Bacteria Sources and Fate REPORT

Bacteria Source, Transport and Fate Study - Phase 1

VOLUME 3

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Preserving The Environment • Improving Water Quality

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This report was compiled by Dr. Sandra McLellan and colleagues at the University of Wisconsin- Milwaukee's Great Lakes WATER Institute

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EXECUTIVE SUMMARY

The growing number of beach closings nationwide has received considerable attention from the press, public health officials, water resource managers, and citizens. Beach closing statistics have been used to illustrate water quality concerns throughout the Great Lakes and marine coastal areas; however, this oversimplification does not offer sound scientific information as to the overall status of water quality in our rivers, lakes, and oceans. The perception that water quality is on the decline has been fueled by the fact that beach closings have increased by almost 100% since the passing of the Federal BEACH Act in October of 2000. In fact, more beaches are being monitored for fecal indicator bacteria, a measure of fecal pollution that may carry disease-causing organisms, and therefore, more closings have occurred. Further, many beach closings are caused by localized sources of pollution and the bacteria levels that are found in the beach area do not reflect regional water quality. Few, if any, beach monitoring programs include an assessment of offshore waters adjacent to beach sites.

In this study, the source, transport, and fate of bacterial pollution in the nearshore waters of Lake Michigan were investigated using a combination of field and laboratory based techniques. One of the primary objectives of this research was to assess the impact of combined sewer overflows (CSOs) and sanitary sewer overflows (SSOs) on levels of the bacterium *Escherichia coli* (*E. coli*) at beaches, as well as evaluate the extent in which regional water quality in Lake Michigan is affected. This three-year study identified potential sources of *E. coli* contamination, as well as the relative amounts of fecal bacteria in the Milwaukee Harbor, outside of the harbor breakwall, and at beaches in close proximity to the harbor. Importantly, the contribution of stormwater to degraded water quality was evaluated, along with an assessment of the sources of fecal pollution in stormwater (human vs. non-human).

The overreaching goal of this study was to better understand the origin and fate of bacterial contamination in regional waterways, beaches, and Lake Michigan. The following questions were addressed:

- What is the contribution of urban stormwater to the overall *E. coli* levels in receiving waters, and what is the source of the *E. coli* in stormwater (e.g. human vs. non-human)?
- Do receiving waters meet bacterial standards and are the standards achievable given current point source and non-point source pollution control strategies? What are the short and long-term impacts of CSOs on water quality in terms of achieving bacterial standards?
- What is the human health risk and persistence of residual pathogens potentially contained in floodwaters and/or in stormwater detention basins?

The microbiological assessments in the BSTF study closely interfaced with hydrodynamic modeling efforts. Data from extensive spatial surveys were used to validate and refine a hydrodynamic model, developed by the engineering and consulting firms of Camp, Dresser & McKee (CDM) and HydroQual Inc., to produce an integrated water quality framework that could be used as a tool in planning efforts. A complete description of the hydrodynamic modeling efforts can be found at "*Assessing Bacteria Source Impacts on Beaches: A Modeling Approach*" (Thuman *et al*., 2004). Overall, our understanding of water quality in the Milwaukee River Basin and Lake Michigan will be greatly enhanced by interfacing advanced biological measurements with hydrodynamic modeling.

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E. coli as an Indicator of Fecal Pollution and Human Health Risk

Currently*, Escherichia coli* is the USEPA recommended indicator for fecal pollution in recreational waters. Recreational waters are considered unsuitable for public use when numbers of *E. coli* bacteria exceed 235 Colony Forming Units (CFU)/100 ml. The organism *E. coli* is used to detect fecal pollution because it occurs in high numbers in the gastrointestinal tract of all warm-blooded animals and is easily detected using microbiological techniques. However, there is a lack of basic scientific knowledge needed to interpret results of elevated *E. coli* levels in surface waters. The transport and survival dynamics of this bacterium in the environment is not well understood, nor is the relationship between *E. coli* levels and pathogen concentrations. The studies described in this report demonstrate that *E. coli* is a short-lived indicator of fecal pollution in Lake Michigan. The rapid disappearance of bacteria outside of the harbor could not be attributed solely to dilution, but also to the occurrence of cell die-off. *E. coli does* not survive at appreciable levels (e.g. above the recreational water quality limit of 235 CFU/100 ml) in the open waters of Lake Michigan and, therefore, is a poor indicator of water quality on a regional or long-term basis.

In general, *E. coli* is considered a better indicator than fecal coliforms, since this organism does not survive as long in the environment as other members of the fecal coliform group (Toranzos and McFeters, 1997). However, in this study it was found that fecal coliforms may actually be a more appropriate indicator organism than *E. coli* for measuring sewage overflow distribution in the Great Lakes, since it appeared to be a more conservative tracer of pollution. In addition, survival rates of fecal coliforms may be more similar to pathogens of concern than *E. coli*, particularly *Giardia* and *Cryptosporidium* which are noted for their long survival times (Rose, 1997).

Distribution of E. coli in Nearshore Lake Michigan

The source, transport, and fate of bacterial contamination in the major tributaries of the Milwaukee River Drainage Basin and the nearshore waters of Lake Michigan were investigated by carrying out extensive spatial surveys and performing both microbiological and molecular analyses. The results of this three-year study identified potential sources of *Escherichia coli (E. coli)*, as well as determined the relative amounts of these bacteria in the rivers, Milwaukee Harbor, outside of the harbor breakwall, and at beaches in close proximity to the harbor breakwall. Each area was monitored before and after rainfall to assess the overall contribution of urban stormwater to elevated *E. coli* levels in relation to CSO and/or SSO events.

Throughout the three years in which spatial surveys were conducted, there were notable trends. *E. coli* levels increased at all sample locations in conjunction with rain events, with average increases ranging from 1 to 4 orders of magnitude. Historically, data collected by MMSD has revealed a positive relationship between stream flow (a direct result of the amount of rainfall) and bacteria levels. In individual surveys, the most significant rise in bacterial concentrations was detected in the confluence of the three major rivers and the channel. The channel is subjected to multiple sources of bacterial pollution because it is the primary drainage point for the entire 850 square mile watershed. Bacterial concentrations in the channel/estuary tended to be highly concentrated in comparison to the harbor and open waters of Lake Michigan because, as the river water moves into the lake and mixes with the harbor and lake water, *E. coli* levels are diluted out of the water column (Figure A). Cell die-off, as well as attachment to particles and subsequent sedimentation, may also play a role in the removal of *E. coli* from the water column.

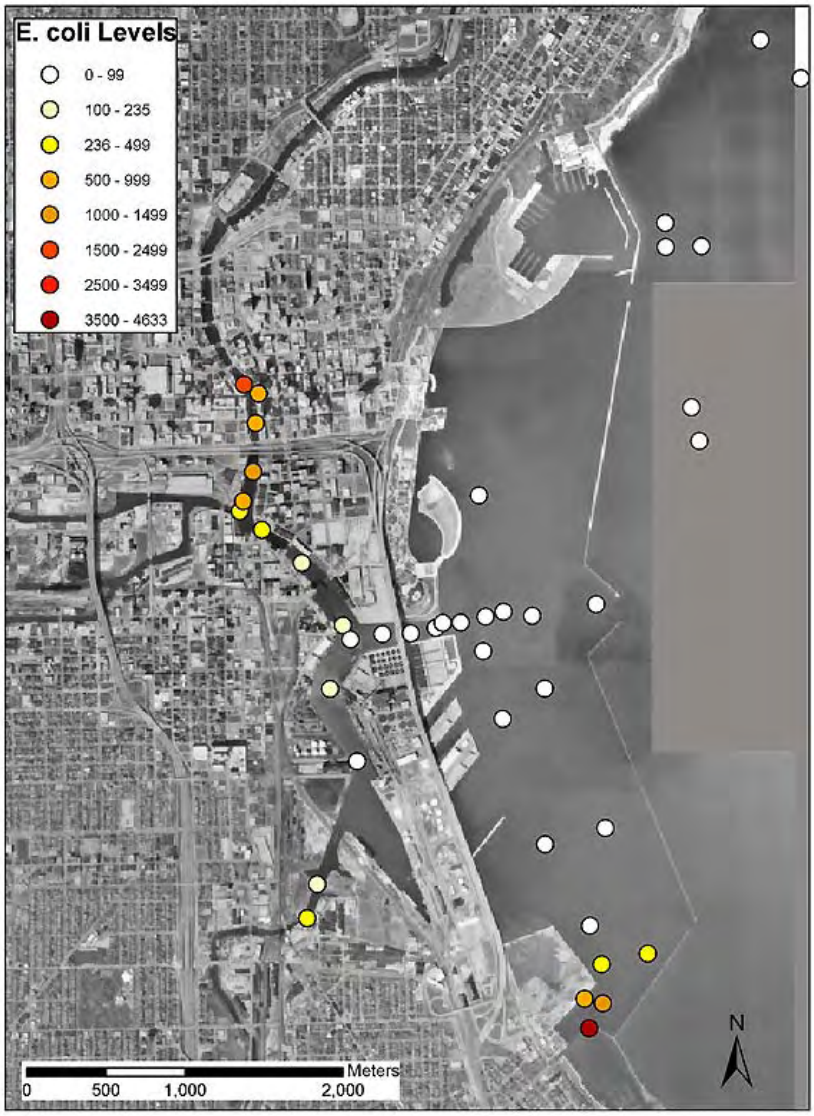

Figure A. Spatial survey of *E. coli* levels in the Milwaukee Harbor and surrounding nearshore area following 0.29 inches of rain on July 5, 2003. Levels above 235 *E. coli*/100 ml were rarely detected in the open waters of Lake Michigan, except inside pollution plumes which occurred following rain events greater than 1 inch of precipitation.

Distribution of E. coli in Lake Michigan Following a CSO Event

Around the Great Lakes Region, CSOs are a major concern to the 150 communities which are serviced by the combined sewer systems; therefore, Milwaukee is not the only community challenged to minimize CSOs and SSOs. Considerable improvements have been made to the Milwaukee wastewater conveyance system in recent years including the completion of the Inline Storage System (ISS/deep tunnel). Since the deep tunnel was put into service in 1993, the number of CSOs has been reduced from over 60 times per year to 2-3 times per year on average (http://www.mmsd.com/wastewatertreatment/).

One of the main objectives addressed in this research project was to characterize the distribution and ultimate fate of enteric bacteria entering Lake Michigan during sewage overflows. Bacterial surveys demonstrated that after rainfall, bacterial pollution travels in a distinct plume with river water as it moves into the harbor and past the breakwall structure. *E.*

coli levels were found to be 30-100 fold higher in the river water plume than samples taken less than 50 meters outside of the plume, a larger decrease than could be accounted for by dilution alone. Numerous environmental variables affect the survival and distribution of the fecal indicator bacteria. The nutrient loads entering Lake Michigan concurrently with fecal pollution may be a large determinate of survival, as higher survival rates have been found for *E. coli* when supplemented with filtered sewage; this effect is strongly modulated by indigenous bacteria competing for the nutrients (Lim and Flint, 1989).

Current bacterial die-off parameters used in hydrodynamic modeling utilize a first order die-off coefficient to account for all of the physical and ecological influences on survival. This study demonstrates that both dilution and die-off can account for the disappearance of bacteria. In addition, the die-off dynamics did not follow a first order decay function. The influences of temperature, sunlight, particles, and predation and the interrelationship among these parameters need to be better defined for a more accurate estimate of survival, particularly in the open waters of Lake Michigan.

Major increases in *E. coli* levels were found in Lake Michigan during the May 2004 CSO/SSO events compared with post-rain samples collected in 2003, a year with much less precipitation. In May of 2004 heavy rains occurred throughout the Milwaukee River Basin accompanied by CSO and SSO events over an 18 day period. The stream flows measured during the 2004 surveys were, on average, an order of magnitude higher than those in 2003. It is difficult to estimate the proportion of *E. coli* that originated from sewage contamination verses urban stormwater, since the heavy rains in 2004 simultaneously increased the amounts of urban stormwater directly released to rivers and caused sewage overflows. However, such information would be important for estimating the health risk associated with the pathogens in the contaminated waters since fecal pollution from human sources is of high concern.

Impact of Milwaukee's CSO on Regional Beaches

A major concern with sewage overflows into the Great Lakes is the potential impact to coastal beaches. Spatial surveys demonstrated that the numbers of *E. coli* decreased drastically once outside the harbor breakwall structure during sewage overflows, and in many cases, *E. coli* did not exceed the recreational water limit of 235 *E. coli*/100 ml (Figure B). Previous studies suggested that the harbor may actually protect the outlying waters of Lake Michigan while acting as a form of primary treatment by containing the majority of pollutants within the breakwall structure and consequently preventing their dispersal into outlying waters (Zanoni *et al*., 1978).

During the May 2004 CSO timeframe, Wisconsin and Illinois beaches experienced numerous closures as a result of elevated *E. coli* levels. Past research demonstrate that elevated *E. coli* levels are often due to localized sources, and regional sources such as sewage overflows, while presenting a serious health risk, do not contribute large numbers of *E. coli* to the beach area. The hydrodynamic model for this study revealed that fecal coliform levels in both the northern and southern part of the model domain near Fox Point and Wind Point respectively are typically less than 100 MPN/100 ml and, therefore, bacteria impacts either north of Fox Point or south of Wind Point were not due to Milwaukee-derived sources of bacteria (upstream river inputs, WWTP and CSO/SSO discharges). Similar conclusions were drawn in a paper presented at the International Association for Great Lakes Research (IAGLR) in Ann Arbor, Michigan, May 2005. A whole lake circulation model demonstrated that water discharged from Milwaukee reached Wind Point, Racine, but did not extend further south, during the time frame when beach closings

were reported in Chicago, Illinois (Schwab *et al*., 2005). Therefore, this paper concluded that Milwaukee's CSOs in May 2004 were not related to the Chicago beach closures during this time period.

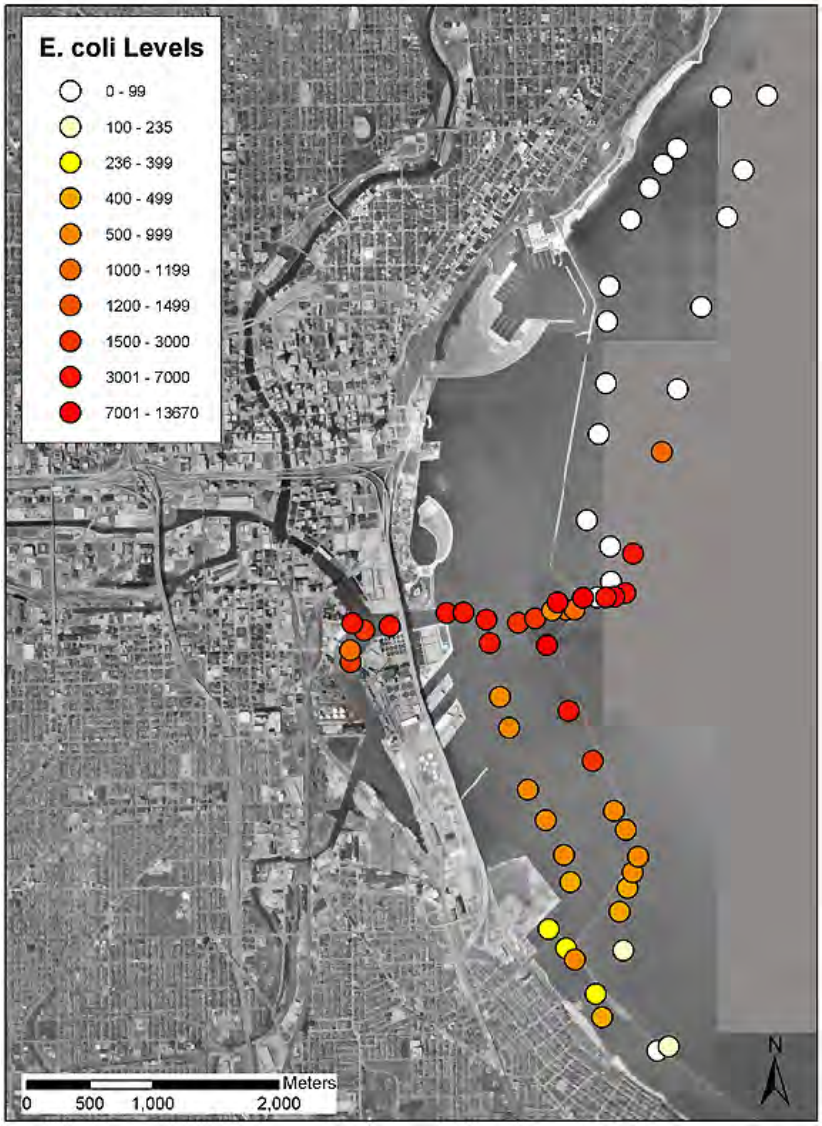

Figure B. Spatial survey of *E. coli* levels in the Milwaukee Harbor and surrounding nearshore area on May 14, 2004. Levels above 235 *E. coli*/100 ml are rarely detected in the open waters of Lake Michigan except in pollution plume. Significant loading of bacteria pollution to the harbor does occur through river discharge to the harbor, as well as stormwater outfalls that drain directly into Lake Michigan. Samples were collected following 1.99 inches of rain and during a CSO. Rainfall estimates for May 2004 were 12.8 inches (Theiler *et al*., 2004). Winds were recorded from the Northeast (NE).

Data collected as part of the BSTF study contributed to these conclusions presented in this paper. These findings highlight the need for better assessment tools to evaluate both localized and regional pollution sources in Lake Michigan. Even though the sewage overflows did not have a direct effect on the numbers of *E. coli* (and therefore the beach monitoring results), the hydrodynamic model would be extremely useful in determining water movement, and therefore, the potential for CSO contamination to reach beaches near the Milwaukee Harbor.

E. coli Levels in Urban Stormwater

Urban stormwater remains one of the most difficult environmental and fiscal challenges in the United States and is a major source of fecal pollution to rivers and subsequently, Lake Michigan. Increases in *E. coli* levels in the major tributaries as well as within the nearshore region of Lake Michigan (e.g. channel/estuary and inner harbor) were detected following rainfall (Figure A). On average, bacterial concentrations were found to be the highest in the rivers and the channel/estuary, with concentrations decreasing as the distance increased eastward from the confluence of the three rivers (Milwaukee, Menomonee, and Kinnickinnic) and into the harbor. Overall, *E. coli* counts dropped below the USEPA recreational limit of 235/CFU 100 ml once outside of the breakwall structure. Extensive hydrodynamic modeling was performed as part of this study in conjunction with CDM and HydroQual, Inc. River flows and lake currents were modeled to determine the extent of bacterial transport in Lake Michigan. The results of hydrodynamic modeling and spatial surveys illustrate how the discharge of urban runoff in Milwaukee River Drainage Basin surface waters is directly correlated to the rise in bacterial concentrations subsequent to rain events and are limited to a distance of 2-5 km from the primary discharge point located within the channel/estuary.

