MONITORING MICROBES IN THE GREAT LAKES

Workshop #1: Monitoring Benefits, Needs, and Methods

Planning and Reporting
by
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Executive Summary

Monitoring and surveillance of non-human pathogens and microbes is new to the Great Lakes even though many environmental monitoring efforts are well established. Recent fish kills and the threat of harmful non-native microbes entering the Great Lakes has motivated serious interest in understanding, assessing, and tracking microbes. This report covers a workshop that was held in late 2008 to define the benefits and needs for a microbe monitoring program, define a set of methods, and explore ways to imbed microbe monitoring in current Great Lakes environmental programs. The primary benefits for this monitoring program include: building understanding and experience, identification of threats and correlates of risk, and allowing proactive management and thoughtful response planning. The scope of microbial monitoring should be aimed at harmful microbes but not human pathogens. The goal adopted at the workshop was to:

Monitor existing and potentially introduced microbes with harmful consequences to the ecosystem, non-human organisms, and human uses of the Great Lakes.

This goal includes diseases of fish and wildlife, direct and indirect ecosystem effects, impacts on human facilities and water uses, and alterations of biotic communities. In short, the dominant aim for monitoring should be an early warning system on harmful microbes.

A vision for a Great Lakes microbe monitoring program is that it should be rapid and robust: fast at reporting results and broad in scope. Thus, the program needs a balance of standardization and adaptability. Guiding principles for a rapid and flexible monitoring program were identified at the workshop:

- Assess occurrence of high-threat introduced microbes
- Track sites associated with vectors, dispersion, and impacts
- Maintain flexibility to adapt to changing needs
- Design sampling from a place-based perspective
- Determine the rate of change in pathogenic microbes
- Detect the presence of a wide range of microbes

We concluded that a collaborative program, shared by Great Lakes agencies and organizations, could balance the demands of standardization of protocols and flexibility in program execution.

The workshop group decided that a representative site selection approach will be most effective and desirable for this type of monitoring. Site selection will give priority to areas with harbors, industrial and power plants, water supply, long-term environmental monitoring, and overall high human use sites. Also important are invasion hotspots, priority species locations, water quality issues areas, and program partner study sites. Approximately 10 sites per lake and associated waters were considered feasible and adequate. Organism sampling will focus on fish at seasonal times of stress (spring, spawning) and aim for representative species collections. Sampling should include multiple capture gear deployed over a range of water depths suitable for representing major trophic classes (predators, prey, planktivores, etc.). Dead and moribund fish and other taxa would be opportunistically collected. Finally, water sampling was regarded as essential and should be collected in a broadly representative way.

Laboratory analyses and procedures cannot be standardized, and extensive expertise is needed to employ the correct procedures for a suspected pathogen or microbial target. There are a limited number of laboratories that can conduct these analyses. Relevant analysis technologies are rapidly advancing. Support will be needed for exploring and employing new technologies and refining the interpretation and threat associated with different microbe findings. With rapid changes in analysis capabilities and target microbes, samples should be archived because they can be used to assess different pathogens and microbes as new issues emerge.

For this proposed monitoring program to be valuable the workshop participants concluded that data and information must reach decision-makers quickly to enable management actions. Speed of reporting and reliability of interpretation will be challenging and a committee of experts could provide initial interpretation for management action. At present there is no lead agency or common point of contact in the Great Lakes region for directing results or findings on microbes and pathogens. A network for information distribution will be needed to receive monitoring results and early warning information.

Introduction

Ecosystems like the Great Lakes support indigenous bacteria and other organisms which often are critical to the maintenance of the physical, chemical and biological properties of the Great Lakes. While some microorganism communities are essential to the proper functioning of the system, others, especially non-native varieties, can be harmful. Little is known about this invisible biological activity, and little is being done to protect native biological communities of the Great Lakes from damage and undesirable change. Of urgent interest are the potential effects of non-native microbes brought to the lakes in ships and by other vectors.

With major support from the Great Lakes Protection Fund, the Northeast-Midwest Institute is leading a team that includes Cornell University, Old Dominion University, the University of Minnesota, the US Geological Survey, and the Great Lakes Commission to develop tools and processes to assess the status of Great Lakes microbial communities and address threats from microbial new-comers. The goal of the integrated research and policy exercise is to generate a practicable approach to assess, detect and manage the risks to the Great Lakes posed by the introduction and spread of non-native microbes, particularly pathogens, by commercial ships. The output of the two year project will be a preliminary risk characterization, development of effective monitoring tools geared at assessing actual microbial threats to the ecosystem, and methods for integrating the tools with current monitoring programs of Great Lakes resource managers. The workshop reported here is one task of this effort and focused on monitoring needs and methods.

