		3
Alberto Mazza	Canada's National Research Council	Y	Y			2
Alex Chik	Ontario Clean Water Agency, Project Manager	Y	Y	Y		3
Ana Serviente	Great Lakes Observing System		Y	Y	Y	2
Anders Kiledal	University of Michigan	Y				1
Andrea Busch	GLWA - Detroit		Y	Y		2
Andrew Gangidine	Cranbrook Institute of Science, Curator of Earth and Space Sciences	Y				1
Andy Bramburger	ECCC		Y			1
Anna Majury	Public Health Ontario Microbiologist	Y	Y			2
Ann-Marie Abbey	Ontario MECP	Y				1
Arthur Zastepa	ECCC		Y		Y	1
Ben Southwell	Lake Superior State University	Y				1
Bernie Mayer	Haliburton Kavartha Health Unit	Y	Y		Y	2
Bill Snodgrass	City of Toronto, ON	Y				1
Brandon Moody	Charlotte County Water Quality Specialist	Y				1
Brian Pecson	Trussell Technologies	Y				1
Carmen Thiel Ebert	UW Oshkosh ERIC Lab Associate Lab Director		Y			1
Carol Waldmann Rosenbaum	Ph.D. student at Michigan State University		Y			1
Carrie Givens	USGS, Microbiologist, Environmental Microbiology Team Lead		Y			1
Cassandra Lofranco	MECP, Manager of Biological Analysis		Y		Y	1
Charles Greer	National Research Council Canada, Team Leader		Y	Y		2
Charles Greer	National Research Council		Y	Y		2
Chris Weisener	Great Lakes Institute for Environmental Research		Y		Y	1
Chrystal Landgraff	Public Health Agency of Canada				Y	1

Name	Affiliation	MST Attendee?	HABs Attendee?	Meta-genomics Attendee?	Synthesis workshop Attendee?	# of Workshops Attended?
Claire Holeton	Ontario Ministry of the Environment, Conservation and Parks. Scientist, Nutrient and Algal Monitoring		Y	Y	Y	2
Cody Sheik	University of Minnesota Duluth, Associate Professor		Y	Y		2
Craig Stow	NOAA GLERL	Y			Y	1
DEEPAK SHARMA	Health Canada, Head Microbiological Assessment Section	Y	Y			2
Dennis McCormac	Ontario Genomics	Y				1
Dwayne Jarman	FDA, HHS	Y				1
Ellen Cooney	Wisconsin Department of Natural Resources, Lake Superior Sediment and Monitoring Coordinator		Y			1
Emily Darby	Trussell Technologies, Engineer	Y				1
Erin Dreelin	Michigan State University	Y				1
Florence Hsu	Kent State University			Y		1
Gabrielle Parent-Doliner	Water Rangers	Y	Y		Y	2
Gertjan Medema	MSU visiting scientist	Y				1
Glen Diagger	University of Michigan	Y				1
Glen Goodman	Ontario First Nations Technical Services Corporation	Y				1
Godlove Ngwa	MECP		Y			1
Greg Boyer	State University of New York		Y			1
Greg Dick	Univ of Michigan and CIGLR Dir.		Y	Y	Y	2
Greg Ford	Swim Drink Fish Canada	Y	Y			2
Gregory T. Kleinheinz	University of Wisconsin-Oshkosh	Y				1
Heather Murphy	Univ of Guelph	Y				1
Heather Richards	Senior Policy and Program Advisor	Y		Y		2
Herb Schellhorn	Mcmaster university	Y	Y	Y	Y	3
Huiyun Wu	Postdoc			Y		1
Jacob Orlandi	ECCC - Great Lakes Rec. Water Indicator Author	Y				1
James Macklin	Agriculture and Agri-food Canada, Research Scientist			Y	Y	1
Janette Anderson	Environment and Climate Change Canada	Y				1
Janis Thomas	Ont Ministry of the Environment, Conservation and Parks; Research Scientist	Y	Y	Y	Y	3