Urban areas are particularly vulnerable to degraded water quality. In many cases, the hydrology of these regions has been severely altered to allow for urban development, which has resulted in an increase in the amount of impervious surfaces. Outfalls that discharged directly into Lake Michigan may negatively impact beach water quality. Essentially, beaches can be highly impacted by local sources, such as direct runoff from impervious surfaces or storm outfalls. Conducting an extensive assessment for human sources of fecal bacteria in stormwater outfalls may be warranted when extremely high levels of *E. coli* are noted.

Microbial Source Tracking Methods

Watersheds, especially urban watersheds, are complex systems subject to both point and non-point sources of pollution making the identification of the source of pollution a complicated endeavor. Currently, numerous bacterial source-tracking methods exist such as testing for antibiotic resistance of fecal indicator bacteria, detection of host specific molecular markers using Polymerase Chain Reaction (PCR), and assessment of chemical tracers such as caffeine. Although each method has its own merits, studies have shown it is more effective to use a combination of source detection methods/techniques rather than rely on one particular test to discriminate between bacterial host sources found in environmental water samples (Scott et al., 2002; Stewart et al., 2003).

Antibiotic Resistance Testing

The occurrence of a known sewage overflow was used to evaluate one potentially useful microbial source tracking method, antibiotic resistance testing. Antibiotic resistance testing source tracking methods have been developed under the premise that in general, humans are exposed to antibiotics more frequently than wildlife. As a result, humans carry a higher proportion of *E. coli* resistant to antibiotics as opposed to wildlife which are not exposed to antibiotics. This study established that there were large differences in the percentage of isolates resistant to antibiotics from human sources compared with non-human sources. As a result, determining the frequency of antibiotic resistance in fecal indicator bacteria from environmental water samples can provide valuable information regarding the proportion of human vs. non-human sources. Antibiotic resistance frequencies of *E. coli* isolates collected during the 2004 CSOs were compared to the resistance frequencies of isolates obtained from

2003 post rain samples, a year in which there were no sewage overflows. Overall, there were no significant differences in percentage of *E. coli* isolates resistant to ten different antibiotics for the 2004 harbor isolates (during a CSO) compared with the 2003 isolates (no CSO). Essentially, this data suggests that while there is an overall increase in fecal indicator bacteria in the harbor during a CSO compared with a non-CSO survey, the proportion of human sources is similar. These results may indicate that in the absence of a documented CSO, there may be unrecognized sanitary sewage inputs such as cross connections between the stormwater and sanitary systems and/or aged sewer infrastructure resulting in leaking sewer lines.

DNA Fingerprinting

Extensive characterization of *E. coli* from sewage, gulls, cows, and other hosts revealed that there is a high amount of genetic diversity among strains in the natural *E. coli* population. However, in some instances, highly related strains were found to be uniquely present in one type of host. These findings would suggest that DNA fingerprinting may be applicable in discrete areas where pollution inputs can be characterized, but is not feasible for investigation of contaminated surface waters in complex systems (McLellan *et al*., 2003).

It was anticipated that the stormwater *E. coli* isolates would display broadly diverse rep-PCR fingerprint profiles reflecting the diffuse nature of the bacterial contamination in urban runoff. However, the diversity among strains isolated directly from stormwater was considerably less than that found in strains isolated from a particular host source. This may indicate that interrelationships among strains is not primarily host dependent since there was a high amount of similarity among strains from stormwater, which is expected to carry fecal pollution from many different sources. Identical strains may indicate possible clonal propagation, and this could account for some of the low diversity. Alternatively, the strains that were detected may be a product of selective die-off and may represent a limited range of persistent strains that can be isolated in the environment.

DNA fingerprinting may not be a cost-feasible methodology to identify and quantify fecal pollution sources given the extensive diversity and under-characterized genetic structure of the natural *E. coli* population. However, these types of analyses offer valuable insight into the potential for surface waters to harbor persistent residual populations that may confound recreational water testing and are useful in understanding the ecology of *E. coli* in the secondary environment (e.g. surface waters) outside the host. Replication of cells outside the host interferes with routine beach monitoring as growth of indicator bacteria in the environment would essentially cause a site to appear to have more pollution than what is actually released at the site. This may occur under specific conditions, such as shallow protected waters (McLellan *et al*., 2001) or in the sand environment (Beversdorf *et al*., ; Kinzelman *et al*., 2004).

Bacteroides Host Specific PCR

As an alternative method for detecting human sources of contamination, samples were analyzed for the *Bacteroides* human specific genetic marker. Previous studies have indicated that *Bacteroides* spp. may be one of the most sensitive fecal indicator genetic markers since *Bacteroides* are present in fecal pollution at a much higher abundance (1000X) than fecal coliforms. In addition, certain species of *Bacteroides* have been found to commonly be harbored in humans, but not other sources of fecal pollution such as cows or gulls (Bernhard and Field, 2000b). The human specific *Bacteroides* genetic marker was detected in sewage influent samples (n=15) from five different treatment plants in Wisconsin, including plants from

Milwaukee, Manitowoc, and Door Counties. This marker was found to be positive when influent samples were diluted to 1:25,000. Further, fecal samples from gulls (n=240) and feed lot manure samples (n=48) were always negative. These results demonstrate that the human specific *Bacteroides* marker is potentially a sensitive and specific marker for human sources of fecal pollution.

Bacteroides, as obligate anaerobes, are not expected to survive for extended periods of time in the environment (Carrillo *et al*., 1985; Kreader, 1998; Resnick and Levin, 1981); however, the relationship between standard measures of water quality (e.g. culturing *E. coli*) and the capability to detect these organisms in surface waters over time is unknown. Therefore, the distribution of *Bacteroides* spp., and other fecal indicator genetic markers, was determined in nearshore Lake Michigan following a sewage overflow, and the results were compared with the levels of culturable *E. coli* measured in the same samples. Results from this study demonstrate that the *Bacteroides* spp*.* genetic marker may be useful for detecting low levels of fecal pollution, particularly in a system such as Lake Michigan where dilution makes it difficult to track pollution inputs from watershed drainage. The simultaneous disappearance of the *E. coli* (detected by PCR) and human specific genetic markers in Lake Michigan suggests that *E. coli* and the human specific *Bacteroides* spp. cells remain intact for similar amounts of time.

Furthermore, because stormwater is a major source of *E. coli* and one of the leading sources of water quality impairment in the U.S. today, tracking sources of bacterial contamination in stormwater is imperative. This study analyzed 56 outfalls throughout the service area for evidence of human sources of fecal pollution, by testing for the *Bacteroides* human specific marker. Many outfalls were repeatedly sampled; the total number of samples analyzed was 147. Overall, 22 of 56 outfall locations were found to have at least one sample positive for *Bacteroides* human specific genetic marker. Approximately 34% of 1st flush samples and 30% of 2nd flush samples from the stormwater outfalls in the MMSD service area tested positive for the *Bacteroides* marker. Honey Creek and Underwood Creek demonstrated the highest incidence of positive *Bacteroides* human specific marker, with 5 of 5 outfalls positive on Honey Creek, and 3 of 4 outfalls tested positive on Underwood Creek. In general, the antibiotic resistance patterns, indicating potential sanitary sewage contamination, corresponded with outfall locations that also were found to be positive for the human specific *Bacteroides* genetic marker. These results suggest that in the Milwaukee River Drainage Basin, sanitary sewage is present in a significant portion of the stormwater system and may be entering the system through leaking pipes or cross connections.

Molecular techniques, e.g. *Bacteroides* host specific PCR, offer promising results for human source identification in environmental water samples. Results from the stormwater outfall screening in 2003-2005 suggest that there is a need for a comprehensive assessment of all stormwater systems within the MMSD service area, in order to identify potential sources (e.g. leaks, cross connections, wildlife) of bacterial contamination within the stormwater infrastructure.

Pathogen Occurrence

Pathogen assessments provide direct evidence as to health risk. Human sources of fecal contamination are known to carry human pathogens; however, little is known about the types of pathogens and ultimately, the health risk that is associated with non-point sources of fecal pollution in urban stormwater. Pathogen assessments concentrated on three pathogens, one

bacterium (e.g. *Salmonella*), and two protozoan (e.g. *Giardia*, *Cryptosporidium*) which have previously been detected in the surface waters of the Milwaukee River Drainage Basin.

PCR protocols were developed and validated by initially analyzing sewage treatment plant influent. Control samples demonstrated that *Giardia* could be detected in a 1:100 dilution of sewage sample. *Cryptosporidium* was detected in 3 of 6 sewage samples, but the signal was weaker and lost at dilutions of 1:10 or 1:100. Samples collected during the May 2004 CSO events were used to assess loading of pathogens into harbor during storm events. Both *Giardia* and *Cryptosporidium* were negative for 31 samples collected in the confluence of the Milwaukee and Menomonee Rivers. Experiments were performed to determine if inhibitors were present in the environmental samples. The presence of inhibitors would decrease the sensitivity of the assay. The limit of detection for *Giardia* in environmental samples in these experiments was 0.01-0.001 ng/sample genomic DNA, which is equivalent to approximately 1000 to a lower limit of 100 cells. Similarly, *Cryptosporidium* could be detected at levels of 0.01 ng/sample genomic DNA. While this limit of detection is sufficient for evaluating sewage samples, low levels of these organisms in Lake Michigan would not be detected.

The public health risk posed by very low levels of pathogens in Lake Michigan is difficult to estimate, since concentrations may not exceed the infectious dose needed to cause illness. However, Lake Michigan serves as the source for drinking water to the City of Milwaukee and other communities, and the *Cryptosporidium* outbreak in 1993 is a sound reminder that drinking water treatment should not be the only protective measure against pathogen exposure in Lake Michigan waters. Rather, contamination of source waters (e.g. CSOs, SSOs, urban runoff, etc.) should be minimized as much as possible to protect public health. Moreover, evidence of human sources of contamination in stormwater strongly indicates that there is a need for a more detailed pathogen assessment of urban stormwater for disease causing organisms, including human viruses. Given the difficulty in direct pathogen assessments in Lake Michigan, stormwater outfalls should be directly tested for pathogens.

MAJOR FINDINGS AND CONCLUSIONS

- CSOs and SSOs contribute high loads of *E. coli* and other indicator organisms to local receiving waters. The proportion of human sources compared with animal sources (derived from both urban and agricultural non-point source runoff), remains difficult to quantify. Spatial surveys clearly demonstrate that levels of indicator organisms are an order of magnitude higher in the harbor following severe rain events accompanied by sewer overflows compared with rain events of <2 inches of precipitation, however the proportion of human sources compared with animal sources appears to be the same (as indicated by antibiotic resistance testing). Importantly, these findings suggest the total amount of human sources of fecal pollution was found to increase during sewage overflows, but the non-point sources also increased on an equal scale. These results indicate that there may be unrecognized sanitary sewage inputs into stormwater systems, and ultimately receiving waters, during all rainfall events.

- Locally, stormwater is a major source of *E. coli* and is considered one of the leading sources of water quality impairment in the U.S. today. Over 13% of the outfalls tested locally demonstrated *E. coli* levels of 100,000 CFU/100 ml or greater. Possible human sources were detected at 22 of 56 of these outfalls. In over half of the outfalls testing positive for the human specific marker, antibiotic resistance testing would suggest that human sources were not the major contributor to the *E. coli* levels, further illustrating that both human and non

human sources of fecal pollution contribute to high *E. coli* levels. These findings highlight the need to better scrutinize the causes of elevated fecal indicator bacteria in urban stormwater.

- Better quantitative assessments are needed in the upstream regions to account for unrecognized sanitary inputs into the watershed. The development of quantitative molecular methods is necessary to track human and non-human signals during different types of storm events. In addition, quantitative methods will be crucial for targeting and prioritizing stormwater outfalls that demonstrate sanitary sewage contamination.

- During a CSO/SSO, *E. coli* levels fall below 235 *E. coli*/100 ml once the pollution plume disperses outside the harbor breakwall. The highest levels of *E. coli* were detected in the channel/estuary, harbor, and at stormwater outfalls. Bacteria levels are diluted out of the water column once the polluted plume mixes with the lake water. Because *E. coli* does not survive well in Lake Michigan, further development and utilization of a hydrodynamic model will be very important when evaluating the ultimate distribution of biological pollutants in Lake Michigan.

- Fecal coliforms are a better indicator organism than *E. coli* in Lake Michigan; however both organisms are relatively short lived in Lake Michigan and therefore poor indicators of pathogens such as *Giardia* and *Cryptosporidium*. More sensitive measures are necessary to determine the human health risk from pathogens associated with CSOs and stormwater runoff into Lake Michigan.

- Source tracking methods are useful in determining host sources (human vs. non-human) of bacteria however; one method is not capable of identifying all the host sources which might contribute to bacterial contamination. It is more effective to combine a variety of source tracking techniques to get a clearer picture of what is occurring in the watershed.

- *Bacteroides* human specific and cow specific genetic markers appear to be both sensitive and reliable in detecting human and agricultural sources of fecal pollution. However, these markers provide only presence/absence information. Methods for the quantification of the human specific marker are recommended so that sites which test positive can be prioritized according to the magnitude of the problem, e.g. sanitary sewage as a minor contributor or major contributor to fecal pollution detected at the site.

- Pathogens need to be measured directly in order to determine the pathogen loads entering Lake Michigan. Initial work conducted by United States Geological Survey (USGS), the Wisconsin Department of Natural Resource (WDNR) and others has been focused on *Giardia* and *Cryptosporidium* methods; however, viruses will be an important group of pathogens to monitor in the future.

FUTURE RESEARCH DIRECTIONS

Based on the results of this research, we recommend the following future research priorities:

- Quantify and understand the impacts of stormwater runoff on water quality.

- Identify stormwater outfalls within the Milwaukee River Drainage Basin that are discharging elevated levels of bacterial contamination into the stormwater system, and subsequently determine if the origin of the pollution is from primarily human or non-human sources.
- Assess stormwater outfalls for human viruses.
- Develop more sensitive methods for detecting sources of sewage contamination. Also, it is essential to expand upon and refine methods for direct pathogen detection in environmental water samples.

PUBLIC COMMUNICATIONS

A list of presentations, interactions and interviews are included in Appendix VI. Public presentations and interviews were used to convey the results of the BSTF study to the public in layperson terms. In addition, current research and up-to-date results from these studies may be found at http://www.uwm.edu/Dept/GLWI/ecoli/.

1.0 INTRODUCTION

The primary goal of the Milwaukee Metropolitan Sewerage District (MMSD) Bacteria Source, Transport, and Fate Study (BSTF) was to differentiate sources of bacterial contamination that contribute to beach closings and negatively impact water quality in the Milwaukee area, as well as identify the transport mechanisms and ultimate fate of bacteria in the harbor and nearshore waters of Lake Michigan. Dr. McLellan's laboratory at the Great Lakes Wisconsin Aquatic Technology Environmental Research Institute (WATER) has completed all of the specific aims of the BSTF Project Phase I. The specific aims of this project focused on the following activities: (1) providing comprehensive data during rain events and rain events accompanied by combined sewage overflows to assist in model validation; (2) surveying stormwater outfalls for biological or chemical markers of sanitary sewage contamination; and (3) evaluating contaminated river/harbor water for pathogens.

Periodic discharges of combined sewer overflows (CSOs) into Lake Michigan during heavy precipitation have been cited as the cause of elevated levels of *Escherichia coli* (*E. coli*) bacteria at beach sites in the Milwaukee area. However, high levels of indicator bacteria in beach areas cannot be correlated with CSOs, making it very difficult to determine the exact impact of regional contamination of nearshore waters on beach water quality.

Unfortunately, the water quality benefits of reduced levels of sewage overflows in recent years remain difficult to quantify in the face of complex land use patterns and other major sources of pollution in the Milwaukee metro area. **It is impossible to estimate the benefits to water quality from the further reduction or elimination of combined sewer overflows (CSOs) without first understanding the proportional impacts of other major sources. Stormwater runoff stands as a major source of bacterial pollution in surface waters, particularly in rapidly urbanizing or already developed areas.** As yet, the impact on local receiving waters and beaches from sewage overflows or stormwater, as well as the resulting health risks, is not quantifiable. Therefore, in order to understand the origin and fate of bacterial contamination in regional waterways, beaches, and Lake Michigan, the following questions were addressed:

- What is the contribution of urban stormwater to the overall *E. coli* levels in receiving waters, and what is the source of the *E. coli* in stormwater (e.g. human vs. non-human domestic)?

- Do receiving waters meet bacterial standards and are the standards achievable given current point source and non-point source pollution control strategies? What are the short and long-term impacts of CSOs on water quality in terms of achieving bacterial standards?

- What is the human health risk and persistence of residual pathogens potentially contained in floodwaters and/or in stormwater detention basins?

The quantification and identification of contaminant loading at various points in the system was accomplished using both field and laboratory based methods. The purpose of this data was to identify the major contributing sources of fecal contamination into the Milwaukee Harbor and, subsequently, the nearshore waters of Lake Michigan. This study design employed multiple approaches to differentiate each potential source into three broadly defined categories: 1) rural, agricultural, and urban runoff from upstream, outside of the MMSD boundaries; 2) urban stormwater runoff within the MMSD boundaries; and 3) CSO and SSO discharges. This type of information is critical to water resource management agencies as they develop pollution reduction and/or mitigation strategies to improve the quality of surface waters within our region.

2.0 BACKGROUND

2.1 *Beach Closings*

Beach closings are a growing problem throughout US coastal regions. Beaches are closed when certain types of indicator bacteria exceed acceptable limits, signifying that fecal pollution is present. The urban coastal environment is particularly susceptible to beach closings due to the intense pressure human activity places on the beach environment. Ironically, it is these highly polluted areas that are in greatest demand for use as recreational beaches.

Coastal waters are complex systems subjected to numerous contaminants; headwaters may experience agricultural runoff, while urbanized estuaries receive sewage overflows and urban stormwater. However, discerning the sources of pollution is not a straightforward endeavor. Assessments that quantify fecal pollution using either *Escherichia coli* (*E. coli*) or fecal coliforms as indicator organisms provide no information as to the source of pollution; yet this is the critical piece of information needed to evaluate health risk, devise management strategies, and direct fiscal resources.

The Beaches Environmental Assessment and Coastal Health (BEACH) Act, signed into law October 15, 2000, is currently being implemented nation-wide. Under this bill, the USEPA is directed to publish performance criteria for pathogens and pathogen indicators by 2005, as well as guidelines for prompt notification of the public. In Wisconsin, the Department of Natural Resources (WDNR) has formulated a model ordinance for local beach monitoring. As monitoring programs are being initiated, beach areas that have not been tested previously are now experiencing high numbers of advisories or closures.