Workshop Purpose

States, provinces and federal agencies of the Great Lakes region have a long history of cooperation for developing and operating basin-scale monitoring. The topic breadth and partnerships which characterize current monitoring programs in the Great Lakes basin lay a strong foundation for new monitoring efforts. The purposes of the first workshop were to:

(1) Identify the needs, and propose a goal and specific benefits for a harmful microbe monitoring program,

(2) Define a set of methods (field, lab, data/information) and operations for a harmful microbe monitoring program spanning the Great Lakes basin in collaboration with existing programs, and

(3) Explore ways to integrate microbe monitoring in current Great Lakes environmental programs.

The workshop was held on 18 and 19 December 2008 at the Crowne Plaza Convention Center in Romulus, Michigan. A directory of project staff and participants is provided in Appendix A.

Workshop Presentations

WHO'S NEXT? A CALL FOR AN EARLY WARNING SYSTEM FOR NEW PATHOGENS IN THE GREAT LAKES BASIN

Gary Whelan
Michigan Department of Natural Resources
Fisheries Division

The Great Lakes are currently in the midst of a pathogen invasion with at least 10 new pathogens being detected since 2000. Currently, fisheries agencies are in a reactionary mode as new pathogens are found in the Great Lakes basin. They are usually detected after they are already well established in fish populations in the Great Lakes basin. Fisheries agencies in the Great Lakes basin need the ability to determine potential and likely new pathogens and need the ability to detect new pathogens before they express disease. The ability to detect pathogens in the water column prior to disease expression will provide much needed prior warning to allow fisheries agencies to determine best courses of action to slow the spread of disease from these pathogens. The continuous monitoring of key Great Lakes port areas could potentially provide important information concerning any ballast water introductions.

PATHWAYS OF RISK

Fred Dobbs, Marty Stokes, and Jim Winton

Old Dominion University and the
Western Fisheries Research Center
US Geological Survey

Pathway analysis is a tool that can help policymakers and water quality managers evaluate options to interdict the introduction of nonindigenous species and to contain those species already arrived. The analysis also may be applied to harmful native species. For the Great Lakes, we are developing such a tool to consider the introduction and translocation of harmful aquatic microbes, including harmful microalgae, pathogens of humans, and pathogens of aquatic animals. In addition to specifying pathways whereby microbes may enter and disseminate throughout the Great Lakes, we also rank their probability of occurrence. Likelihood estimates vary according to the particular microorganisms under consideration, but overall, recreational boating and commerce in animals emerge as pathways of greatest concern. In comparison, ships' ballast water ranks high only for the potential introduction and spread of harmful microalgae.

EXAMINING MICHIGAN'S E. COLI DATABASE AND PATHOGEN MONITORING APPROACHES

Joan Rose
Water Research, Michigan State University

Escherichia coli (E. coli) contamination continues to cause beach closings and advisories in the Great Lakes as well as drives the total maximum daily load (TMDL) identification of impaired water. Monitoring the waters of the state has been undertaken by Michigan Department of Environmental Quality with Michigan State University compiling E. coli data for the state. The main objective of the analysis was to determine the extent of water quality standard violations, identify waters which are most at risk for beach closings, and evaluate the validity of the current state and federal policies for regulating E. coli in surface waters. The database includes E. coli data collected from 1998-2006 as part of beach monitoring, TMDL values, and other state water quality information. For all sites, the median and 95th percentile estimates based on the monthly and daily data using the Hazen method were calculated to gain an understanding of typical E. coli values for Michigan waters. In addition, alternative monitoring strategies including microbial source tracking and pathogen monitoring are ongoing for use in a risk-

based framework to assist with policies. The recommendations from the CWS Pathogens Water Fellows program for monitoring will be presented.

THE NATIONAL WILD FISH HEALTH SURVEY IN THE GREAT LAKES BASIN (AND BEYOND)

Kenneth Phillips
US Fish & Wildlife Service
LaCrosse Fish Health Center

The U.S. Fish and Wildlife Service (USFWS) began the National Wild Fish Health Survey (NWFHS) in 1997 as a response to the whirling disease outbreak that occurred in Montana and other western states in the 1990s. The NWFHS has been used by the USFWS to conduct surveillance for viral hemorrhagic septicemia virus (VHSV) in the Great Lakes basin.