Name	Affiliation	MST Attendee?	HABs Attendee?	Meta-genomics Attendee?	Synthesis workshop Attendee?	# of Workshops Attended?
Jerry Chao	Ontario Ministry of the Environment. Technologist		Y			1
Jessica Strand	Mashkiiziibii Natural Resources Department, Environmental Specialist	Y				1
Jill Crumb	TreeFrog Environmental, Ecologist/President		Y			1
Jingrang Lu	EPA, Research Biologist		Y		Y	1
Joan Rose	Michigan State University/ IJC HPAB	Y	Y	Y		3
Joel Stokdyk | Biologist	U.S. Geological Survey | Upper Midwest Water Science Center	Y				1
John Ravenscroft	US EPA	Y				1
Jon Allan	IJC Water Quality Board	Y			Y	1
Jorge Santo Domingo	CESER/WID/BCB		Y		Y	1
Julia Hatcher	ECCC - Great Lakes Project Officer	Y				1
Julie Kinzelman	City of Racine, WI	Y				1
Katelyn McKindles	University of Michigan, Postdoctoral Fellow			Y		1
Kathey-Lee Galvin	IJC				Y	1
Katie Stammler	Essex Region Conservation Authority - Source Water Project Manager		Y			1
Kevin McDermott	Public Health Ontario-Research Technician	Y	Y	Y		3
Kevin Oshima	USEPA	Y				1
Kyle Davis	Municipality of Lakeshore Water Compliance Coordinator		Y		Y	1
Lars Schreiber	National Research Council Canada				Y	1
Lauren Lynch	U.S. Geological Survery, Biologist		Y			1
Laurie Chan		Y				1
Lori Phillips	Agriculture and Agri-Food Canada; Research Scientist in Microbial Ecology			Y		1
Mahesh Patel	City of Toronto, ON	Y				1
Marc Habash	University of Guelph, Associate Professor	Y		Y	Y	2
Mark Mattson	Swim Drink Fish Canada	Y				1
Martha Gerig	Michigan Sea Grant, Extension Educator		Y			1
Mary Anne Evans	USGS, Research Ecologist		Y			1
Matthew Robson	Supervisor, Organic Contaminants, Ontario Ministry of the		Y			1

Name	Affiliation	MST Attendee?	HABs Attendee?	Metagenomics Attendee?	Synthesis workshop Attendee?	# of Workshops Attended?
	Environment, Conservation & Parks					
Maureen Coleman	University of Chicago, Associate Professor			Y	Y	1
Megan McCusker	Environment Canada				Y	1
Mike McKay	University of Windsor - GLIER	Y	Y			2
Nathalie Fortin	National Research Council of Canada, Research council officer		Y	Y		2
Ngan Diep	MECP, Great Lakes Advisor			Y	Y	1
Nicolas Tromas	UdeM/LS2N Researcher		Y	Y		2
Norman Barth	IJC	Y				1
Orin Shanks	US EPA	Y			Y	1
Peter Lenaker	USGS - Physical Scientist	Y				1
Rajesh Seth	University of Windsor	Y				1
Rebecca Klaper	UW-Milwaukee		Y			1
Rene Sahba Shahmohamadloo	U Guelph		Y			1
Rich Haugland	US EPA	Y			Y	1
Richard Villemur	INRS Centre Armand-Frappier Santé Biotechnologie			Y		1
Richard Whitman	USGS-Retired	Y				1
Rick Rediske	Grand Valley State University	Y				1
Rodney Bouchard	Union Water Supply System - General Manager	Y	Y			2
Ruth Briland	Ohio Environmental Protection Agency, Environmental Specialist		Y			1
Ruth Etzel	IJC HPAB	Y				1
Ryan Graydon	US EPA	Y				1
Ryan Newton	University of Wisconsin-Milwaukee	Y				1
Ryan Sorichetti	Ontario Ministry of the Environment, Conservation and Parks - Great Lakes Scientist		Y			1
Sandra McClellan	University of Wisconsin - Milwaukee	Y			Y	1
Sandy Edelsward	Public Health Ontario - Program Coordinator, Drinking Water Testing	Y				1
Sara Hudson	City of Ashland Parks and Recreation	Y				1
Sarah Bartlett	NEW Water, Water Resources Specialist		Y			1
Sarah Dorner	Polytechnique Montréal		Y		Y	1
Satoshi Ishii	University of Minnesota	Y				1