Despite the fact that we have a much greater understanding of the quality of water at recreational beaches, the data collected is inadequate for beach managers to identify causes of elevated fecal indicator levels, which impairs their ability to address the problem. Even in regions in which extensive beach testing has occurred, pollution sources at these beaches remain unknown. While more extensive beach monitoring may provide better protection of public health, the data generated in these programs only highlights our coastal problems and does little to advance our understanding of how to mitigate the sources of pollution.

2.2 *Escherichia coli (E. coli) as an Indicator of Fecal Pollution and Human Health Risk*

E. coli is an enteric bacterium present in high numbers in the gastrointestinal tract of almost all warm-blooded animals and is a sensitive measure of fecal pollution which may carry pathogenic bacteria, viruses, and protozoa. Both *E. coli* and enterococci, another enteric bacterium, are recommended by the U.S. Environmental Protection Agency (USEPA) as indicator organisms for freshwater. Most Great Lakes states use *E. coli* for water quality standards (USEPA, 2003), where levels above 235 CFU (colony forming units)/100 ml are deemed unsafe in recreational waters. Epidemiological studies have demonstrated a predictive relationship between the levels of *E. coli* in water and rates of gastrointestinal and other illnesses in swimmers (Dufour, 1984; Pruss, 1998; Wade *et al*., 2003). This relationship may be complicated by the fate of pathogens once they leave the host. Survival of *E. coli* strain O157:H7 outside the host environment may be at least 5 weeks at 5^{o}C (Geldreich *et al*., 1992), while some *Salmonella* and *Shigella* spp. decrease in numbers by 50% within 24 hours once introduced to well water (McFeters *et al*., 1974). The survival of enteric bacteria is influenced by turbidity (and subsequently particle attachment), sunlight, nutrients, and the metabolic capacity of the organism (Pommepuy *et al*.,

1992). Overall, the relationship between the survival characteristics of indicator organisms and pathogens is not well characterized.

Gastroenteritis is the most commonly reported ailment associated with water-borne pathogens, although eye, ear and skin infections also occur. Disease may be caused by a broad variety of bacteria (*Salmonella*, *Vibrio cholera*, *Shigella*), viruses (hepatitis A and other enteroviruses, hepatitis E, rotavirus, Norwalk viruses) or parasites (*Cryptosporidium*, *Giardia*, and *Leptospira*). Human sources of fecal contamination can be introduced to waterways by sanitary and combined sewer overflows (CSOs); such sources of fecal pollution are considered a serious health risk since they are likely to carry human pathogens (Scott *et al*., 2002; USEPA, 2004). Animals can also serve as vectors for important pathogens, such as the waterfowl hosts for *Salmonella* spp. and healthy domestic cattle that may carry *E. coli* strain O157:H7 and *Cryptosporidium parvum*. The origin of fecal pollution is relevant since the human health risk may vary depending upon the source (NRC, 2004).

2.3 *Approaches to Source Identification*

Recent reviews on microbial source tracking indicate that no single method is adequate to clearly identify all host source contributions to contaminated water and further method development is necessary (Scott *et al*., 2002; Simpson *et al*., 2002; Stewart *et al*., 2003). Therefore, a multi-tiered approach might be more effective than a single approach (Boehm *et al*., 2003). DNA fingerprinting approaches have employed either ribotyping or rep-PCR to characterize fecal indicator bacteria and have demonstrated an association of genetically similar strains with different host sources (Dombek *et al*., 2000; Parveen *et al*., 1999). However, the extent of host specificity across the whole population remains controversial (Gordon, 2001). In recent studies, the usefulness of DNA fingerprinting for assessment of clonal populations has been noted, which suggests environmental replication of the cells (Kinzelman *et al*., 2004; McLellan *et al*., 2001). In addition, there has been found a high amount of genetic diversity among strains in the natural *E. coli* population, which would suggest that DNA fingerprinting may be applicable in discrete areas where inputs can be characterized, but is not feasible for investigation of contaminated surface waters in complex systems (McLellan *et al*., 2003).

Evaluating the antibiotic resistance traits of indicator organisms is another approach to source identification and may be useful because this approach is founded on a well-supported hypothesis that host animals (e.g. humans or certain agricultural animals) that are exposed to antibiotics will harbor antibiotic resistant bacteria more frequently than hosts not exposed to antibiotics. Studies have demonstrated an increased proportion of fecal indicator bacteria with antibiotic resistance traits associated with human sources of fecal pollution (Harwood *et al*., 2000; Parveen *et al*., 1997). Most likely this approach will need to take into account the geographic region, the antibiotic usage in the human population, and other suspected sources in the watershed (Harwood *et al*., 2000).

More recently, methods that are not dependent on culturing and analyzing indicator organisms have been described. Culture-independent, molecular-based methods are designed to detect genetic targets in organisms that occur in certain hosts. Viruses by nature are highly host specific, and source identification methods have been based upon detection of human enteroviruses or adenoviruses (Jiang *et al*., 2001; Noble *et al*., 2003). Other approaches detect virulence genes that produce toxins in pathogenic *E. coli* that are harbored in specific hosts (Chern *et al*., 2004). These methods are useful when the fecal contamination is derived from a large pool of individuals that includes infected hosts.

Perhaps the most promising genetic targets to be used with culture independent methods are those that are based upon fecal anaerobes within the order *Bacteroidales*, where certain species have been found to be host specific (Bernhard and Field, 2000a; Kreader, 1995). PCR assays have been developed that target the 16S rDNA genes of "total" *Bacteroides* spp., e.g. the conserved region, as well as unique sequences found in both human specific and cow specific spp. (Bernhard and Field, 2000b). Fecal anaerobes such as *Bacteroides* and *Bifiobacterium* have long been suggested as alternative indicators to the fecal coliforms group (Fiksdal *et al*., 1985). Fecal anaerobes make up the majority of bacteria in the gastrointestinal tract of humans and may be present at 1000-fold higher densities than the fecal coliform group (Fiksdal *et al*., 1985). The advent of molecular based methods has made it more feasible to detect these organisms in contaminated waters (Field *et al*., 2003). In addition, field testing has demonstrated the usefulness for detecting human and cattle sources of fecal pollution (Bernhard *et al*., 2003; Boehm *et al*., 2003; Bower *et al*., 2005). This approach is particularly advantageous because: 1) the host specific organism is widespread and thought to be present in most hosts within a host specific group, unlike viruses and toxin producing *E. coli* that might only be present in a small subpopulation of the host source, and 2) culture-independent methods make it feasible to test for several targets within a single sample.

3.0 METHODS

3.1 *Study Area*

This nearshore area of Lake Michigan was selected for study because the harbor is the primary discharge point for an 850 square mile river basin that includes agricultural, suburban and urban land use, with no other major watershed inputs entering Lake Michigan within 10 km. The Milwaukee Harbor has three openings to Lake Michigan designated as the north gap, main gap, and south gap. The Milwaukee, Menomonee, and Kinnickinnic rivers are the primary tributaries in this drainage basin with their confluence located within the combined sewer service area in the City of Milwaukee. These three rivers discharge into Lake Michigan a short distance from the confluence point inside the harbor breakwall. The study area included sites immediately above the confluence of the three major rivers (Milwaukee, Menomonee, and Kinnickinnic), the channel leading to the harbor, the Milwaukee Harbor, and outside of the harbor as far out as 8 km from the harbor breakwall (Figure 1). In addition, the study area included sites 0.5 km off of two urban beaches in close proximity to the harbor, which were potentially the most susceptible to sewage overflows and agricultural and stormwater runoff carried by river water. Additional sampling was conducted at South Shore Beach within the study time frame. These sampling regions were defined based on hydrological characteristics, specific conductance measurements, and distance from shoreline.

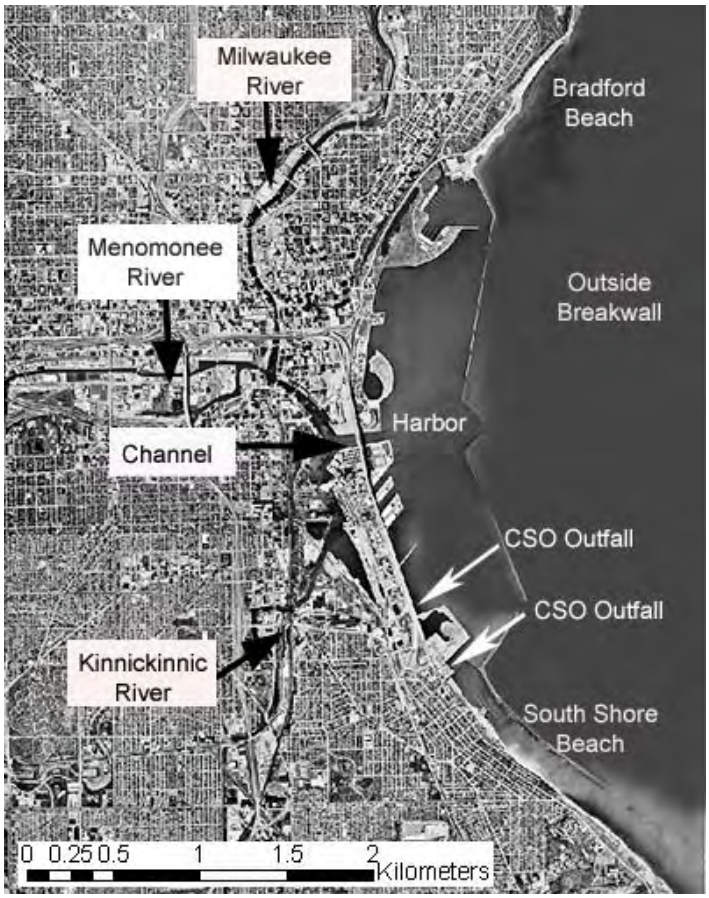

Figure 1. BSTF sample regions.

The harbor potentially receives bacterial contamination from a combination of sources including upstream runoff from rural and agricultural lands, urban stormwater released to rivers, and combined sewer overflow (CSO) and/or sanitary sewer overflow (SSO) discharges which have occurred on average two to three times per year for the past five years. The majority of the CSO outfalls, approximately 117, are located on the lower reaches of the Milwaukee, Menomonee, and Kinnickinnic Rivers (Figure 2), with only two outfalls on Lake Michigan. SSOs are discharged into these same rivers further upstream, either from portions of the MMSD separated sewer system, or within individual municipalities. In most urban areas, stormwater is conveyed in separated sewer systems and discharged directly to receiving waters with no treatment. However, older sections of many cities around the Great Lakes are serviced by combined sewers, which are designed to capture both sanitary sewage and stormwater for conveyance to a wastewater treatment plant. While this configuration minimizes stormwater

impacts on receiving waters during most rain events, the large volumes of stormwater generated during heavy precipitation can exceed the system's capacity, resulting in both stormwater and sanitary sewage being released as part of a combined sewer overflow (CSO). In addition, separated sanitary sewers also may be overwhelmed if infiltrated with large volumes of rainwater or if a mechanical failure occurs, resulting in a sanitary sewer overflow (SSO).

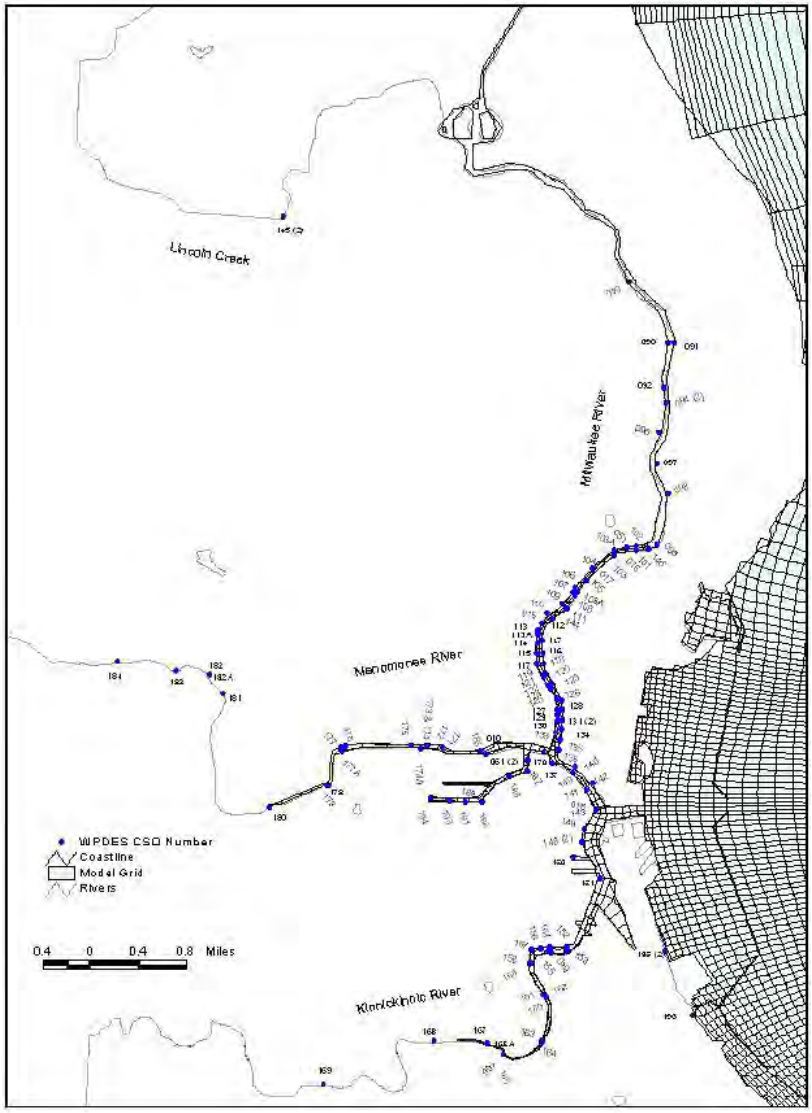

Figure 2. MMSD CSO locations along the Milwaukee, Menomonee, and Kinnickinnic Rivers.

CSOs and SSOs generally occur following extreme rain events. For example in May 2004, a series of large storm events resulted in record rainfalls occurring in southeastern Wisconsin, with an average of 8.18 inches of rain falling throughout the Milwaukee metropolitan area, accounting for a 5.12-inch departure from normal rates (http://www.nws.noaa.gov 2005). During a two week period, the MMSD service system experienced 3 CSO events and 5 SSO events.

Sanitary sewer systems in the surrounding area also exceeded capacity and 14 municipalities reported SSOs during this same time frame (Table 1).

Table 1. Communities with reported SSO discharges from May 10th to May 25th 2004 located within the Milwaukee River Drainage Basin.

Permittee	Volume MG
Bayside, Village	0.0720
Brookfield Fox Water Pollution Control	0.0265
Brown Deer, Village	0.3508
Cedarburg, City	0.2403
Cudahy, City	0.0795
Elm Grove, Village	0.0378
Fox Point, Village	0.0020
Hales Corners, Village	0.1896
Mequon, City	0.4181
Milwaukee, City	3.2880
South Milwaukee, City	1.7000
Theinsville, Village	1.7408
West Allis, City	0.0720
Whitefish Bay, Village	0.1044

(Source: Wisconsin Department of Natural Resources)

Milwaukee is not the only community challenged to minimize CSOs and SSOs. In the US, it is estimated that over 770 communities and 40 million people are serviced by combined sewer systems (http://cfpub.epa.gov/npdes/). Approximately 150 of these communities are within the Great Lakes drainage basin. In the Great Lakes states, major cities with combined sewer systems include Toledo, Buffalo, Cleveland, Detroit, metropolitan Chicago, and Milwaukee. The majority of these cities discharge sewage into the Great Lakes with one exception, certain areas within the City of Chicago. In the 1900s the Chicago Sanitary and Ship Canal was constructed to reverse the flow of the Chicago River from eastward to westward, thereby diverting storm and treated wastewater away from Lake Michigan and into the Mississippi River system (http://dnr.state.il.us/owr). However, during high storm flow events, the locks are opened and the Chicago River is allowed to flow into the lake. This occurs, on average, once per year (Lake Michigan Technical Committee, 2000).

3.2 Sample Collection

Approximately 480 environmental water samples were collected during 12 spatial surveys in the months of May through August 2003. Water samples were collected from four discrete regions: the Milwaukee Harbor, outside the breakwall in the open waters of Lake Michigan, at beach sites, and along the Milwaukee, Menomonee, and Kinnickinnic rivers (Figure 1). Antecedent precipitation, for the 24 hours preceding sample collection, ranged from 0.00 inches to 1.14 inches (Table 2). Sixty stormwater samples were also collected from MMSD automated inline stormwater sites at various points within the drainage basin beginning in July and concluding in October (Appendices I & II).

Table 2. 2003 harbor spatial surveys with corresponding precipitation data (precipitation measured in inches accumulated within the 24 hours preceding sample collection).

Date	Precipitation	No. of Samples Collected
05/29/03	0.09	50
06/10/03	Trace	9
06/11/03	Trace	10
06/16/03	0.00	50
06/26/03	0.02	12
07/05/03	0.25	50
07/07/03	1.14	48
07/10/03	0.08	50
07/15/03	0.37	50
08/04/03	0.39	50
08/05/03	0.00	50
08/06/03	0.01	50

In year two of the study, 10 harbor spatial surveys (190 water samples) and 3 South Shore Beach surveys were performed from May 10 to June 10, 2004 (Table 3). Sample regions included the channel/estuary (confluence of the rivers and main channel), the harbor within the breakwall, outside of the breakwall within the pollution plume, outside of the breakwall outside of the plume, and South Shore Marina (Figure 1). Additional water sampling took place at South Shore Beach within the study timeframe. These study areas were defined according to hydrological characteristics, specific conductance measurements, and distance from shoreline. Stormwater samples (138) were collected on eight individual surveys from both MMSD automated inline stations and individual stormwater outfalls located along the Menomonee and Kinnickinnic rivers between March and November 2004 (Appendices I & II).

Table 3. 2004 harbor spatial surveys and South Shore Beach surveys with corresponding precipitation data (precipitation measured in inches accumulated within the 24 and 72 hours preceding sample collection).