PATHOGEN SURVEILLANCE: RESULTS OF A PILOT STUDY I

James Casey, Paul Bowser and Mark Bain
Veterinary Medicine and Natural Resources
Cornell University

In the spring of 2008 a study was conducted to test the feasibility of rapid field sampling and laboratory analyses for detecting pathogens in the Great Lakes. Water and fish were collected at 30 Great Lakes coastal sites (USA) from Sault Ste. Marie (MI) to the St. Lawrence River (NY). Lab analyses used quantitative reverse transcription-polymerase chain reaction (qRT-PCR) and cell culture assays for detection of viral hemorrhagic septicemia virus (VHSV). The pathogen was detected at most sites indicating rapid field surveys can be used to assess pathogen distribution independently of investigating outbreaks.

PATHOGEN SURVEILLANCE: RESULTS OF A PILOT STUDY II

Marty Stokes, Fred Dobbs, and Jim Winton
Old Dominion University and the
Western Fisheries Research Center
US Geological Survey

The team developed a set of primers and probes for quantitative PCR of a suite of fecal-indicator and pathogenic microorganisms (bacteria, cyanobacteria, and protists) found in the Great Lakes. Targeting the 16S and 18S ribosomal RNA genes, these primer/probe sets are designed for use in rapid testing of genomic DNA extracted from field samples and can be used for identification as well as quantification of specific organisms. Positive controls using extracted laboratory cultures and commercially available genomic DNA have shown good results. Assays of DNA extracted from a subset (n=9) of the spring, 2008 field samples detected 7 of the 8 target species, with at least 3 organisms found in each sample.

Workshop Outcomes

Benefits of a Monitoring Program

In the Great Lakes region and elsewhere, little is known about the distribution and threat posed by most pathogens and other harmful microbes. There is a lack of data on the environmental conditions associated with disease events, and concentrations of pathogens that result in disease outbreaks. Investigations into pathways of introduction of new harmful microbes tend to occur after a prominent invasion event with inadequate data, so the vector is often misidentified. Information on the vulnerable taxa for many pathogens is often only compiled as large kills or sharp declines in abundance bring evidence. In addition, viruses and bacteria evolve quickly, leading to a changing capacity to induce disease symptoms, be transmitted, and cause mortality. Only the data and understanding provided by routine monitoring will resolve these knowledge deficiencies.

The ability to detect harmful microbes in the environment has advanced greatly in the last several years. In particular, it is now possible and practical to identify the presence and relative abundance of microbes without appearance of disease or outbreaks. This capability can allow management in the Great Lakes region to move from a reactive posture to a proactive one enabling anticipation of what could happen and how to respond. Having expectations of what could happen, predicting events, and providing early warnings to the public are important in actual management. Advance planning would likely save on emergency action costs by allowing mitigation capacity to be developed, and enabling the deployment of effective responses when necessary. Results, findings, and experience gained from an established monitoring program would likely change our thinking and understanding of pathogens and microbes, how they colonize the Great Lakes system, and what can be done about nuisance taxa. Thus a major benefit is gaining experience and knowledge on what is a new challenge for Great Lakes conservation and management.

The workshop group saw three major forms of benefit from a monitoring program:

- A way to build understanding and experience,
- Greater understanding of the relationship between occurrence and environmental risk associated with harmful microbes, and
- Capacity to undertake proactive management and response planning.

Program Goals and Objectives

The workshop included considerable discussion of a consensus vision on goals and objectives for a monitoring program. In terms of the taxonomic scope, the participants concluded that it should exclude human pathogens. The complexity, regulatory demands, costs, and analytic facility requirements rise tremendously if human health becomes a purpose. Participants urged that instead, the monitoring program should focus on other forms of harmful microbes, particularly wildlife pathogens, but also those that can have environmental effects. Viral hemorrhagic septicemia virus (VHS) is an important example of a wildlife pathogen of pressing concern to the region (Figure 1) and has motivated interest in microbe monitoring. The workshop developed goal was to monitor existing and potentially introduced microbes with harmful consequences to the ecosystem, non-human organisms, and human uses of the Great Lakes. As stated, this goal includes diseases of fish and wildlife and other harmful microbes which cause direct and indirect ecosystem changes that degrade human uses and biotic communities.