Name	Affiliation	MST Attendee?	HABs Attendee?	Metagenomics Attendee?	Synthesis workshop Attendee?	# of Workshops Attended?
Sean Backus	Environment and Climate Change Canada	Y				1
Shannon Briggs	State of Michigan EGLE	Y				1
Sophie Crevecoeur	Environment and Climate Change Canada, research scientist		Y	Y	Y	2
Stacey MacFarlane	Macomb County Health Department	Y				1
Steven Corsi	USGS	Y				1
Steven Wilhelm	The University of Tennessee, Professor		Y	Y	Y	2
Subba Rao Chaganti	University of Michigan		Y		Y	1
Sue Watson	Retired, adjunct, Trent University, part time consultant		Y			1
Susan Weir	Ontario	Y				1
Tami Sivy	Saginaw Valley State University	Y				1
Teresa Brooks	Health Canada Senior Evaluator	Y	Y	Y	Y	3
Tom Edge	Adjunct Professor, McMaster University	Y	Y	Y		3
Tom Speth	US EPA	Y				1

Appendix B. Workshop Summaries of Findings and Recommendations.

Provided as separate files.

Appendix C. Summary of Some Genomic Studies in the Great Lakes (non-comprehensive)

Study	Target	Location	Method	Results	Laboratory
MacGregor, et al. (1997)	Archaea	Lake Michigan (sediment)	Hybridization of 16S rRNA	Peak archaeal rRNA abundance in oxic zone, and below it; 6 species identified	Center for Great Lakes Studies, University of Wisconsin-Milwaukee
Kim, et al. (2015)	Viral communities	Ballast water from ships across GL, arriving at Port of Duluth-Superior	16S rDNA PCR with 27F/1492R to ensure viral nucleic acids, Illumina HiSeq	Viral taxonomic profile of ballast water (uncultured virus diversity)	Research Technology Support Facility, Michigan State University
Fujimoto, et al. (2016)*	Bacteria communities (free living, particle associated)	Muskegon estuary, Lake Michigan	Target V4 Region of 16S rRNA gene, Illumina MiSeq	139 operational taxonomic units used to determine spatiotemporal bacterial community variability	Joint Genome Institute
Denef, et al. (2017)	Bacterioplankton	Lake Michigan	V4 Region 16S rRNA gene, Illumina MiSeq	Effect of invasive dreissenid mussel impacts on bacterial assemblages	Joint Genome Institute, University of Michigan Medical School
Paver, et al. (2019)*	Bacterial & archaeal picoplankton	Lakes Huron, Ontario, Michigan, Erie, Superior	V4-V5 regions of 16S rRNA, Illumina MiSeq	Hydrologic connectivity and local selective pressures and depths shape microbial communities	Joint Genome Institute
Butler, et al. (2019)	Bacteria and archaea	Keweenaw Waterway	V4-V5 regions of 16S rRNA, Illumina MiSeq	Ice formation may cause microbial community composition shifts, increasing diversity during ice cover	(Michigan Tech mentioned for sampling help, but not metagenomics work)
McKindles, et al. (2020)	Microcystis viral genomic fragments	Western Lake Erie (Toledo water intake)	NovaSeq 6000	Compared viral communities between 2014 + 2019 events, captured lysis event	University of Michigan Advanced Genomics Core, Ann Arbor, MI
Shahraki, et al. (2021)	Bacteria communities	Lake Erie and Lake St. Clair	Amplified V5-V6 region of 16S rRNA	Spatial variation, distinct temporal and seasonal variation in taxonomic groups	Environmental Genomics Facility, Great Lakes Institute for Environmental Research, Windsor University
Grim, et al. (2021)	Cyanobacterial mats bacteria communities	Middle Island Sinkhole (Lake Huron)	Illumina HiSeq 2000, producing pair-end reads	Specific microbial groups were linked to metabolic processes and tight oxygen/sulfur coupled cycling in mats	University of Michigan DNA Sequencing Core
Palermo, et al. (2021)	Viral communities	Hamilton Harbour, Lake Ontario	0.45 um pore size filters, Nextera Flex DNA, NextSeq 550	Important to analyze both larger (>0.45 um) and smaller (<0.45 um) viral fractions	Microbial Genome Sequencing Centre (MiGS), Pittsburgh, PA
Chaudhary, et al. (2021)*	Bacteria communities	Offshore/coastal, Kalamazoo River mouth, Lake Michigan	V3 to V4 primers of 16S rRNA, Illumina MiSeq	Evaluated bacterial community structure; role of t-DOM in bacterial metabolism	University of Illinois at Chicago Sequencing Core
Zepernick, et al. (2022)	Planktonic communities	Lake Erie	Illumina NovaSeq S4 2 × 151-nucleotide indexed run protocol	Confirmed diatoms were a transcriptionally active component of the winter microbiome in Lake Erie	Department of Energy Joint Genome Institute