Date	Precipitation (previous 24 hours)	Precipitation (previous 72 hours)	No. of Samples Collected
05/10/04	0.68	1.08	20
05/11/04	0.32	0.68	7
05/13/04	0.79	0.79	4
05/14/04	1.99	2.33	90
05/18/04	0.82	0.82	9
05/19/04	0.23	0.82	13
05/20/04	0.04	0.27	4
05/21/04	1.19	1.19	6
05/22/04	2.20	2.24	6
05/28/04	0.00	0.00	16
06/02/04	0.00	0.21	6
06/10/04	1.12	1.12	9

All harbor and beach water samples were collected using a 1-liter grab sampler before, during, and after rain events. Samples were collected from the surface (0 meters) of the water column and immediately transferred into sterile 500 ml polypropylene bottles. A subset of sites was sampled in duplicate or triplicate to determine variability within sample site; in general, less than a 10% variance in *E. coli* levels was found. All water samples were stored on ice and in the dark until filtered. Water quality parameters were also recorded for each harbor and river survey sample locations using a hand-held YSI 600XL sonde (YSI Inc. Yellow Springs, OH). The water quality parameters obtained include Dissolved Oxygen (D.O.), temperature, conductivity, and depth of sample. In addition, Global Positioning System Coordinates (GPS) were documented for each sample location by a handheld GPS 76 marine navigator (Garmin, Olathe, KS) and then downloaded into a Geographical Information Systems (GIS) program, ArcGIS version 8.0 (ESRI Redlands, CA) for geographical referencing purposes.

Over the course of this three-year investigation, approximately 300 stormwater samples were obtained from 70 individual sites, consisting of 17 MMSD inline sampling stations and 53 stormwater outfalls along the Menomonee, Kinnickinnic, and Milwaukee rivers as well as their associated tributaries. Stormwater samples were collected from MMSD automated inline sampling stations which operated during storm events at the final collection point of underground storm sewers before the outfall that discharges to the Menomonee, Milwaukee, and Kinnickinnic Rivers as well as those that discharge directly into Lake Michigan. The inline samplers collected two flow-weighted composite samples for each station per storm event, one from “first flush” stormflow (first two hours of the rain period) and one from “second flush” stormflow (two to four hours after the beginning a rain event). The samples were removed from the automated samplers within 8 hours of the beginning of precipitation and placed on ice in the dark until analysis. Stormwater samples collected directly from stormwater outfalls were obtained using a 1-liter grab samples when the outfalls were running. All stormwater samples were stored on ice and in the dark until filtered.

A total of 106 stormwater samples were collected during the 2005 sampling season. Samples were obtained from MMSD inline monitoring stations as well as stormwater discharge points on the Milwaukee, Menomonee, Honey Creek, Underwood, and Kinnickinnic rivers (Appendix I). Stormwater samples were obtained from automated inline samplers and stormwater outfalls on 9 separate occasions over the course of 6 months. The stormwater discharge sites were specifically chosen based on evidence of uncharacteristically high fecal indicator levels from MMSD monitoring data and our results from 2003 and 2004.

3.3 *E. coli and Fecal Coliform Identification and Enumeration*

Water samples were analyzed within 6 hours of collection using two enumeration techniques developed by the USEPA, the original method of *E. coli* enumeration, (Method 1103.1) and the modified method for *E. coli* (Method 1603) (USEPA, 1986; USEPA, 2002). In the original method, presumptive identification of *E. coli* colonies was made by observing yellow, yellow-brown, or yellow-green colonies on m-TEC media (Difco BD, Franklin Lakes, NJ). These results were confirmed by testing a subset of bacterial isolates from the m-TEC plates for β-glucoronidase activity using 4-methyl-umbelliferyl-β-D-glucuronide (MUG) media (Remel, Lenexa, KS). The MUG-positive isolates were then cultured on agar plates and confirmed for indole production using a colorimetric spot test of p-dimethylaminoc innamaldehyde (Remel, Lenexa, KS). *E. coli* concentrations were calculated using the percentage MUG-positive isolates. In the modified method, bacteria were grown on modified m-TEC agar which contains a chromogen (5-bromo-6-chloro-3-indolyl--β-D-glucuronide) that is catabolized to glucuronic acid and a red-magenta compound by *E. coli* with β-D-glucoronidase activity (USEPA, 2002). The false-positive and false-negative rates generated for environmental water samples using the modified method for *E. coli* are <1% and 4% respectively (USEPA, 2002). Fecal coliforms levels were determined by membrane filtration using m-FC agar (Difco BD, Franklin Lakes, NJ) based on the protocol described in USEPA Report #600/8-78-017 (USEPA, 1978).

3.4 *E. coli Mortality Rate Constants*

Dilution effects vs. bacterial decay were assessed by observing the correlation between *E. coli* concentrations in water samples and specific conductance measurements, using regression analysis tools in Sigma Plot 8.0 (SPSS, Chicago, IL). For calculation of bacterial decay constants, *E. coli* levels were corrected for dilution effects using specific conductance measurements. The dilution factor was derived from the percentage of river water in the sample volume, which was calculated using the formula:

$$\% \text{ river water} = \frac{\text{Con}_{\text{measured}} - \text{Con}_{\text{lake}}}{\text{Con}_{\text{estuary}} - \text{Con}_{\text{lake}}}$$

where Con is specific conductance in mS/cm. Specific conductance was used as a time surrogate since dilution is a reflection of residence time in the lake. Data from the May 14, 2004 (Appendix III) spatial survey was used to calculate the bacterial decay rate according to the formula:

$C_t = C_0 e^{-bt}$ where:

Where: C_t = Specific conductance at time point (t)

C_0 = Specific conductance at time (t) = 0

b = number of bacteria

t = time point

3.5 Antibiotic Resistance Testing

Antibiotic resistance testing was performed on a total of 6425 *E. coli* isolates; 2447 stormwater isolates, 1465 from 2003 harbor data (rain no CSO), and 2513 isolates from the harbor and nearshore waters during a CSO. *E. coli* isolates from water samples were tested for antibiotic resistance and compared to *E. coli* isolates obtained from sewage and from host animals which

were not exposed to antibiotics. For water samples, *E. coli* isolates were obtained from either m-TEC or modified m-TEC primary isolation plates by inoculating individual colonies into 96-well microtiter plates containing MUG media. Following confirmation of *E. coli* identification by indole testing as described above, isolates were transferred to 96-well microtiter plates containing LB broth (Difco BD, Franklin Lakes, NJ) and incubated overnight at 37°C. *E. coli* isolates were stored in 96 well plates at –80°C in LB supplemented with 25% filter sterilized glycerol. As part of previous studies in the laboratory, *E. coli* isolates had been obtained from various sources of fecal pollution and were used for comparison with *E. coli* collected from contaminated water samples (McLellan *et al*., 2003; Salmore *et al*.). Overall, *E. coli* isolates were collected over a three-year period from 46 sewage treatment plant influent samples from two different treatment plants servicing the Milwaukee Metropolitan area, 1252 different gull fecal samples from 14 beach sites along Lake Michigan, and 15 different farm sites.

Ten antibiotics were chosen based on their widespread use in the human population. Antibiotic stock solutions were prepared using sterilized water and stored at –20°C in the dark. Stock solutions of antibiotics were aliquoted into sterilized LB media, which was mixed and poured into petri dishes. Antibiotic plates were stored at 4°C to prevent degradation of the antibiotics. *E. coli* isolates were stored in 96 well plates in a 50% solution of LB broth (Fisher Scientific, Fair Lawn, NJ) and filter sterilized glycerol (Sigma Aldrich, St. Louis, MO) at –80°C. Isolates were thawed on ice and then transferred to the antibiotic plates using a 96-well pin replicator (V & P Scientific, San Diego, CA). The following final concentrations used are as follows: ampicillin (20 μg/ml, 40 μg/ml), chlorotetracycline (25 μg/ml, 50 μg/ml), kanamycin (50 μg/ml, and 100 μg/ml), nalidixic acid (25 μg/ml, 50 μg/ml), neomycin (50 μg/ml, 100 μg/ml), oxytetracycline (25 μg/ml, 50 μg/ml), penicillin G (150 units/ml, 300 units/ml), streptomycin (25 μg/ml, 50 μg/ml), sulfathiazole (1000 μg/ml, 4000 μg/ml), and tetracycline (25 μg/ml, 50 μg/ml). Isolates were recorded as 0 = no growth, 1= intermediate resistance, and 2 = resistant. Isolates were considered resistant if the colony, measured with a ruler, was 50% larger than the isolate grown on the control plate. *E. coli* colonies that exhibited some growth, but less than 50% of an increase in diameter in relation to the control plate, were considered to be sensitive to the selected antibiotic.

3.6 *DNA Fingerprinting*

Polymerase chain reaction (PCR) was used to amplify target DNA from bacterial isolates to generate fingerprint patterns. The target DNA was the repetitive extragenic palindrome (rep) sequence, a non-coding region found repeatedly interspersed in bacterial genomes (Versalovic *et al*., 1991). Approximately 2 μL of whole cell preparations at 1 O.D. provided templates for each 25 μL PCR reaction. Primers employed to generate amplified fragments included REP1R and REP2I primers (Versalovic *et al*., 1991). PCR and cycling parameters were performed as described by Rademaker and de Bruijn (Rademaker and de Bruijn, 1997). Reactions were run for 30 cycles with a 42°C annealing temperature on a PTC-225 thermocycler (MJ Research, Waltham, MA) to perform the amplifications. One sample of *E. coli* strain K12 and three wild type *E. coli* isolates from a previous run were included in every PCR set-up as controls to determine if variability in PCR amplification occurred. The banding patterns of the control strain K12 and the repeated wild type strains were analyzed concurrently with the test samples (procedure described below) to assure consistency in reaction products. Rep-PCR runs that did not demonstrate reproducibility of patterns in the control strains were discarded.

Separation of amplified genomic fragments was accomplished via gel electrophoresis using 1% agarose gels made with 1X TAE and run at 70V for 16 hours at 4°C. A 1 kb molecular weight

marker (Invitrogen, Grand Island, NY) was run in three to four lanes of each gel as an external reference standard in order to allow for the correction of gel irregularities due to electrophoresis. Gels were stained with 0.6 μg/mL ethidium bromide in 1X TAE and visualized under UV light. Banding patterns were digitally photodocumented using an EpiChemi II Darkroom bioimaging system (UVP, Inc., Uplands, CA).

3.7 *Bacteroides DNA Extraction and PCR Analysis*

In general, water sample volumes that were filtered for DNA extraction ranged from 200 to 300 ml for CSO samples, 100 to 200 ml for stormwater samples, and 300 to 500 ml for beach samples, with the exception of SSO samples collected below the outfall, where 50 ml was used. All samples were filtered onto a 0.45 μm nitrocellulose filter and frozen at -80^{o}C for DNA extractions. The frozen filters were broken into small fragments with a metal spatula. The DNA was extracted using the Qbiogene FastDNA Spin Kit for Soil (Qbiogene, Inc, Irvine, CA) as specified in the manual, except the cells were mechanically lysed using the MiniBeadBeater-8 Cell Disruptor (BioSpec Products, Bartlesville, OK) on the homogenation setting for 1.5 minutes at room temperature. The DNA was eluted in 75 or 100 μl of sterile, distilled water. The numbers of culturable *E. coli* per filter were calculated from the cell counts on m-TEC or modified m-TEC media. The DNA concentration was determined using the NanoDrop ND-1000 Spectrophotometer (NanaDrop Technologies, Wilmington, DE).

Bacteroides spp*.* (Bac32F/708R), *Bacteroides* human specific (HF183F/708R), and *Bacteroides* cow specific (CF128F/708R) primer sequences were based on the 16S rDNA gene as described previously (Bernhard and Field, 2000b). *Bacteroides* cow specific (CF193F/708R) primer was also used in reactions with feedlot manure samples, but was not utilized for analysis of environmental samples due to variability and nonspecific amplification products with this reaction. *E. coli* specific primers (*uidA*1318F/1698R) were designed to target the β-glucuronidase gene using *uidA* sequence of strain K12 (NC 000913).

PCR analysis was conducted with either 1-2 μl of undiluted DNA samples, or samples diluted to a range of 10-40 ng/PCR reaction. All samples were amplified with *Bacteroides* spp*.*, human specific *Bacteroides;* cow specific *Bacteroides* and *E. coli* β-glucuronidase primers. The 25 μl PCR reactions consisted of the Qiagen *Taq* PCR Master Mix Kit (2X concentrated 5U/μl *Taq*, 3mM $MgCl_2$ and 400 μM of each dNTP) and each primer was present at a final concentration of 0.35 μM. All PCR reactions were run in the MJ Research PTC-Quad thermal cycler (Watertown, MA).

3.8 *Caffeine Analysis*

Caffeine methods using LC/MSD (Liquid Chromatograph/Mass Selective Detector) are under development. WATER is currently working on running standards and developing a solid phase extraction. Results are pending at this time due to unforeseen complications with the extraction portion of LC/MSD method, specifically relating to the need for additional clean up methods following the initial extraction to get a clear signal on the LC/MSD. The method involves sample collection of inline stormwater after storm events, addition of ^{13}C-labeled caffeine, concentration on a new mixed-mode solid –phase extraction medium and elution with methanol. One liter of water is concentrated to 1 mL and analyzed using HPLC with an electrospray ionization-ion trap mass spectrometer as the detector. Extracted ion monitoring is done at 195 m/z and 198 m/z for the labeled caffeine. Methods for caffeine analysis in complex water samples will continue to be developed so that this approach may be used in conjunction with biological analysis to estimate the human contribution to contaminants in stormwater run off.

Approximately 50 stormwater samples have been prepared for caffeine analysis. Stormwater samples were collected in 1.5-liter glass and polypropylene containers, stored on ice and protected from sunlight. Upon arrival to the laboratory 1-liter of each sample was transferred into a glass bottle and autoclaved at 120 °C for 15 minutes. The sterilized water samples were then filtered through a Stericup 0.22-micron presterilized vacuum driven disposal filtration (Millipore Corp, Billerica, MA) and spiked with 50 µl per 1000 ml of a 10 µg/ml of $^{13}C_3$ caffeine stock solution. Samples are stored in the dark at 4°C.

3.9 *Pathogen Analysis*

Pathogen analysis was carried out for *Giardia*, *Cryptosporidium*, and *Salmonella* in water samples from stormwater, the harbor during a CSO, and select river water samples. All DNA extractions were performed as described above for *Bacteroides* spp. analyses (Table 4).

Table 4. PCR primers designed for the amplification of specific pathogens targets.

Organism	Primer Sequences used in PCR[1]	Target	Product Size in bp[2]	Reference
Salmonella spp.	(F)* 5′ACAGTGCTCGTTTACGACCTGAAT3′ (R) 5′AGACGACTGGTACTGATCGATAAT3′	*invA*	243	(Chiu and Ou, 1996)
	(F) 5′ACTCCTTGCACAACCAAATGCGGA3′ (R) 5′TGTCTTCTGCATTTCGCCACCATCA3′	*spvC*	570	
C. jejuni & *C. coli*	(F) 5′AATCTAATGGCTTAACCATTA3′ (R) 5′GTAACTAGTTTAGTATTCCGG3′	16S	854	(Linton *et al*., 1997)
Giardia	(F) 5′CATAACGACGCCATCGCGGCTCTCAGGAA3′ (R) 5′TTTGTGAGCGCTTCTGTCGTGGCAGCGCTAA3′	giardin	218	(Rochelle *et al*., 1997)
Crypto spp.	(F) 5′TTCTAGAGCTAATACATGCG3′ (R) 5′CCCATTTCCTTCGAAACAGGA3′	small subunit rRNA	1325	(Xiao *et al*., 2001)
	(F) 5′GGAAGGGTTGTATTTATTAGATAAAG3′ (R) 5′AAGGAGTAAGGAACAACCTCCA3′		864	

[1] DNA sequences of Primers
[2] Base Pair of nucleotides
* Forward (F) and Reverse (R) primers

4.0 RESULTS AND DISCUSSION

The primary purposes for conducting the Bacterial Source, Transport, and Fate (BSTF) study were to assess the impact of bacteria sources on area beaches and on a more regional basis, as well as evaluate whether impacts could potentially occur outside of the Milwaukee area.

As part of this study, a monitoring program was implemented to investigate the use of bacterial source tracking techniques for determining the primary sources of bacterial pollution in the study area as well as define nearshore sources of bacteria. These data were used to validate and refine a hydrodynamic model, developed for this study by Camp, Dresser, and McKee (CDM) and HydroQual (2003). The estuary water quality modeling is intended to create a tool to evaluate water quality and water movement (circulation/dispersion) in the estuary, harbor and nearshore area of Lake Michigan to provide a quantitative assessment of bacteria source impacts on area beach water quality. Examples of the hydrodynamic model results are shown in Figures 3 and 4 (additional model simulations found in Appendix V). Overall, hydrodynamic model results showed that the fecal coliforms distributed approximately 5 km from the harbor, before they were not longer detectable. Data collected in our laboratory for model validation closely matched model predictions. A full description of the hydrodynamic modeling for the harbor can be found in "Assessing Bacteria Source Impacts on Beaches: A Modeling Approach" (Thuman *et al*., 2004).

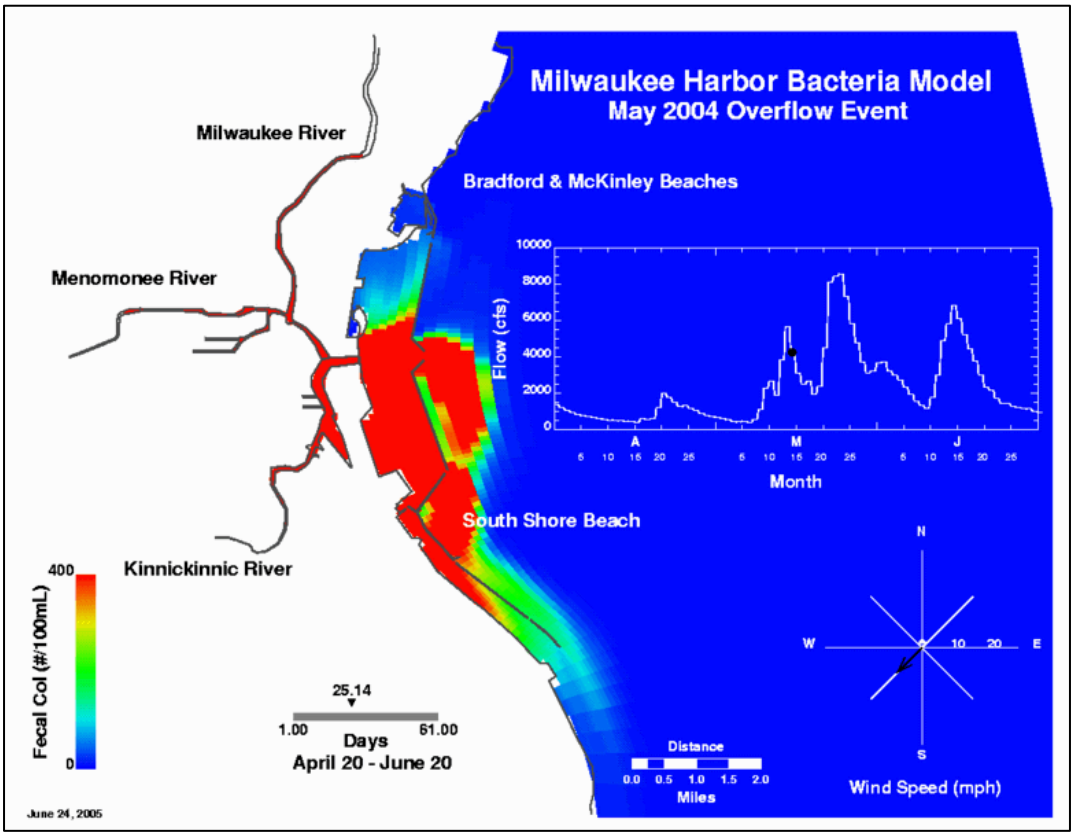

Figure 3. Fecal Coliforms (FC) concentrations following heavy rainfall between May 13-14, 2004 and 30 hours after peak of the hydrograph (day 24 of the model). Winds recorded out of

the NE with average speeds between 10 to 20 mph. The plume extends outside the harbor breakwall but remains within <1 mile (1.6 km) from the breakwall edge.