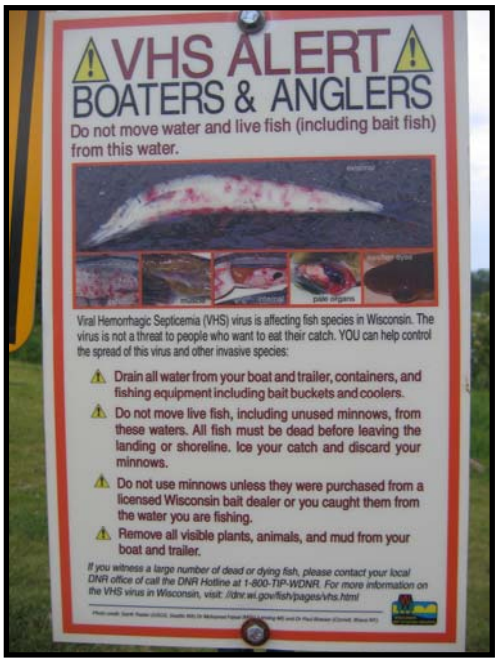

Figure 1. Public warning sign from Wisconsin's shoreline of Lake Superior. [Emily Cornwell, Cornell U]

Throughout the workshop, it was noted that the dominant aim for monitoring should be an early warning system for harmful microbes in the Great Lakes ecosystem. Though important, prevention of microbe introductions was not considered a realistic aim for this effort. Rather, tracking the presence and dispersion for existing and new, potentially harmful microbes was seen as the right focus. Early warning of an outbreak, infestation, or ecosystem impact will require knowledge of factors associated with such events, and that is also seen as a benefit of a monitoring program.

Workshop attendees also stressed the need for the monitoring effort to be "rapid, relevant and robust." That is, the program should produce results quickly; in days or weeks rather than months and seasons. Robust means a broad scope of coverage: comprehensive of microbes of interest and inclusive of key vectors of introduction. Identification of critical control points (introduction hotspots, centers of dispersion, etc.) for monitoring locations is a way to approach

the coverage of vectors while ensuring the need for quick results. Simultaneously striving for speed of results and breadth of coverage will demand strategic decisionmaking and maximization of efficiencies. Still, some compromises are expected in actual program operation.

It was also noted that a monitoring program must deliver clear information based on a consistent set of methods. However, there was also broad support for maintaining flexibility and adaptability in order to address new threats and findings. Doing both would require some portion of the program to be standardized while ensuring the capability to react to emerging threats and new needs. A collaborative program could help bridge standardization of protocols and flexibility in program execution. Collaboration in program operation has always been an aim because monitoring in the Great Lakes has historically been shared across agencies, organizations, and technical capabilities. By merging different organizational capabilities, program managers may find a balance of standardization and adaptability.

Finally, the participants stressed the need for practicality. The monitoring methods should entail analytical processes that are low cost and feasible to apply. The program also should be organized in the simplest way that can accomplish objectives. Methods that are low in cost to apply were another priority, as well ease of application, such as portable or automated options for sampling and detection. The use of indicators such as *E. coli* in beach monitoring for human pathogen threats was rejected for this program since indicators were not seen as relevant when microbial threats are continually changing and new organisms are always of concern.

The guiding principles for developing program specifications rely on four ideas. First, the program should be generic in structure but specific in focus. Second, the program should use monitoring methods that deliver results quickly and at low cost. Third, the program should be organized in the simplest way that can accomplish objectives. Finally, the program needs to maintain a distinction between what appears interesting from a research perspective and what is needed for effective management and policy. To summarize, the guiding principles ranked in order of priority for a successful monitoring program are:

- Assess occurrence and abundance of high-threat introduced microbes
- Track sites associated with vectors, dispersion, and impacts
- Maintain flexibility to adapt to changing needs
- Design sampling from a place-based perspective
- Determine the rate of change in pathogenic microbes, and
- Detect the presence of a wide range of microbes.

Sampling Design and Methods

A fundamental decision that will need to be made for any monitoring program is whether to select sampling sites by either a random or representative approach. Random designs provide statistically representative data when done properly and without many constraints on selections. Representative site selection can be more relevant for specific interests and issues because sites include areas for specific reasons. Random designs often require more sites for broad coverage, while representative sites can usually be fewer, be more targeted to a specific issue, and selected to include sites of special interest. The workshop group strongly concluded that a representative site selection approach will be most effective and desirable for this type of monitoring program.