Appendix D. Detailed Laboratory Network Table

Institution	Lab Leads at Institution	MST	HABs	Metagenomics
McMaster University	Tom Edge, Herb Schellhorn	x	x	x
Ontario MECP	Susan Weir	x		
U.S. EPA	Santo Domingo, Orin Shanks, J. Lu	x	x	x
University of Minnesota	Satoshi Ishii	x		
University of Wisconsin-Milwaukee	Sandra McLellan	x		
University of Windsor - GLIER	Daniel Heath, Mike McKay,	x	x	x
Public Health Ontario and Queen's University	Anna Majury	x		
Oakland University	Dave Szlag	x		
City of Racine Public Health	Stephan Kurdas	x		
USDA-USGS	Mark Borchardt	x		
University of Illinois Chicago School of Public Health	Abhilasha Shrestha	x		
USGS Michigan Bacteriological Research Laboratory	Carrie Givens	x		
University of Guelph	Marc Habash, Lawrence Goodridge	x		
University of Michigan	Gregory Dick, Rao Chaganti, Vincent Denef, Kevin Bakker, Krista Wigginton, Chuanwu Xi	x		X
Univ of Wisconsin Oshkosh	Greg Kleinheinz	x		
Michigan Department of Environment, Great Lakes, and Energy	Shannon Briggs	x		
Central Michigan University	Michael J. Conway	x		
Ferris State University	Sky Pike	x		
Grand Valley State University - Annis Water Resources Institute	Richard Rediske	x		
Hope College	Aaron Best	x		
Kent County Health Department	Leslie Griswold	x		
Lake Superior State University	Benjamin Southwell	x		
Macomb County Public Works	Vince Astorino	x		
Michigan State University	Joan Rose, Irene Xagoraraki	x		

Institution	Lab Leads at Institution	MST	HABs	Metagenomics
Northern Michigan University	Josh Sharp	x		
Northwest Michigan Health Department	Daniel Thorell	x		
Oakland County Health Division	Kimberly Snowdon	x		
Saginaw Valley State University	Tami Sivy	x		
Traverse City	Art Krueger	x		
Wayne State University	Jeffrey Ram	x		
White Water Associates Inc.	Bette Premo	x		
Polytechnique Montréal	Sarah Dorner	x		
INRS Centre Armand-Frappier	Richard Villemur	x		
Université du Québec à Trois-Rivières	François Guillemette	x		
University of Waterloo	Trevor Charles	x		
Trent University	CJ Kyle	x		
Health Sciences North Research Institute	Gustavo Ybazeta	x		
McGill University	Dominic Frigon	x		
National Research Council Canada	Charles Greer			x
Agriculture and AgriFood Canada	Lori Phillips			x
INRS-Centre Armand-Frappier Santé Biotechnologie	Richard Villemur			x
University of Chicago	Maureen Coleman			x
University of Tennessee	Steven Wilhelm		x	x
Algal Taxonomy and Ecology Inc	Hedy Kling		x	
Great Lakes Water Authority	Mary-Lynn Semegen		x	
National Research Council Canada	Charles Greer		x	x
Environment and Climate Change Canada	Sophie Crevecoeur		x	x
Bowling Green State University	George Bullerjahn		x	x