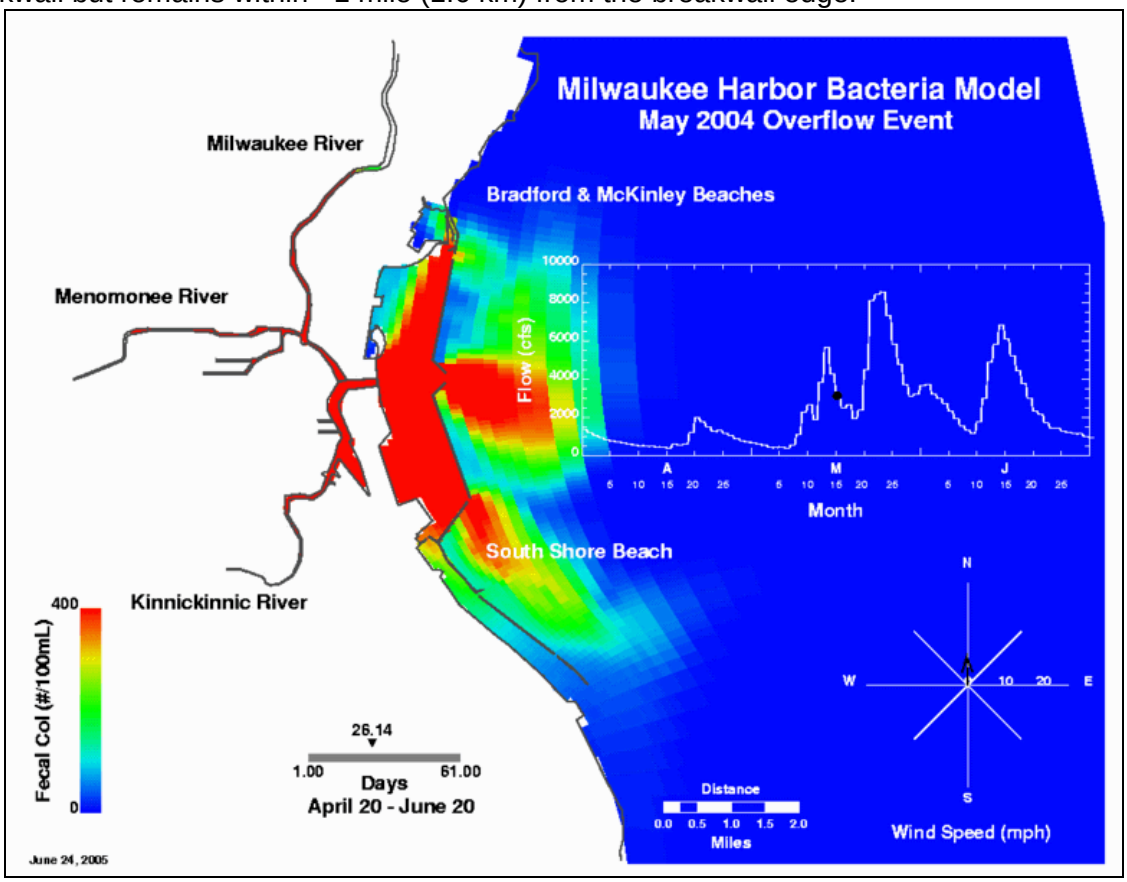

Figure 4. Fecal coliform (FC) levels 54 hours (day 26 of the modeling period) after the May 13th, 2004 peak in the hydrograph (day 24 of the model) following a wind shift (winds out of the South at roughly 10 mph). Model validation data collected during this time frame closely matched FC levels predicted by the hydrodynamic model (Appendix V).

4.1 *E. coli and Fecal Coliform Disappearance*

Research conducted by the USEPA determined that *E. coli* is the best method for assessing the potential risk of acquiring a gastrointestinal illness from recreational waters in freshwater systems (USEPA, 1986). Prior to 1986 the USEPA recommended the use of fecal coliform bacteria as a measure of recreational water quality; however, further epidemiological studies revealed that there was a poor correlation between fecal coliform bacteria concentrations and swimmer associated illnesses (USEPA, 1986). Fecal coliform data was collected in year one of this study in conjunction with *E. coli* data to determine the relative survival characteristics of both organisms under the same environmental conditions, and the relevance of each organism to assessing water quality in Lake Michigan.

Fecal coliforms are defined as organisms that demonstrate strong lactose fermentation (which produces gas) and grow at 44.5°C. We have found that for newly introduced contamination, *E. coli* is the major fecal coliform; however, as the numbers of fecal coliforms decline, the ratio

shifts and the predominant organisms found are *Klebsiella pneumoniae*, *Enterobacter cloacae*, and *Enterobacter sakasakii*. Further, we have found that beta-glucuronidase activity is the most useful test used to distinguish *E. coli* from other fecal coliforms (Byamukama *et al*., 2000; McLellan *et al*., 2001). The beta-glucuronidase enzyme activity is determined by cleavage of MUG. In past studies we have observed a clear signature: for newly introduced contamination such as stormwater and sewage discharges taken directly from outfalls, the fecal coliform composition was 60% to ~80% MUG-positive fecal coliforms. However, as contaminated water moved down the major rivers and into the open waters of Lake Michigan, the ratio of MUG-positive isolates decreased to <10%, and near 1% in the open waters of Lake Michigan.

From the harbor and nearshore sites, fecal coliform and *E. coli* levels were compared for baseflow and post-rain samples collected in 2003 (Table 5). The proportion of fecal coliform levels that were *E. coli* was approximately an order of magnitude higher inside the breakwall than that found outside of the breakwall, demonstrating that other fecal coliforms persist longer in the open waters of Lake Michigan.

Table 5. Percentage of *E. coli* comprising FC levels in rivers and Lake Michigan

Region	% *E. coli* comprising fecal coliforms levels ± 1sd	
	Baseflow (n=95)	Post rain (n=162)
Rivers[1]	9.7 ± 8.5	32.5 ± 21.4
Channel	9.1 ± 5.8	11.3 ± 8.2
Harbor	4.8 ± 3.7	13.9 ± 11.8
Lake	0.6 ± 2.2	3.0 ± 3.3

[1] Samples were collected in the lower reaches of the Milwaukee, Menomonee, and Kinnickinnic Rivers within 1 km of the confluence.

Bacterial decay rates for fecal coliforms in Lake Michigan have been calculated to be 90% disappearance in 6-8 hours (Zanoni *et al*., 1978), which is fairly rapid considering estimates for base die-off rates (Mancini, 1978) and results from *in situ* die-off experiments (Easton *et al*., 1999). In this study, *E. coli* disappeared more quickly than fecal coliforms; there were dramatic differences in the numbers of *E. coli* contributing to the fecal coliform levels as pollution distributed >1-2 km from the harbor.

In general, *E. coli* is considered a better indicator of fecal contamination than fecal coliforms, since this organism does not survive as long in the environment as other members of the fecal coliform group (Toranzos and McFeters, 1997). However, in this study we found that fecal coliforms actually were the more appropriate indicator organisms for measuring sewage overflow distribution in the Great Lakes, since they appeared to be a more conservative tracer. In addition, survival rates of fecal coliforms may be more similar to pathogens of concern than *E. coli*, particularly *Giardia* and *Cryptosporidium* which are noted for their long survival times (Rose, 1997).

4.2 *E. coli Disappearance: Dilution Effects vs. Die-Off*

Overall, *E. coli* levels were reduced as conductivity measurements decreased throughout the Milwaukee Harbor and nearshore waters of Lake Michigan (Figure 5A). There was a poor linear relationship between *E. coli* levels and specific conductance as the river plume dispersed into

Lake Michigan (r^2 = 0.51), where the reduction in *E. coli* levels was greater than the amount of dilution. For example, samples containing 60-80% river water were found to contain between 12-17% (540-780 *E. coli*/100 ml) of the mean concentration detected in the channel (4500 *E. coli*/100 ml). This demonstrated that the rapid disappearance of bacteria outside of the harbor cannot be attributed solely to dilution, but that cell die-off also occurred. In order to evaluate if the bacterial decay followed a first order function, the log of *E. coli* concentrations (corrected for dilution) was plotted against conductivity as a surrogate for time (Figure 5B). The data did not display a linear fit for exponential decay, as would be expected with a first order decay constant. These results are similar to what has been noted for fecal coliform die-off in sediments (Davies *et al*., 1995) and indicator bacteria and pathogens found *in situ* river water (Easton *et al*., 1999).

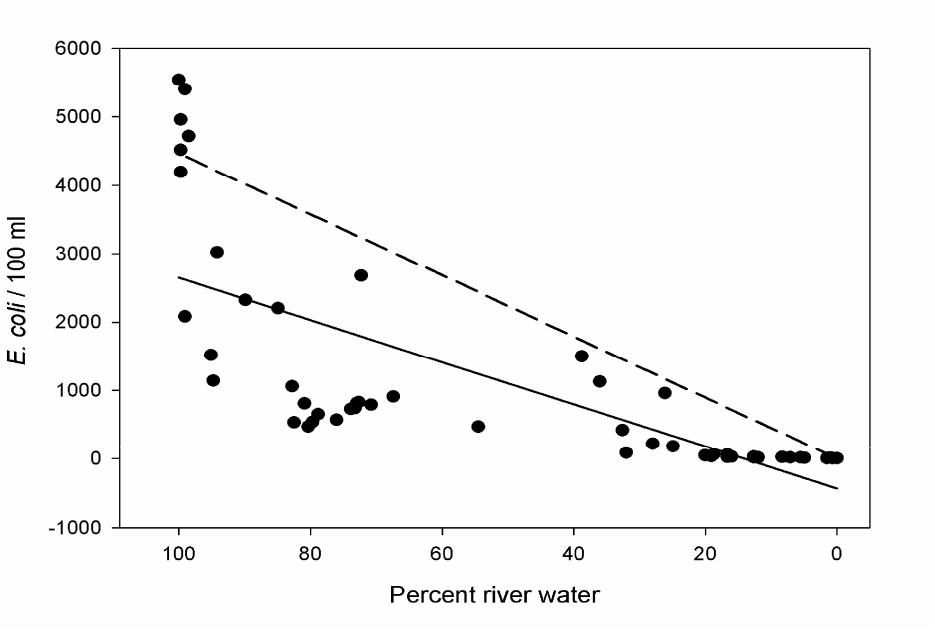

Figure 5: (A) *E. coli* levels in relation to conductivity measurements; (---) trend line is the predicted *E. coli* levels if dilution was the only factor accounting for bacterial loss. Regression analysis indicated by the solid line did not demonstrate a linear fit (r^2=0.51).

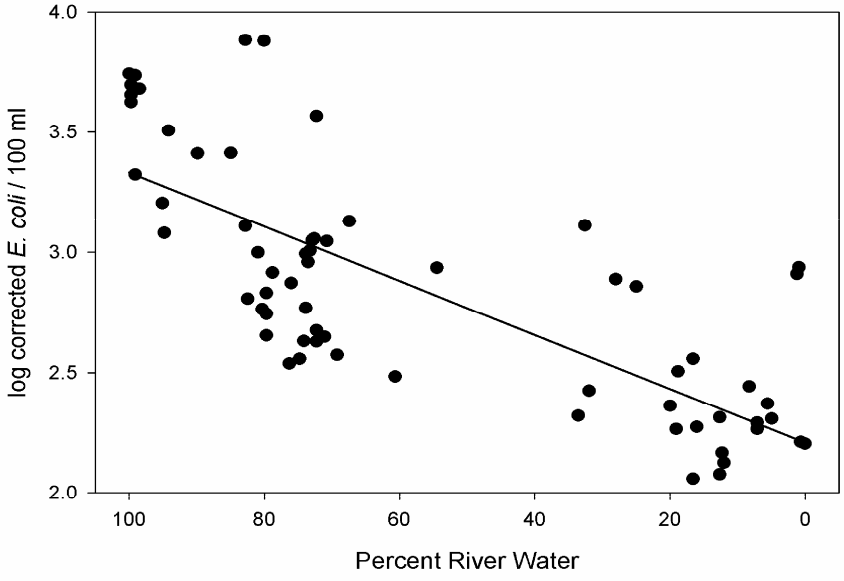

Figure 5: (B) *E. coli* levels corrected for dilution effects. Data demonstrates a poor fit to a first order decay constant (r^2=0.65).

4.3 *E. coli and Fecal Coliform Results*

4.3.1 *Spatial and temporal distribution of E. coli in absence of CSO*

E. coli levels increased at all sample locations in conjunction with rain events, with average increases ranging from 1 to 4 orders of magnitude. Historically, data collected by MMSD has revealed a positive relationship between stream flow (a direct result of the amount of rainfall) and bacteria levels is shown in Figure 6 (Thuman *et al*., 2004)**.** In the individual river and harbor surveys, the most significant rise in bacterial concentrations was detected in the main river channel with *E. coli* levels measured as low as 9 CFU/100 ml following 0.09 inches to as high as 11,000 CFU/100 ml post 0.39 inches of rain (Table 6). The channel is subjected to multiple sources of bacterial pollution because

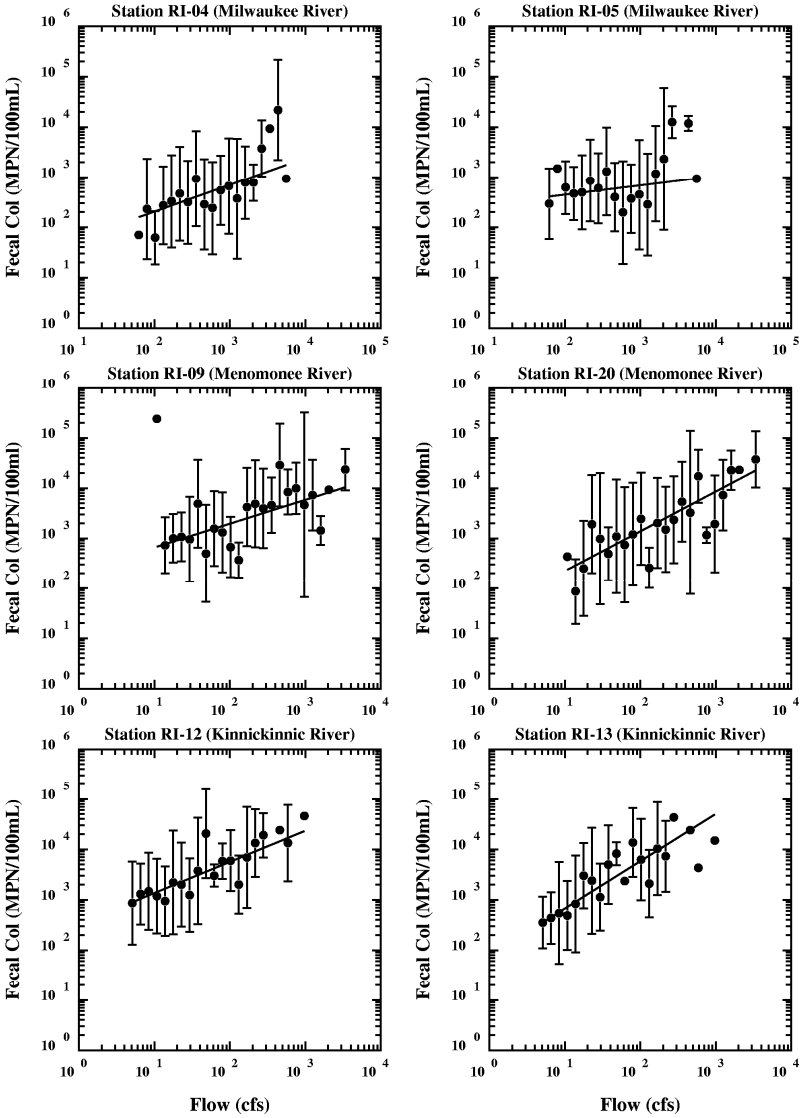

Figure 11 Three Rivers Bacteria/Flow Relationships (1990-2001)
(Mean +/- standard deviation)

it is the primary drainage point for the entire 850 square mile watershed. Bacterial concentrations in the channel/estuary tended to be highly concentrated in comparison to the harbor and open waters of Lake Michigan because, as the river water moves into the lake and mixes with the harbor and lake water, *E. coli* levels are diluted out of the water column. Attachment to particulate matter and subsequent sedimentation may also play a role in the removal of *E. coli* from the water column.

E. coli measurements detected inside the channel were found to be the highest overall, followed by the rivers, harbor, and South Shore Beach (Table 6). *E. coli* counts in the harbor were reflective of conditions in the rivers and channel, increasing and decreasing in concurrence with precipitation events. However, once outside the breakwall, *E. coli* levels typically remained within USEPA guidelines (<235 CFU/100 ml) with the exception of a few select water samples collected on 7-7 and 7-10, 2003. It is important to note that these particular water samples were collected just outside of the main and south gaps and inside the river plume subsequent to 1.14 and 0.08 inches of rain, respectively (Table 6).

South Shore Beach behaved in a similar fashion with the highest *E. coli* readings detected closest to shore, and levels dropping off as the nearshore water mixed with the lake water. These findings are consistent with previous research that documented contamination at South Shore Beach was a result of local shoreline sources washing into the water, rather than the result of delivery of contamination from offshore waters (McLellan and Salmore, 2003). *E. coli* levels at South Beach fluctuated with rainfall, where bacterial levels increased up to 3 orders of magnitude between pre and post rain conditions.

Table 6. Comparative ranges of *E. coli* (CFU/100 ml) levels per region for water samples collected during the 2003 sample season, with corresponding cumulative precipitation (inches) within the 24 period preceding sample collections.