The total number of sampling sites was also discussed. Cost and program efficiency considerations suggest that a monitoring protocol should limit the number of sampling sites; the group estimated that approximately 10 per lake and associated waters should be sufficient. These select sites will be considered as 'sentinel sites' that should be dispersed but associated with specific human uses. Human activity was raised as a driving consideration in site selection. Thus, priority should be given to areas with commercial harbors, industrial and power plants, long-term environmental monitoring, and overall high human presence. Invasion hotspots or biological entry and exit points were also a key consideration. Other site selection criteria could include priority species locations, water supply sources, water quality issues, and program partner study sites. Using the approach adopted at the workshop, the actual sites will need to be selected in association with partner monitoring programs and representatives of data users' organizations.

Most of the discussion regarding sampling of organisms for pathogens and microbes was focused on fish. While other taxa are possible targets, these were not discussed at the meeting. As noted above, the concept of indicator species, the traditional approach for human pathogen monitoring in water, was declined as a viable approach. Fish sampling was seen as the best direct means for biological monitoring of wildlife pathogens.

Fish sampling should be done during times of stress (spring, spawning) and should focus on capture of representative species at monitoring sites. Active capture methods such as seining, electrofishing, and trawling were seen as better than passive capture methods like gill nets, traps, and bait and hooks. Whatever combination of gear is used, sampling should span water depths and trophic classes (predators, prey, planktivores, etc.) of fish at a site. Sampling should focus on collecting a representative sample at each site to avoid bias. This task will require

experienced and trained staff since skill is involved to obtain unbiased and representative samples. Many current fishery study and monitoring programs already have well developed methods and effective field staff. Finally, it was noted that delivering the fish live for analyses is preferred but fish samples on ice is also effective.

Dead and moribund fish and other taxa should be opportunistically collected in a monitoring program even though these samples would not be used to represent sampling sites along with the other, live fish. These dead organisms are much more likely to be carriers of nuisance and pathogenic microbes and could be helpful in identifying emerging pathogens of concern. This level of field discretion and flexibility was regarded as potentially important to rapidly document new problems.

Beyond sampling fish, sampling water was also regarded as essential for microbe monitoring. Again, water should be collected in a broadly representative way. Samples should be integrated to serve as a composite sample for a site on a specific date. There is an interest in minimizing fish mortality in a monitoring program, and using high-fish-contact water may be a way to reduce wild fish losses. Capturing and holding collected fish in water for extended periods may be an effective alternative for detecting pathogens with a minimum of fish mortality. However, more research is needed on this approach since it is a novel and relatively untested.

Analysis Methods

Workshop deliberations on analysis methods produced some conclusions and aims for operation of a monitoring program. However, laboratory procedures for microbe detection vary by taxa. The traditional approach for viruses has been to detect presence through cell culture of select tissues and if positive, confirm the identity by quantitative reverse transcription-polymerase chain reaction (qRT-PCR, a molecular analysis technology). Bacteria detection involves biochemical screening and identification by serology or PCR.

Genomic techniques like qRT-PCR require use of specific gene primers and probes, genomic databanks, and verification of laboratory standards and results. Consequently, laboratory procedures cannot be standardized and substantial expertise is needed to employ the correct procedures for a suspected pathogen or microbial target. There are a limited number of laboratories that can conduct these analyses and design proper laboratory procedures. Standard sets of laboratory procedures are documented and regularly revised for specific microbial detections by expert groups (e.g., Office International des Epizooties 2006, Fish Health Section, American Fisheries Society 2007). Any microbial monitoring program in the Great

Lakes region should access these standardized methods and employ them to the greatest extent possible.

Current microbial detection technologies are advancing very rapidly and require laboratory expertise to track changes, monitor improvements in testing, and regularly acquire new devices and hardware. Molecular procedures like qRT-PCR are quickly becoming central to a wide range of procedures and uses. However, it is not clear that all needs can be met by this testing approach. Metagenomic analyses are just emerging in practice and may soon be able to screen large numbers of microbial taxa. As these procedures are used and refined, much more capability and sensitivity can be expected. Therefore, this aspect of a monitoring program needs to be implemented at specialized facilities and supported for employing continually changing technologies and information. For these reasons, samples should be archived because changes in capability can provide new uses of sample material and new pathogens can be tested on stored material.

Use of Results and Findings

Throughout the workshop, the dominant interest was having a monitoring program act as an early warning system for the occurrence and potential outbreak of harmful microbes excluding human pathogens. Achieving this aim will require rapid reporting of microbe detections. Criteria to interpret the number of occurrence or measures of abundance are needed to mark a threshold for concern or signal for action. Interpreting changes in distribution, abundance, and trends was seen as necessary for findings and demonstrating the importance of monitoring. However, there was a strong interest in making results available quickly and letting agencies and users draw conclusions. These competing views of transmitting early warning evidence and simple reporting of data might be reconciled by developing an experts committee that can judge and disseminate monitoring results rapidly and provide initial interpretation.

For a monitoring program to be valuable we concluded that data and information must reach decision-makers quickly and enable management actions. There is no lead agency or common point of contact in the Great Lakes region for directing results or findings on microbes and pathogens. However, there are several options to be further explored. Also, public information on microbial threats and introductions can be valuable to transition agency management away from the reactive mode seen at times of distress. It was recognized by the group that to achieve high impact with monitoring results the information provided must be timely, reliable, and interpreted in terms of threat and need for action. Much more than a rapid posting of data will be needed and a group of experts charged with first level interpretation will be required.

Models for this kind of information sharing include programs designed to inform the public and governments about threats posed by weather, human health, economic change, and others.

Program Development Support

Microbe monitoring in the Great Lakes is a new endeavor because it addresses fish, wildlife, and ecosystem health and the impact of novel organisms entering the region. Methods, tools, protocols, and interpretation of results will evolve through time and especially at the start of a basinwide program. Recent technological developments in molecular detection methods have allowed the consideration of a monitoring program that can be feasible and informative. This class of technology is rapidly developing and some effort and funds will be needed to track developments, test new technologies, and build improved capabilities into the program through time. Aside from detection sensitivity improvements and new organism capability, improvements in testing speed are expected and will need to be incorporated for cost-effective and timely findings. Also, critical specifications and performance standards for test procedures need to be developed so laboratories provide consistent data through time and across program partners. Finally, efforts should be made to closely integrate and evaluate coordination of all methods and procedures. Therefore, funding will be needed to allow experimentation, investigation, technology development, and modeling beyond the work of routine monitoring. Two types of special support were seen as needed: research funding and technology development funds.

In addition, program support should afford flexibility to execute special investigations. Introduction of new organisms and outbreaks of novel pathogens are expected, and the opportunity to direct effort at these events will be important for overall program accomplishments. Research on predictive models and criteria signaling impending outbreaks or regional dispersal will be another key need. This support will repay dividends in informing effective response capacity and alerting agencies and the public to emerging threats.

Comparison of Approaches

Monitoring Methods and Programs

Microbe and pathogen monitoring and testing programs were reviewed to compare their attributes with the workshop outcomes for a Great Lakes microbe monitoring program. The efforts reviewed, the sources of information, and a characterization of the program purpose are shown in Table 1. One entry was added for the Great Lakes microbe monitoring program. Each reviewed program has some relevance to our monitoring plan although there are important differences. The review below allows our effort to be viewed relative to other programs for judging consistency and feasibility of program plans.

Of the nine programs reviewed, two were methodological reference works developed by international or American scientific groups (OIE - Office International des Epizooties, World Organization for Animal Health; AFS - American Fisheries Society, Fish Health Section) primary aimed at aquaculture facility testing. Another two were focused on monitoring Great Lakes region waterways for human contact recreation (MSU - Michigan water monitoring plan, Michigan State University; U.S. EPA – U.S. Environmental Protection Agency, Microbiological Monitoring). Two monitoring programs had strong overlap with our proposed program but were more targeted to VHSV (APHIS – U.S. Department of Agriculture Animal and Plant Health Inspection Service; AQUAVETPLAN - Australian Aquatic Veterinary Emergency Plan). One set of related monitoring programs were focused on aquatic animal health (mainly salmonid fishes) in productions facilities (Nordic countries fish disease surveillance). Finally, two programs had broad aims of monitoring microbes and pathogens at a national (USA) scale for wild fish populations (USFWS – U.S. Fish and Wildlife Service, National wild fish health survey) and as an element of water quality (USGS – U.S. Geological Survey, Microbiological Monitoring as part of the National Water-Quality Assessment Program, NAWQA). Workshop outcomes for a Great Lakes microbe monitoring program identified a purpose (Table 1) that has elements of the other programs but is unique in combining the aims of gaining new knowledge, threat identification, and proactive management.

Table 1. The purpose of monitoring and methods programs with similarities to the proposed Great Lakes microbe monitoring program. Sources for each program are shown and common acronyms of the host organization or program.