Date	Total Precipitation	Rivers	Channel/ Estuary	Harbor	South Shore & Beach/Marina	Outside Breakwall
05/29/03	0.09	0-200	9-30	0-12	1-465	0-11
06/10/03	0.00	--	315-370	18-380	--	23
06/11/03	0.00	--	85-120	27-51	25	--
06/16/03	0.00	60-275	37-145	2-34	2-4	0-2
06/26/03	0.01	--	29-34	3-24	--	3
07/05/03	0.29	43-1500	45-80	3-4633	--	1-11
07/07/03	1.14	55-6700	320-1580	55-1625	180-800	175-635
07/10/03	0.08	1100-4650	120-2200	89-1270	235-460	177-320
07/15/03	0.37	87-400	20-217	1-99	86-400	0-9
08/04/03	0.39	355-6500	405-11000	6-565	100-150	2-11
08/05/03	0.00	--	650-1200	10-300	110-130	4-80
08/06/03	0.01	--	--	18-396	--	0-10

-- No data collected for this sample region during a specific spatial survey.

4.3.2 *Spatial and Temporal distribution of E. coli following a CSO event*

Record rainfalls occurred in southeastern Wisconsin during the month of May 2004, with an average of 8.1 inches of rain recorded at the General Mitchell International Airport weather station (http://www.nws.noaa.gov, 2005). The Milwaukee River drainage basin received the majority of its rainfall during two periods, May 7-14 and May 20-23. The greatest amount of rain took place on May 21 and 22, 2004, with a total of 1.64 inches of rainfall within a 24-hour period. Dense data rainfall estimates were obtained from radar data in order to attain a more accurate representation of rainfall estimates per square kilometer throughout the MMSD service region (Theiler *et al*., 2004). Based on this analysis, during the May 2004 rain events the study area received a maximum, average, and minimum amount of 12.8, 9.0, and 5.7 inches of rain, respectively for the month (Theiler *et al*., 2004). An additional analysis was conducted in order approximate the precipitation loads in separated and combined sewer areas. Monthly precipitation averages during this time period for the separated sewer area was 8.75 inches, while the combined sewer area received 9.5 inches (Theiler *et al*., 2004). According to these results, the combined sewer area received more rainfall compared to the separated sewer area during May 2004.

Increases in discharge rates for the Milwaukee, Menomonee and the Kinnickinnic Rivers coincided with individual rain events (Figure 7). River discharge rates to Lake Michigan were obtained for the Milwaukee, Menomonee and the Kinnickinnic Rivers using USGS gauge stations 4087000, 4087120, and 4087159 (http://waterdata.usgs.gov/wi/nwis/rt). During 2004, three CSO events occurred during periods of the heaviest rains. The first CSO ran from May 13 until May 16, totaling 55.5 hours in length and was approximately 515 million gallons (MG). The second CSO, the smallest of the three discharges, totaled 26.8 MG and ran for 7 hours from May 17 to 18. The third CSO occurring from May 21 to May 25 was the longest and largest of the three, totaling 93.75 hours with an estimated volume of 547 MG. The majority of the CSOs were discharged into the rivers where more than 45 CSO outfalls are located; only two outfalls empty directly into Lake Michigan, one within South Shore Marina, the other on the south end of the harbor (Figure 2). In total, approximately 1,088 MG of a combination of stormwater and sanitary sewage were discharged into local surface waters during this 18-day time period. In addition five SSOs occurred during the same time period (Figure 7). Approximately 102 MG of sanitary sewage were discharged into area waters between May 10 to May 18 and 372 MG during the period of May 19 to May 24. All of the SSO events occurred in the northern portion of MMSD's service area (approximately 10 to 15 km from the confluence) and discharged into the Milwaukee River. In addition to these discharges, SSO's occurred in 14 municipalities (Table 1), with an estimated 4554 million gallons (MG) discharged during the period of May 10th to May 25th 2004 (information provided by the Wisconsin Department of Natural Resources).

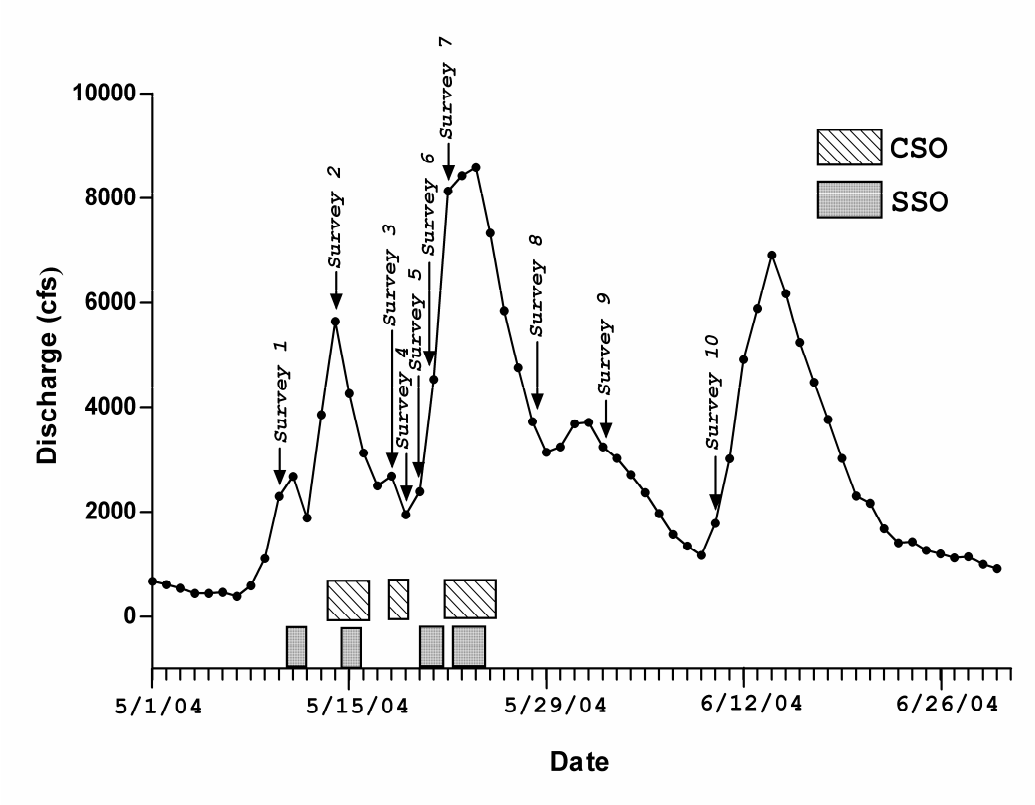

Figure 7. Composite hydrograph of the Milwaukee, Menomonee, and Kinnickinnic Rivers as they discharge to the Milwaukee harbor. Sample survey dates in relation to SSO and CSO events are shown. Two SSO events overlapped on 5-22 to 5-23, 2004. Fourteen municipalities also reported SSOs during this time including; Bayside, Brookfield, Brown Deer, Cedarburg, Cudahy, Elm Grove, Fox Point, Hales Corners, Mequon, Milwaukee, South Milwaukee, Theinsville, West Allis, and Whitefish Bay (Municipality information provided by the Wisconsin Department of Natural Resources, 2004).

E. coli levels increased in combination with rainfall and the onset of a CSO and/or SSO event in all sampling regions. *E. coli* concentrations varied considerably across sampling regions during the study (Table 7). The first three surveys were conducted 1-3 days prior to the initial CSO and in conjunction with SSO events in the northern portion of the Milwaukee River Drainage Basin, approximately 10-15 km from the estuary. Water samples collected 12 hours after the start of the first SSO event showed *E. coli* levels in the estuary and harbor ranged from 122-760 CFU/100 ml and 47-780 CFU/100 ml, respectively. With increasing distance from the confluence, *E. coli* levels decreased to <200 CFU/100 ml at the main and south breakwall gaps of the harbor. South Shore Marina experienced the highest *E. coli* levels during this timeframe, with the greatest numbers of bacteria detected at the north end of the Marina, adjacent to a slip area where a stormwater outfall and a combined sewer outfall are located. *E. coli* levels in water collected immediately below the stormwater outfall were found to be 1490, 4500, and 5500 CFU/100 ml on the three days prior to the first CSO event, when only stormwater was discharged. *E. coli* levels in this same area increased an order of magnitude during the CSO discharges, containing a mixture of stormwater and sanitary sewage. However, it is uncertain if this increase in bacteria numbers from the CSO could be attributed to sanitary sewage or stormwater since heavy rains caused significantly more stormwater to enter the system.

During the first CSO (May 13 – 16), *E. coli* levels increased for each sampling region in relation to pre-CSO conditions and were primarily contained within the pollution plume. For example, on May 14, *E. coli* levels were found to average 3300 CFU/100 ml, 3000 CFU/100 ml, and 5600 CFU/100 ml, within estuary/channel, harbor, and outside the breakwall within the plume, respectively, but decreased to 92 CFU/100 ml outside the breakwall within 0.5 km of the harbor (Table 7). In all, seven bacterial spatial surveys were conducted subsequent to the CSO/SSO events of May 2004. All surveys showed a similar trend of *E. coli* levels decreasing with increased distance from the shoreline and confluence of the three rivers. The geometric mean for *E. coli* densities during the CSO and SSO events exceeded the USEPA recommended limit of 235 CFU/100 ml for recreational waters in the channel/estuary (725 CFU/100 ml), the harbor (312 CFU/100 ml), and South Shore (285 CFU/100 ml). In contrast, *E. coli* counts outside of the breakwall in the open waters of Lake Michigan were found to be below the recommended criteria, with geometric mean concentrations of 39 CFU/100 ml. In general, *E. coli* levels fell below 10 CFU/100 ml (considered background levels) at distances of 2-5 km from the harbor breakwall.

Table 7. Comparative ranges of *E. coli* (CFU/100 ml) within regions of the Milwaukee Harbor and nearshore Lake Michigan during a CSO event along with total rainfall (inches) within a 24-hour period.

Date	Amount of Precipitation[3]	Channel	Harbor	South Shore Beach	Stormwater Outfall[1]	Outside Breakwall Within Plume[2]	Outside Breakwall Outside Plume 2-5 km from harbor
05/10/04	0.96	122-760	47-780	--	(1,296-1,490)	--	--
05/11/04	0.32	--	--	368-610	(3,000-4,500)	--	--
05/13/04	0.79	--	--	117-180	(5,000-5,500)	--	--
05/14/04	1.99	700-8,333	167-13,667	67-4167	50,000	0-7,000	0-590
05/18/04	0.82	2,620-3,100	40-960	--	--	520	210
05/19/04	0.23	--	--	150-587	(507)	173-277	9-132
05/20/04	0.04	--	83	--	--	81-83	2-10
05/21/04	1.19	207-1587	579	--	--	--	--
05/22/04	2.20	2,175-4,755	1132-1,895	--	--	--	--
05/28/04	0.00	7-61	0-32	--	--	4-18	--
06/02/04	0.00	100 -105	180	--	--	--	0
06/10/04	1.12	--	--	80-1,580	(8,700)	--	--

-- No data collected for this sample region during a specific spatial survey.

[1] Samples taken below a combined sewer outfall, which is located adjacent to a stormwater outfall. Parentheses indicate that samples were collected when there was no CSO discharge occurring.

[2] Specific conductance readings >0.45 mS/cm indicative of river water and used to distinguish samples taken outside the breakwall but within the pollution plume.

[3] Precipitation estimates were obtained from the National Weather Service Milwaukee/Sullivan official weather station located at General Mitchell International Airport and do not reflect precipitation totals for the entire service area as there were reports of significantly more rain elsewhere in the MMSD service area (Theiler *et al*., 2004).

E. coli levels in the sample regions were higher during the CSO/SSO events in 2004 than that observed in these same regions in 2003 following rain events with no sewage overflows (Figures 8A and 8B). During six surveys conducted in 2003 with 0.07 to 1.14 inches of rain, geometric mean *E. coli* levels were found to be lower than 2004 in the estuary (567 CFU/100 ml), the harbor (91 CFU/100 ml), and South Shore (126 CFU/100 ml). Geometric mean levels in Lake Michigan outside the breakwall were 19 CFU/100 ml. *E. coli* levels for each sampling region during baseflow conditions were within USEPA guidelines with average levels of 116 CFU/100 ml, 15 CFU/100 ml, 4 CFU/100 ml, and 1 CFU/100 ml from the estuary/channel, harbor, South Shore Marina, and outside of the breakwall, respectively.

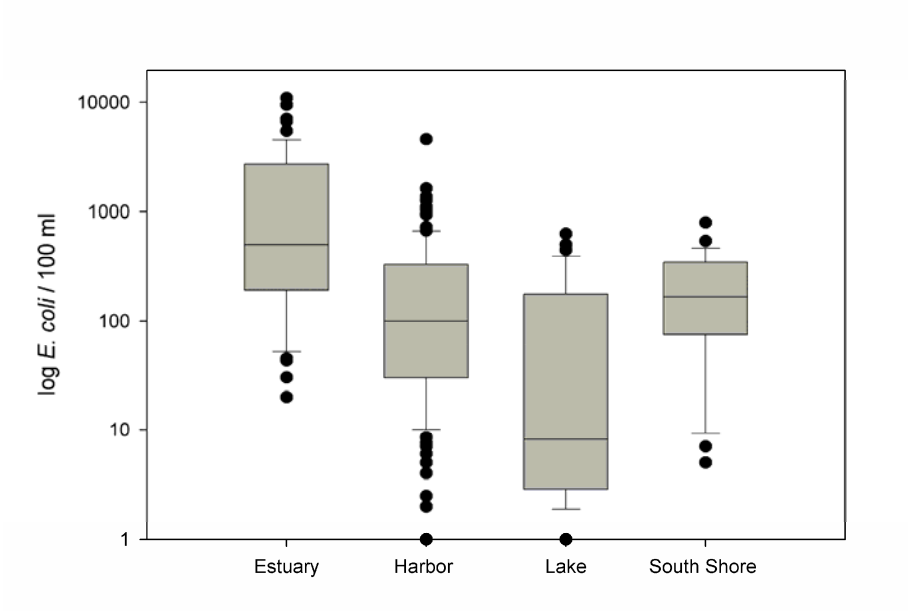

Figure 8A. *E. coli* levels in sampling regions in 2003 following rain events with no sewage overflows.

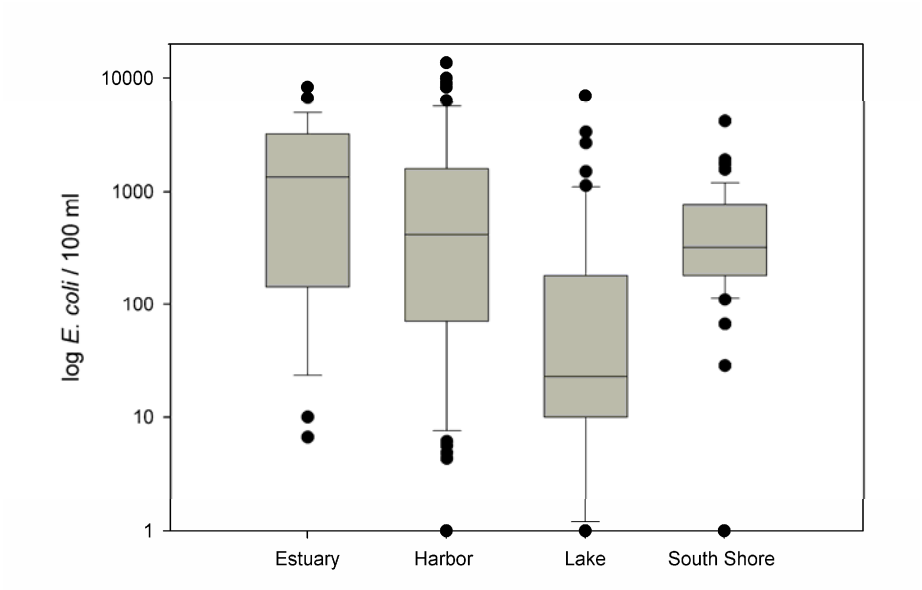

Figure 8B. *E. coli* levels in sampling regions 2004 following rain events measured during combined and sanitary sewage overflows.

4.3.3 *Impact of Milwaukee's CSO on Regional Beaches*

Extensive sampling surveys during the May 2004 CSO events demonstrated that *E. coli* could not be detected at appreciable levels, e.g. 10 CFU or greater/100 ml, at distances >5 km from the Milwaukee Harbor breakwall. During this same timeframe, Wisconsin and Illinois beaches experienced numerous closures due to elevated *E. coli* levels. Past research conducted in the Milwaukee area, as well as studies done by investigators in other parts of the country (Boehm *et al*., 2003; McLellan and Salmore, 2003; Scopel *et al*.), demonstrate that elevated *E. coli* levels are often due to localized sources, and regional sources such as sewage overflows, while presenting a serious health risk, do not contribute large numbers of *E. coli* to the beach area. Similar conclusions were made in a paper presented at the International Association for Great Lakes Research in Ann Arbor, Michigan in May 2005, where a whole lake circulation model demonstrated that water from Milwaukee could theoretically reach Wind Point, Racine, but not further south, during the time frame of the beach closings in Chicago (Schwab *et al*., 2005). This paper concluded that Milwaukee's CSOs did not close Chicago's beaches. Data collected as part of the BSTF study also contributed to the paper. This finding highlights the need for better assessment tools to evaluate both localized and regional pollution sources.

While the sewage overflows did not have a direct effect on the numbers of *E. coli* (and therefore the beach monitoring results), the hydrodynamic model will be extremely useful in determining water movement, and therefore, the potential for CSO contamination to reach beaches.

4.3.4 *Urban stormwater E. coli levels*

Managing the quantity and quality of urban stormwater runoff remains one of the most difficult environmental and fiscal challenges in the United States (Heaney *et al*., 1999). Urban areas are particularly vulnerable to degraded water quality. In many cases, the hydrology of these regions has been severely altered to allow for urban development, which has resulted in an increase in the amount of impervious surfaces, and subsequently, a drastic rise in the volume of runoff that ultimately can cause CSOs in some areas. One solution to this problem has been to utilize separated sewer systems, rather than combined sewers. This design is advantageous with respect to minimizing sewage overflows; however, the system does send stormwater, and its accompanying pollutants with it, directly to the receiving waters, a detrimental effect on water quality (Bannerman *et al*., 1993; Bickford *et al*., 1999; Faulkner *et al*., 2000; USEPA, 2000; Wyer *et al*., 1997).