Program	Source	Purpose
OIE - Office International des Epizooties, World Organization for Animal Health	1	Standard methods for cultured organisms at production facilities
AFS - American Fisheries Society, Fish Health Section	2	Standard methods for health inspections on aquatic animals
MSU - Michigan water monitoring plan, Michigan State University	3	Plan for improving water monitoring to best standards with human threat focus
USFWS – U.S. Fish and Wildlife Service, National wild fish health survey	4	Monitoring and testing methods for determining the distribution of specific pathogens in wild fish
APHIS – U.S. Department of Agriculture Animal and Plant Health Inspection Service	5	Identify VHSV distribution and need for control measures
USGS – U.S. Geological Survey, Microbiological Monitoring	6	National scale microbiological water quality monitoring as part of National Water-Quality Assessment Program (NAWQA)
U.S. EPA – U.S. Environmental Protection Agency, Microbiological Monitoring	7	Monitoring and testing of recreational waters; mostly beaches for human health threats
AQUAVETPLAN - Australian Aquatic Veterinary Emergency Plan	8	Viral hemorrhagic septicemia virus (VHSV) detection for control action implementation and planning
Nordic countries fish disease surveillance	9	The four Nordic countries have national surveillance and disease control for aquatic animals
Proposed Great Lakes microbe monitoring	This report	Monitoring to build understanding, identify threats and risks, and allow proactive management planning

Program Goals and Objectives

A review of the goals and objectives of the nine programs (Table 2) shows a large extent of overlap with our proposed monitoring program. Common goals and objectives are developing information on pathogen threats, distributions, and response planning. These objectives are similar whether the focus is on pathogen problems for human water contact, aquaculture production, or a specific organism (e.g., VHSV). Our agenda is somewhat broader in focus with consideration of local issues for site selection and program adaptability. However, all programs seek to provide scientifically sound information for prevention, proactive management, and response planning.

Table 2. Program goals and objectives compared to the proposed Great Lakes microbe monitoring program.

Program	Goals and Objectives
OIE	Standard methods reference source
AFS	Specify scientifically supported methods for disease detection across species, pathogens, and settings
MSU	Monitoring that depicts pathogen problems, identifies sources, describes movements, and indicates control strategies
USFWS	Monitoring to avoid large fish losses, provide knowledge for prevention and response, compare states and regions, and used in fish management
APHIS	Conduct test-based surveillance, estimate risks, map VHSV, develop VHSV detection and screening methods
USGS	Provide long-term data on pathogens and indicators, and assemble information to explain trends in water quality
U.S. EPA	Guide effective testing for human pathogen indicators relative to mean concentration limits used for human waterway use decisions
AQUAVETPLAN	Detect first occurrence of VHSV in Australia and implement control measures to minimize impacts
Nordic countries	Monitoring for disease detection, eradication, control, and to promote disease free areas for fish production
Proposed Great Lakes microbe monitoring	Assess occurrence of high threat microbes, track sites associated with vectors; maintain adaptability, use a place-based sampling design, determine the rates of microbe change, and detect a wide range of microbes

Sampling Design and Methods

The nine comparative programs and our monitoring plans are all focused on fish and water (Table 3) although some include other aquatic organisms in a general way. The programs that are aimed at water quality and human health (USGS, MSU, USGS, and EPA) rely on water samples for monitoring. Most others that are aimed at fish production facilities that have disease symptoms or moribund individuals. The OIE recommendations mention wild fish stocks for testing and specify a sample of 150 fish. APHIS specifies 170 specimens or fewer if moribund individuals are collected. Some programs estimate the number of fish samples need to have a 95% probability of detection. Water is collected by dispersed sampling at 9 or 10 locations at a site but commonly pooled for a composite sample for a day and a site. Our plan is to collect fish and water at a level compatible with covering at least one site a day.

The programs vary in fish sampling approach. Most emphasize representative sampling at a site as is in our program plan. Others target fish with symptoms of disease or dead and moribund individuals. Our plan was to collect these non-representative fish opportunistically in order to maintain program flexibility. When wild fish are sampled the methods used are to collect a representative sample at a site, much like water sampling designs. Our plan is consistent with this approach.

Analysis Methods

Laboratory procedures for microbe detection vary by taxa and all programs recognize a range of techniques are needed and a high level of laboratory expertise. However, some general descriptors of the analysis approaches used in the programs are provided in Table 4. Most programs rely heavily on cell culture of tissues from diseased or suspected fish as a starting analysis. Then these programs use a few testing procedures (molecular, serological, immunoassays) to confirm the identity of pathogens. Our program plan stands out in contrast to the other programs by emphasizing molecular techniques and specifying that archiving of samples will be a program priority. Otherwise, all programs draw on a similar set of laboratory procedures that would change depending on the target of the analysis.

Table 3. Sampling designs and methods specified in programs used to compare with the proposed Great Lakes microbe monitoring program.

Program	Methodological Approach and Key Attributes
OIE	Fish from production facilities; 10 or statistically estimated number of moribund or symptomatic fish. For wild or mixed assemblages of fish; 150 representative individuals.
AFS	Fish numbers expected for a 95% detection probability in facilities
MSU	Use disease indicators to identify high risk sites and then identify pathogens in water and investigate possible sources
USFWS	Wild fish captured using active and passive gears to collect representative sample of live fish
APHIS	Capture of 170 fish of susceptible species or 35 moribund fish (best) in hydrologic zones that have clearly a different mix of VHSV exposure, high risk facilities, and known VHSV positive waterways
USGS	Less than 10 water samples (1 L) at 3 to 4 sites per study river basin
U.S. EPA	Collect by hand 9 water samples dispersed in bathing areas from 0.075 to 3 m depth with two collections per a day. Composite made of each set of samples for testing.
AQUAVETPLAN	Suspected fish specimens sent to central testing laboratory
Nordic countries	Sampling for mostly salmonid fishes in production facilities. Collection of fish done by veterinary laboratories or agencies in each countries.
Proposed Great Lakes microbe monitoring	Use about 10 sites in each lake and associated waters selected to be dispersed but inclusive of human activities, long-term science activity, and invasion hotspots. Fish and water sampled in a broadly representative way with opportunistic recovery of dead and moribund animals.

Table 4. Laboratory analysis methods specified in programs used to compare with the proposed Great Lakes microbe monitoring program. In all cases the programs recognize that a range of methods are needed to detect different microbes and pathogens. The summary information provided here reflects the most commonly used testing approach in each program.

Program	Methodological Approach
OIE	Cell cultures used for screening and isolation and then molecular techniques for confirmation
AFS	Cell culture methods for screening and then molecular or serological methods for confirmation
MSU	Cell culture methods followed by molecular techniques
USFWS	Cell culture methods for screening and then molecular or serological methods for confirmation
APHIS	Cell culture methods followed by molecular techniques
USGS	Both molecular and cell culture methods for testing
U.S. EPA	Standard methods for *E. coli* and Enterococci bacteria
AQUAVETPLAN	Cell culture methods followed by molecular or immunoassay techniques
Nordic countries	Cell cultures used for screening and isolation and then molecular techniques for confirmation
Proposed Great Lakes microbe monitoring	Develop a select set of broadly capable testing laboratories that can tailor traditional and molecular procedures to a range of target organisms. Emphasis expected on new and emerging molecular analysis methods and archiving of samples for future and new testing.

Use of Results and Findings

The handling of data and results are not often specified with clarity for the monitoring and testing programs (Table 5). For some programs (OIE, AFS) this aspect is not addressed. Other programs (U.S. EPA, Nordic countries) treat results as confidential information for use in planning responses that are more regulatory in nature (closing beaches, quarantining facilities). However, most programs put some attention on data sharing and distribution of findings. Generally, web databases are planned and these may be restricted to participating agencies. Public information and reaching managers is an aim in most programs. Our plan is more specific in that it is focused on releasing data immediately in a public manner in order to facilitate rapid interpretation of its meaning and value.

Table 5. Planned use and distribution of data and findings in programs used to compare with the proposed Great Lakes microbe monitoring program.

Program	Approach to handling results and data
OIE	Not covered by protocols
AFS	Not covered by protocols
MSU	Develop database and models to predict threat conditions
USFWS	Web accessible database of sampling and results
APHIS	US and Canadian web sites for results. Share data with agencies and local managers.
USGS	Data stored in national database with national trends and results published
U.S. EPA	Compare test results to safety criteria for human waterway use decisions
AQUAVETPLAN	Positive detection of VHSV will be used in a public awareness campaign and for response actions
Nordic countries	Testing records retained by agencies
Proposed Great Lakes microbe monitoring	Quickly and widely release both data and scientifically interpreted findings in a manner that maintains reliability, judgment of threat, and need for action

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Appendix A

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