Inline stormwater samples were collected throughout the MMSD service area (Table 8) as part of the MMSD Stormwater Monitoring Program and provided to the laboratory for assessment for markers of human fecal pollution. A more complete listing of stormwater outfall locations is listed in Appendix I. In general, stormwater samples (collected directly from the system with inline monitoring equipment) yielded *E. coli* levels greater than 10,000 *E. coli*/100 ml. MMSD inline stormwater samples collected from the Wauwatosa area (e.g. SWWA13, SWWA17, and SWWA20) ranged from 350 to 2,500,000 *E. coli* per 100 ml, while *E. coli* levels from stormwater samples collected from outfalls within Wauwatosa city limits (e.g. Underwood Creek, Honey Creek, and the Menomonee River) ranged from <10 to 80,000 *E. coli* per 100 ml. *E. coli* levels from Milwaukee stormwater outfall sites which discharge to the Milwaukee River, Menomonee River, and Lincoln Creek, spanned from <10 to 50,0000 *E. coli* per 100 ml with the maximum *E. coli* reading detected at SWMI06. Interestingly, the majority of stormwater samples collected from local residential areas SWGF10 and SWNB11 and from an industrial park in Franklin (SWFR03) had *E. coli* levels

exceed 10,000 CFU/100 ml. Overall, *E. coli* levels did not differ considerably between 1st and 2nd flush samples. *E. coli* levels are summarized in Table 10.

Table 8. MMSD Stormwater Monitoring Sites (2003 to 2005)

SITE ID	LOCATION
SWMI01	Lincoln Memorial Dr. and Carferry Dr.
SWMI02	1700 N Lincoln Memorial Dr. @ Lafayette Hill Rd.
SWFR03	54th and Ashland
SWMI04	3500 S. Lake Dr. @ Bay View Park
SWMI05	1200 E. Singer Cir.
SWMI06	Milwaukee County. Zoo
SWMI07	4345 N 47th St.
SWMI08	Hampton and Lincoln Cr. Parkway
SWWB09	4939 N. Newhall
SWGF10	Boerner Botanical Gardens
SWNB11	13380 Eagle Trace and Timber Ridge
SWMI12	3275 S. 72nd St.
SWWA13	Ridge Blvd. and Harding Blvd.
SWSF14	Lake Dr. and Tesch Ave.
SWMI15	42nd and Mt. Vernon
SWMI16	Marquette Interchange
SWWA17	71st and Chestnut St
SWMI18	Miller Park East Parking Lot @ Sausage House
SWMI19	Lincoln Memorial Dr. and Picnic Point
SWWA20	Dana Ct. and 83rd St.

Two stormwater outfalls that discharge directly into Lake Michigan, SWMI04 and SWWB09, were found to have bacteria levels between 10^3 to 10^5 CFU/100 ml. These particular outfalls may negatively impact beach water quality due to the close proximity of the discharge point to the beach. Essentially, beaches can be highly impacted by local sources, such as direct runoff from impervious surfaces or storm outfalls. In addition, it may be warranted to conduct an extensive assessment for human sources of fecal bacteria in these stormwater outfalls (see "**4.6 *Bacteroides* spp. …in Lake Michigan**").

4.4 *Antibiotic Resistance Patterns of E. coli Isolates*

Antibiotic resistance source tracking methods have been developed under the premise that, in general, humans are exposed to antibiotics more frequently than wildlife. As a result, humans carry a higher proportion of *E. coli* resistant to antibiotics as opposed to wildlife which are not exposed to antibiotics. The differences in the percentage of isolates resistant to antibiotics in human vs. non-human sources can provide valuable information regarding the potential sources of fecal contamination in environmental water samples.

Harbor Water

The occurrence of a known sewage overflow was used to evaluate this potentially useful microbial source tracking method, antibiotic resistance testing. Antibiotic resistance (AR) frequencies for *E. coli* isolates obtained from sewage were compared with *E. coli* collected

from post-rain harbor samples in 2003 (in the absence of a CSO), and from post rain samples accompanied by a CSO/SSO in 2004. Post-rain AR results for both 2003 and 2004 exceeded those of the gull, cows, and stormwater isolates, for all antibiotics. Resistance to ampicillin, penicillin G, and tetracycline was common in *E. coli* from harbor water, whereas very few *E. coli* isolates from non-human sources were found to be resistant to these antibiotics. Resistance to kanamycin, nalidixic acid and neomycin was rarer, with a relatively low frequency of resistance found in sewage, with AR results equal to roughly 10%, 12%, and 8% respectively. Interestingly, harbor isolates obtained in 2003, when there was no sewage overflow, exhibited similar resistance patterns to isolates collected in 2004, with no significant difference between the years ($p<0.05$). Approximately 31% of isolates obtained in the harbor during non-CSO conditions were resistant to ampicillin (concentration = 80 μg/ml) while 32% of harbor CSO isolates were also resistant to ampicillin at the same concentration.

These findings would suggest there was no net increase in the proportion of human sources, or more precisely, host sources (including animals) exposed to antibiotics in 2003 samples and 2004 samples (Table 9). Essentially, this data suggests that while there is an overall increase in fecal indicator bacteria in the harbor during a CSO compared with a non-CSO survey (Figure 8a and b), the proportion of human sources is similar. These results may indicate that in the absence of a documented CSO, there may be unrecognized sanitary sewage inputs such as cross connections between the stormwater and sanitary systems and/or aged sewer infrastructure resulting in leaking sewer lines.

Table 9. Percentage of AR of *E. coli* Isolated from Environmental Water Samples

Antibiotic[1]	Concen-tration (μg/ml)[2]	Sewage[3] (n = 1252)	Hosts no Known Exposure to Antibiotics (n = 2356)	Stormwater Outfalls (n = 2447)	Harbor Non-CSO Days 2003 (n = 1465)	Harbor CSO Days May 2004 (n = 2513)
Ampicillin	40	54	7	19	50	44
	80	50	6	15	31	32
Chlorotetracycline	25	27	10	10	13	18
	50	21	8	6	8	13
Kanamycin	50	12	2	2	5	5
	100	10	2	1	2	3
Nalidixic Acid	25	14	1	3	7	8
	50	12	1	1	4	5
Neomycin	50	10	2	<0.5	1	2
	100	8	2	<0.5	<0.5	2
Oxytetracycline	25	30	12	9	18	18
	50	27	10	7	12	14
Penicillin G	90	32	7	18	49	56
	180	32	8	19	39	33
Streptomycin	12.5	23	11	16	18	21
	25	18	7	7	10	11
Sulfathiazole	2000	18	6	4	11	15
	4000	--	7	4	7	10
Tetracycline	25	28	9	9	13	18
	50	24	6	7	10	13

[1]Antibiotics tested are those most commonly used in the human population.
[2]Antibiotic were tested at two concentrations each with stock solutions listed in μg/ml.
[3]*E. coli* isolates were obtained from Jones Island and South Shore Treatment Plant influent samples over a three year period (n=35 samples).

Stormwater

In addition, AR testing was performed on a number of stormwater samples. AR results varied depending upon the outfall location. For example, samples from the Wauwatosa stormwater outfall site SWWA13 (located at the intersection of Ridge and Harding Boulevards) were 20% resistant to ampicillin while from stormwater outfall SWWA17 (71st and Chestnut) only 8% of isolates tested were resistant to ampicillin. Both of these outfalls discharge directly to the Menomonee River. AR patterns from numerous Milwaukee stormwater sites had increased resistance to ampicillin (e.g. SWMI01, SWMI04, SWMI12, and SWMI18) with 55%, 43%, 87%, and 48%, respectively. These percentages are similar to what is found in sewage, where 54% of the *E. coli* isolated from sewage had been found to be resistant to ampicillin. In contrast, only 12% of the *E. coli* isolated from hosts such as gulls, cows, and dogs that are not exposed to antibiotics was found to be resistant to ampicillin. The elevated frequencies of antibiotic resistant *E. coli* could be an indication that there may be sanitary sewage leaking in the stormwater system. In general, stormwater outfall *E. coli* isolates with elevated antibiotic frequencies also tested positive for *Bacteroides* human specific species, which is further described in the following table (Table 10).

Table 10. Stormwater data collected from 2003 to 2005[1] *E. coli* levels and the percentage of *E. coli* isolates resistant to various antibiotics

Stormwater Sample Sites	Range of *E. coli* Levels (CFU/100 ml) 1st Flush	2nd Flush	% Ampicillin Resistance[2]	% Kanamycin Resistance[2]	% Nalidixic Acid Resistance[2]	% Tetracycline Resistance[2]	*Bacteroides* Human Specific Species/# of samples tested 1st	2nd
SWMI01	800-5,000	<10-30,000	<50	9	5	1	0/2	0/2
SWMI02	25-7,000	3-200	6	3	4	2	0/2	0/2
SWFR03	100-17,750	7-66,000	12	5	1	2	1/5	1/4
SWMI04	1,200-380,000	1,490-12,780	43	7	13	26	2/4	2/3
SWMI06	<10-138,500	<10-500,000	16	1	1	13	0/6	0/4
SWMI07	1,400-435,000	700-10,000	21	<0.5	10	30	2/5	2/4
SWWB09	120,00-622,700	3170-42,500	12	1	<0.5	4	3/5	2/3
SWGF10	13-21,000	12-23,000	--	--	--	--	0/3	0/3
SWNB11	90-225,000	200-250,000	11	<0.5	1	4	0/4	1/4
SWMI12	100-336,000	500-15,400	<50	2	2	12	3/6	2/4
SWWA13	350-575,000	1970-1,200,000	20	4	1	10	1/3	1/3
SWMI15	100-3,100	70-2,700	9	0	0	9	1/5	1/5
SWMI16	<10-66,125	5380-20,000	26	7	3	6	1/4	0/2
SWWA17	12,000-870,000	1100-2,570,000	8	0	0	2	2/4	1/5
SWMI18	3,600-350,000	840-32,600	48	8	7	2	4/5	2/3
SWMI19	<10	46,000	--	--	--	--	0/1	0/1
SWWA20	1,050-39,000	950-18,000	--	--	--	--	0/1	1/1
Bradford Beach[3]	636-27,080	--	24	1	2	5	0/12	--
Honey Creek[4]	<10-60,000	--	2	0	0	<0.5	5/5	--
Underwood Ck[4]	100-55,750	--	24	<0.5	0	3	3/4	--
Menomonee[4]	350-80,000	--	--	--	--	--	2/4	--
Kinnickinnic[4]	30-16,700	--	--	--	--	--	2/4	--
Sewage	250,000		54	12	14	28	100% positive	
Hosts- no antibiotic exposure	--	--	7	2	1	9	100% negative	

-- No data

[1] Antibiotic resistance results were based on data collected and analyzed in 2003 and 2004.

[2] Antibiotics concentrations: ampicillin (40 μg/ml), kanamycin (50 μg/ml), nalidixic acid (25 μg/ml), and tetracycline (25 μg/ml).

[3] Stormwater samples collected during 2004 to 2005 from outfalls 1-7 located along the western edge of the Bradford Beach, spanning the entire length of the beach, BBO1 at the southernmost point and BBO7 at the northernmost point. The antibiotic results were exclusively from Bradford Beach Outfall #7.

[4] Stormwater samples were collected from multiple discharge points along Underwood Creek, Honey Creek, Menomonee River, and Kinnickinnic River (For complete list of sample sites see Appendix I).

4.5 *Diversity of E. coli from Hosts and Environmental Water Samples*

Bacteria display a very high genetic diversity in their DNA, much more so than plants or animals. For example, there is more DNA homology (e.g. matching sequences) between ants and elephants than two strains of *E. coli*. Some past research suggests that while there is a great deal of diversity, there may be similarities among *E. coli* strains within the same ecological niche, such as in the gastrointestinal track of humans as compared with gulls or cows. This hypothesis was tested by DNA fingerprinting of a large number of strains from sewage, gulls, cows, and other host sources (Table 9). For comparison, DNA fingerprinting also was performed on *E. coli* isolated from stormwater outfalls, beaches, rivers, and Lake Michigan.

Overall, *E. coli*, collected directly from animals and sewage (which is representative of the human population) were found to have a great deal of diversity. For example, of 490 sewage strains tested, 202, or 41% of the strains tested, were found to have unique fingerprints and were unrelated to any other stains found in the sewage (Figure 9). Additional strain types having the same fingerprint were found in very limited numbers. Gulls were found to have the highest amount of diversity, where 50% of the strains tested were a unique pattern. This means that for every new strain analyzed from gulls, approximately half of them were found to be unlike any other strain from

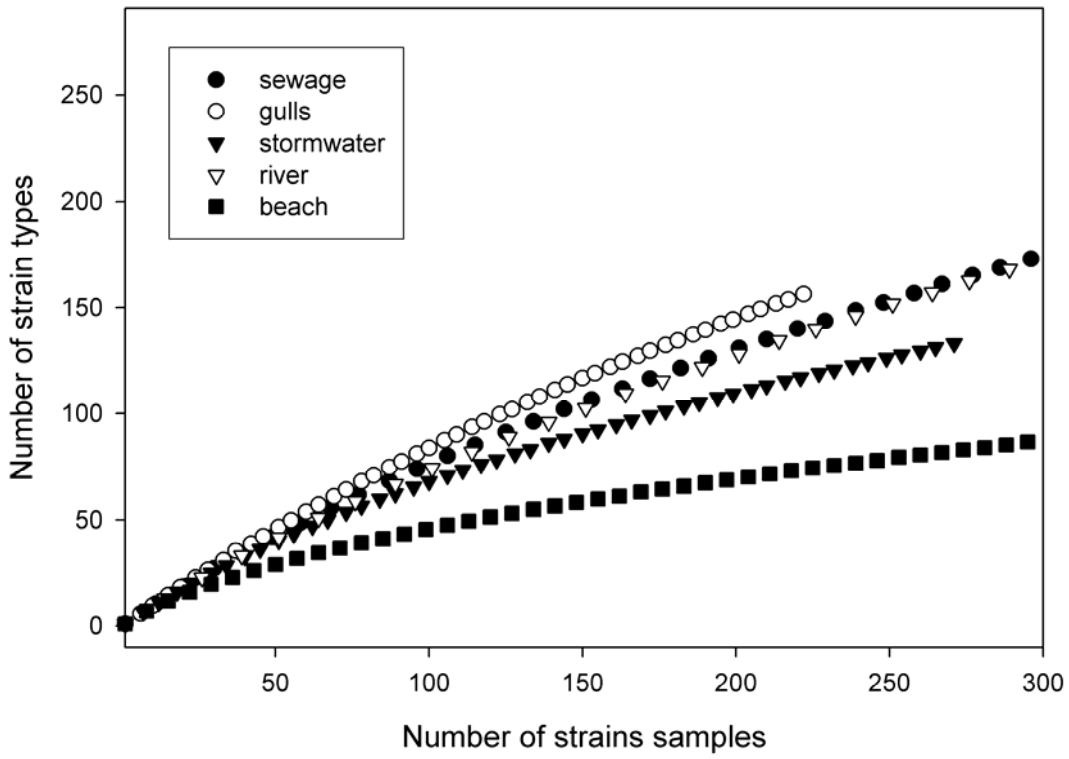

Figure 9. Assessment of the sampling saturation of possible *E. coli* strains from host and environmental groups. A rarefaction curve was generated form iterative sampling to determine the abundance of strain types found in each group (gulls, sewage, stormwater, river, or beach) for the number of strains sampled. Third order regression lines were found to have an $r^2 \geq 0.99$ for all five series. This means there is more diversity among *E. coli* isolates in gulls and sewage than in beach water.

gulls previously characterized. Overall, the high diversity does not make it feasible to discern general patterns in genetic relationships among strains that can be extrapolated to a large geographical area. Despite this, “micro” associations were found, e.g. highly similar strains from gulls at the same beach, or a subset of highly similar strains that were found consistently in sewage and no other source.

The analysis of environmental strains gave unexpected results. It was anticipated that the stormwater *E. coli* isolates would display broadly diverse rep-PCR fingerprint profiles reflecting the diffuse nature of the bacterial contamination in urban runoff. However, the diversity among strains isolated directly from stormwater was considerably less than that found in strains isolated from a particular host source (McLellan, 2004). Even less diversity was found in river water and beach water (Figure 9). This may indicate that interrelationships among strains is not primarily host dependent since there was a high amount of similarity among strains from stormwater, which is expected to carry fecal pollution from many different sources. Identical strains may indicate possible clonal propagation, and this could account for some of the low diversity. However, many of the identical strains were found at different sites, or on different days. Alternatively, and the most likely explanation, the strains that were detected may be a product of selective die-off and may represent a limited range of persistent strains that can be isolated in the environment.

DNA fingerprinting may not be a cost-feasible methodology to identify and quantify fecal pollution sources given the extensive diversity and under-characterized genetic structure of the natural *E. coli* population. These types of analyses offer valuable insight into the potential to create persistent residual populations that may confound recreational water testing. However, this approach is useful in understanding the ecology of *E. coli* in the secondary environment (e.g. surface waters) outside the host. Replication of cells outside the host or persistence of residual strain types interferes with routine beach monitoring as growth of indicator bacteria in the environment would essentially cause a site to appear to have more pollution than what is actually released at the site. This may occur under specific conditions, such as shallow protected waters (McLellan *et al*., 2001) or in the sand environment (Beversdorf et al., ; Kinzelman et al., 2004).

Table 11. Diversity and relative abundance of *E. coli* strains found in hosts and environmental water samples

	No. of isolates (n=2315)	Source	
		Type of sample[1]	Geographical and temporal sample distribution
Sewage	n=490	wastewater treatment plant influent from two treatment plants	25 flow weighted samples from metropolitan Milwaukee over 27 months[2]
Gull	n=230	fecal samples collected from beach sites	7 beach sites on Lake Michigan Southwestern shore, 35 collection days over 27 months[3]
Cattle	n=103	100 ml samples from feed lot detention systems	4 farm sites in Southwestern Wisconsin; two collection days per site
Stormwater	n=295	inline, flow weighted samples from stormwater conveyance system	stormwater system in metropolitan Milwaukee, discharges to two major rivers that drain to Lake Michigan
River water with stormwater[4]	n=513	flow weighted samples during storm events (two sites), or 1 L grab samples in triplicate for each site (transect of ten sites)	12 river sites on two major tributaries that drain to Lake Michigan; 15 collection days over 24 months
River water CSO[5]	n=134	flow weighted samples during storm events (one site), or 1 L grab samples in triplicate for each site (transect of four sites)	5 sites within the combined sewer system collected during 3 separate CSO events over a 24 month period
Beach water	n=353	1 L grab sample in triplicate for each site	2 Lake Michigan beaches in metro Milwaukee, 5-10 sites per beach, 15 collection days
Gull pond	n=23	1 L grab sample in triplicate	stormwater detention pond located near a landfill site in metro Milwaukee

[1] One isolate per fecal sample was used for individual host animals; multiple samples were taken at each cattle feedlot detention system.

[2] Twenty sewage isolates collected in the UK sanitary sewage conveyance system were also analyzed.

[3] Approximately 75% of the gull isolates were collected from three of the sites within a five-mile radius in the Metro Milwaukee area; two of these sites were used to collect beach water samples.

[4] Isolates were collected from river water that received stormwater discharges and no reported sanitary discharges.

[5] Isolates were collected from river water sites during a combined sewer overflow (CSO).

4.6 *Total & Human Specific Bacteroides spp. in Environmental Water Samples*

The prevalence of *E. coli* was assessed along with total, human and cow specific *Bacteroides* genetic markers in surface waters of Lake Michigan impacted by multiple pollution sources following major storm events. Detection of total, human specific, and cow specific *Bacteroides* found in surface waters were linked to the detection of *E. coli* by PCR and the abundance of *E. coli* measured by culture based methods.

The detection of specific markers compared to culturable *E. coli* levels across sites over the 60 day sample period are shown in Table 10. Conductivity measurements approximate the amount of (contaminated) river water in relation to (uncontaminated) lake water and the decrease in conductivity across sample locations indicates the amount of dilution. The rivers in this system, following precipitation events, have been found to range from 0.560 to 0.690 mS/cm, while Lake Michigan with no river water influences is typically measured as 0.281 to 0.223 mS/cm. All samples were positive for total *Bacteroides* spp. genetic markers, even in the absence of other evidence of fecal pollution, e.g. detectable river water or culturable *E. coli*. Other genetic markers (human and cow specific) were variable in relation to the amount of river water and culturable *E. coli*.

CSO/SSO

River water samples collected at three separate locations during an SSO event demonstrated the highest levels of *E. coli*; both *Bacteroides* spp. and human specific *Bacteroides* were detected (Table 12). Interestingly, one site located in the suburban part of the watershed showed the presence of cow specific *Bacteroides*; however, it could not be determined if this signal originated from the SSO discharge or from the river which receives runoff from stormwater outfalls located throughout the area.

During periods of heavy rain and subsequent SSO/CSO events, upstream river water from the agricultural land use in the basin would be expected to mix with urban stormwater and sewage discharge. Interestingly, the cow specific genetic marker was detected following two major rain events in most samples within the harbor, but this marker was no longer detectable once water was discharged outside the harbor to Lake Michigan, even though *E. coli* levels remained elevated and the human specific marker could still be detected. This would suggest that the cow specific marker is at lower concentrations than the human specific marker and that agricultural runoff presumably does not contribute to the majority of fecal pollution levels (Table 12).

During the SSO/CSO events, human specific markers were detected at sites in nearshore Lake Michigan with > 200 CFU/100 ml of *E. coli*. *E. coli* levels at distances of more than 2 km from the harbor contained < 200 CFU/100 ml *E. coli*; these samples were not analyzed for genetic markers except for those samples collected near South Shore Beach. Notably, four beach samples collected during the SSO/CSO events (two at South Shore Beach and two 100 m from shore of South Shore Beach, Table 13) and eight samples collected in the days that followed the SSO/CSO (Table 12) were found to have *E. coli* levels below the USEPA recommended limit of 235 CFU/100 ml for recreational water, but were positive for the human specific *Bacteroides* genetic marker. Because these samples contained known sewage contamination, these results demonstrated that the human specific genetic markers are either more sensitive, and/or persist longer than culturable *E. coli*.

Human specific *Bacteroides* genetic markers were detected 9 days post CSO, but not 7 days post CSO; within this same time frame 0.2 inches of rainfall occurred, contributing additional stormwater to nearshore Lake Michigan. Likewise, the human specific genetic marker was detected >60 days post CSO following a rain event of 0.3 inches. These findings suggest that human source of fecal pollution may not be limited to reported sewage overflows, but that unrecognized sanitary sewage inputs, e.g. leaking sewer line or cross connections, may also contribute to contamination in Lake Michigan.

Importantly, the human specific genetic marker was detected at beaches during a sewage overflow when *E. coli* was 110 -170 CFU/100 ml, below the recommended limit for *E. coli* in recreational water. This illustrates how information as to the source of pollution might protect public health more effectively than the reliance on standard measures of fecal indicators.

Stormwater

Over stormwater samples were analyzed for evidence of sanitary sewage contamination by testing for the *Bacteroides* human specific genetic marker (Table 10). Based on these analyses, approximately 34% of 1st flush and 30% of 2nd flush stormwater samples tested positive for the *Bacteroides* human specific marker. Overall, 22 of 56 stormwater outfalls tested showed evidence of human sources of fecal pollution in one or more samples. These results suggest that in the Milwaukee River Drainage Basin sanitary sewage is present in a significant portion of the stormwater system and may be entering the systems through leaking pipes or cross connections.

In general, the antibiotic resistance patterns, indicating potential sanitary sewage contamination, corresponded with outfall locations that also were found to be positive for human specific *Bacteroides* genetic marker (Table 10). The consistent findings between two independent source tracking methods further illustrate the need for a more extensive assessment of urban stormwater system.

Perhaps noteworthy, is that two stormwater outfalls which discharged within close proximity to beach sites demonstrated evidence of human sources of contamination. Results from SWMI04 showed both an increase in antibiotic resistance frequencies of *E. coli* isolates and positive PCR results for human specific *Bacteroides* marker, suggesting that sanitary inputs may be the major contributor to overall *E. coli* levels detected in this outfall (1,200 to 380,000 CFU/100 ml). On the other hand, SWWB09 did not demonstrate elevated antibiotic resistance frequencies of *E. coli* isolates with *E. coli* counts of (3,170 to 622,700 CFU/100 ml), however, did produce positive PCR results for the human specific *Bacteroides* marker, which suggests that sanitary inputs may be present, but does not account for the majority of the *E. coli* levels. (Refer to Table 10.)

Table 12. Occurrence of host specific markers in Lake Michigan following contamination with combined sewer overflows, sanitary sewage overflows, and river basin drainage containing urban and agricultural runoff.

Event Sample date	Region[1]	Specific Conductivity[2] mS/cm	*E. coli* CFU/100 ml Mean (range)	Number of samples positive by PCR/ Number of samples tested			
				E. coli	*Bacteroides* spp.[5]	Human *Bacteroides*	Cow *Bacteroides*
SSO	River sites (below outfall)	Not measured	11,500 (7,000-20,000)	3/3	3/3	3/3	1/3
SSO/CSO[3]	CSO outfall	0.445	49,000	1/1	1/1	1/1	0/1
	KK River[4]	0.532-0.560	210, 2245	2/2	2/2	2/2	0/2
	Channel	0.430-0.521	3370 (1140-5500)	16/16	16/16	16/16	13/16
	Harbor	0.283-0.517	4450 (590-13,000)	23/23	23/23	23/23	18/23
	Lake Michigan 0.5-2.0 km from harbor	0.282-0.335	1400 (220-3300)	10/10	10/10	9/10	7/10
1 day post CSO	Harbor	0.550-0.589	210, 270	2/2	2/2	1/2	0/2
	Lake Michigan 0.5-2.0 km from harbor	0.403-0.557	132, 173	2/2	2/2	2/2	0/2
	Lake Michigan 3.0-6.0 km from harbor	0.229-0.237	15, 18	0/2	2/2	0/2	0/2
	Lake Michigan 8.0 km from harbor	0.223 (lake water)	7, 9	0/2	2/2	0/2	0/2
3 days post CSO	Channel	0.435	55	1/1	1/1	1/1	0/1
7 days-post CSO	Harbor (at main gap)	Not measured	80	0/1	1/1	0/1	0/1
9 days post CSO	Channel	Not measured	105	1/1	1/1	1/1	1/1
	Harbor (at main gap)	Not measured	120	1/1	1/1	1/1	1/1

Event Sample date	Region[1]	Specific Conductivity[2] mS/cm	*E. coli* CFU/100 ml Mean (range)	Number of samples positive by PCR/ Number of samples tested			
				E. coli	*Bacteroides* spp.	Human *Bacteroides*	Cow *Bacteroides*
23 days post CSO	Channel	0.500	300	0/1	1/1	0/1	0/1
	Harbor (at main gap)	0.365	920	1/1	1/1	0/1	0/1
	Lake Michigan 3.0-6.0 km from harbor	0.295-0.330	8, 16	0/2	2/2	0/2	0/2
>60 days post CSO	Harbor	0.636-0.679	27, 35	2/2	2/2	2/2	0/2
	Lake Michigan 0.5-2.0 km from harbor	0.325	6	1/1	1/1	0/1	0/1

[1] Study area is detailed in Figure 1.

[2] The specific conductivity of river water from the Milwaukee River basin after rain events typically has been found to range from 0.560 to 0.690 mS/cm. Lake Michigan (with no river water influences) has been found to be 0.281 to 0.223 mS/cm.

[3] Samples containing <1000 *E. coli* from the Lake Michigan nearshore sites outside of the harbor were not analyzed for genetic markers on one of the sample days, with the exception of samples collected 100 m from shore of as part of beach sampling conducted during the CSO (data shown in Table 11).

[4] The Kinnickinnic (KK) River drains an urban watershed and is expected to contain no agricultural runoff.

[5] Samples were cloned and sequenced for identification of *Bacteroides* spp.

Table 13. Occurrence of fecal indicator bacteria genetic markers in beach water samples at two urban beaches on Lake Michigan.

Description	Type of Sample	Sample days[1]	*E. coli* CFU/100 ml	Number of samples positive by PCR/ Number of samples tested			
				E. coli	***Bacteroides* spp.**	**Human *Bacteroides***	**Cow *Bacteroides***
Beach site 1 Urban beach	beach water	n=3	500-5400	2/2	2/2	1/2*	0/2
	0.5 km from shore		8, 16	0/2	2/2	0/2	0/2
	outfalls above beach[2]		460-15,900	9/9	9/9	8/9*	0/9
	surface parking lot runoff		2500-3900	2/2	2/2	0/2	0/2
Beach site 2 Urban beach	beach water >235 CFU/100 ml	CSO n=2 no CSO (n=3)	587-4500	4/4	4/4	4/4*	0/4
	beach water <235 CFU/100 ml		110-170	2/2	2/2	2/2	0/2
	100 m from shore		150-1500	5/5	5/5	5/5*	0/5
	outfall 0.5 km from beach[3]		49,000 (CSO) 1190-8700	4/4	4/4	3/4*	0/3
	gull roosting site		4400	1/1	1/1	0/1	0/1
	surface parking lot runoff [4]		300-50,000	1/1	1/1	0/1	0/1

[1] Samples were collected within 24 hours of rain events; samples taken during combined sewer overflow events are indicated as CSO. Not all sites were sampled each survey. Multiple samples were collected on a single day.

[2] Outfalls that discharge to the beach are designed to convey only stormwater

[3] An outfall located 0.5 km from the beach site is designed to discharge combined sewage during heavy rains and only stormwater when no CSO events are occurring.

[4] Only one of the sample dates was analyzed for *Bacteroides* and *E. coli* genetic markers.

* Positive human specific *Bacteroides* genetic markers in outfall and beach samples occurred on the same sample date.

4.7 *Caffeine Method Development*

Caffeine methods using LC/MSD (Liquid Chromatograph/Mass Selective Detector) are under development. WATER is currently working on running standards and developing a solid phase extraction. Results are pending at this time due to unforeseen complications with the extraction portion of LC/MSD method, specifically relating to the need for additional clean up methods following the initial extraction to get a clear signal on the LC/MSD. Methods for caffeine analysis in complex water samples will continue to be developed so that this approach may be used in conjunction with biological analysis to estimate the human contribution to contaminants in stormwater run off.

4.8 *Pathogen Detection in River and Harbor Water Samples*

Pathogen assessments provide direct evidence as to health risk. Human sources of fecal contamination are known to carry human pathogens; however, little is known about the types of pathogens and ultimately, the health risk that is associated with non-point sources of fecal pollution in urban stormwater. Pathogen research was focused on one bacterial pathogen, *Salmonella*, and two protozoan pathogens, *Giardia* and *Cryptosporidium,* as all three have been found previously in the Milwaukee River Drainage Basin. Evaluation of urban stormwater for pathogens is complicated by the possibility of sanitary sewage contamination of the system, either through leaking sewer pipes or illicit cross connections. As these methods are developed in the Milwaukee River system, different treatment strategies (e.g. floodplains/detention systems) could also be assessed for efficacy of reducing not just indicator organism levels, but actual pathogen occurrence.

PCR protocols were developed and validated by initially analyzing sewage treatment plant influent, and samples spiked with known concentrations of bacteria (*Salmonella*) or DNA (for *Giardia* and *Cryptosporidium*). Sewage control samples demonstrated a strong signal for *Giardia*. It was initially anticipated that it would be difficult to detect *Giardia* because of the genetic heterogeneity in strains; however, the methodology employed using the giardian gene target, was able to detect *Giardia* in a 1:100 dilution of the sewage sample. *Cryptosporidium* was detected in 3 of 6 sewage samples, but the signal was weaker and lost at dilutions of 1:10 or 1:100.

Samples collected during the May 2004 CSO events were used to assess loading of pathogens into the harbor during storm events. Both *Giardia* and *Cryptosporidium* were negative for 31 samples collected in the confluence of the Milwaukee and Menomonee Rivers. Experiments were then conducted to determine if inhibitors were present in the environmental samples. Spiking experiments demonstrated that the limit of detection for *Giardia* in environmental samples by this method was 0.01-0.001 ng/sample genomic DNA, which is equivalent to approximately 100-1000 cells. Similarly, *Cryptosporidium* could be detected at levels of 0.01 ng/sample genomic DNA. Further work is focused on (1) improving the filtration methods to increase the volumes of water (from 1 L to 50 L), (2) improving DNA isolation methods in environmental samples to remove inhibitors, and (3) the implementation of internal controls to normalize and quantify PCR results.

Results from the Milwaukee Health Department from 2004 have shown that high levels of *Giardia* can be detected in grab samples of blended effluent (approximately 8 to 500 cells per L). *Cryptosporidium* analyses showed levels two orders of magnitude lower (<1 cell per liter). These findings demonstrate that *Giardia* rather than *Cryptosporidium* might be a better protozoan target for tracking the fate of protozoan pathogens in the harbor. Since these samples were taken as “worse case scenarios”, e.g. during blending events, environmental

water samples in the harbor would be expected to be at lower concentrations, again suggesting that more sophisticated filtration methods need to be developed for determining actual pathogen load in this system. These studies primarily focused on bacterial pathogens, and two protozoa; however, viruses constitute an equal if not greater health risk. As part of future initiatives and efforts to evaluate pathogens loads, viruses should be included.

Further, in order to evaluate the pathogen load into nearshore Lake Michigan and track the die-off of specific viruses and protozoan, integrated samplings will be necessary. One possible mechanism to collect these samples is to utilize the USGS station (currently non-operational). This would allow us to correlate pathogen loads with *E. coli* levels and extrapolate pathogen distribution estimates using the bacterial transport model. Current collaborations involve similar sampling strategies in partnership with Steve Corsi, USGS, and Dr. Mark Borchart, Marshfield Clinic (specializing in clinical and environmental virology), as part of an Oceans and Health grant funded by NOAA.

Initial work to identify pathogens on floodplains demonstrated negative results for all three pathogens. Further work is continuing to evaluate solid matrices (soils and sediments) for pathogens. Methods have been adapted to analyze sediments and soils; however, further optimization as outlined above is necessary to distinguish truly negative samples from those inhibited by the sample matrices. In 2006 (spring) two storm events will be sampled in a representative detention system. Screening source water by PCR for the pathogens listed above will also be performed.

5.0 CONCLUSIONS

- CSOs and SSOs contribute high loads of *E. coli* and other indicator organisms to local receiving waters. The proportion of human sources compared with animal sources (derived from both urban and agricultural non-point source runoff), remains difficult to quantify. Spatial surveys clearly demonstrate that the levels of indicator organisms are an order of magnitude higher in the harbor following severe rain events accompanied by sewer overflows compared with rain events of <2 inches of precipitation, however the proportion of human sources compared with animal sources appears to be the same (as indicated by antibiotic resistance testing). It is important to note that these findings suggest the total amount of human sources of fecal pollution was found to increase during sewage overflows, but the non-point sources also increased on an equal scale. These results indicate that there may be unrecognized sanitary sewage inputs into stormwater systems, and ultimately receiving waters, during all rainfall events.

- Stormwater is a major contributor of *E. coli* and one of the leading sources of water quality impairment in the U.S. today. Over 13% of the outfalls tested demonstrated *E. coli* levels of 100,000 CFU/100 ml or greater. Of the outfalls tested for possible human sources using *Bacteriodes,* 22 of 56 (approximately 39%) tested positive. In over half of the outfalls testing positive for the human specific marker, antibiotic resistance testing would suggest that human sources were not the major contributor to the *E. coli* levels. These findings highlight the need to better scrutinize the causes of elevated fecal indicator bacteria in urban stormwater.

- It is very difficult to distinguish between urban stormwater pollution and CSO/SSO discharge because both often occur simultaneously, and severe rainfalls greatly increase the amount of contaminated stormwater entering these systems. In order to further delineate the relative proportions of stormwater contamination versus CSO/SSO, better quantitative assessments are needed in the upstream regions to account for unrecognized sanitary inputs into the watershed. The development of quantitative molecular methods is necessary to track human and non-human signals during different types of storm events. In addition, quantitative methods will be crucial for targeting and prioritizing outfalls that demonstrate sanitary sewage contamination.

- During a CSO/SSO, *E. coli* levels fall below 235 *E. coli*/100 ml once the pollution plume disperses outside the harbor breakwall. The highest levels of *E. coli* were detected in the channel/estuary, harbor, and at stormwater outfalls. Bacteria levels are diluted out of the water column once the polluted plume mixes with the lake water. Previous studies have suggested that the breakwall acts as a protective barrier, preventing the first flush of the storm from mixing with the lake waters by containing the plume within the harbor breakwall (allowing bacteria levels to die-off) before moving out into the open waters of Lake Michigan. Because *E. coli* does not survive well in Lake Michigan, further development and utilization of a hydrodynamic model will be very important when evaluating the ultimate distribution of biological pollutants in Lake Michigan.

- Fecal coliforms are a better indicator organism than *E. coli* in Lake Michigan; however both organisms are relatively short lived in Lake Michigan and therefore poor indicators of pathogens such as *Giardia* and *Cryptosporidium* which pose serious health risks. More sensitive measures are necessary to determine human health risk from pathogens associated with CSOs and stormwater runoff into Lake Michigan.

- Source tracking methods are useful in determining host sources of bacteria; however, one method is not capable of identifying all the host sources which might contribute to pollution. It is more effective to combine a variety of source tracking techniques to get a clearer picture of what is occurring in the watershed.

- Due to the complications of sample extraction procedures, utilizing caffeine as a source tracking method is still being developed. In lieu of this procedure, the technique, *Bacteroides* spp. and human specific *Bacteroides* PCR, was developed to detect the human specific signal of fecal pollution in environmental water samples.

- *Bacteroides* human specific and cow specific genetic markers appear to be both sensitive and reliable in detecting human and agricultural sources of fecal pollution. However, these markers provide only presence/absence information. Methods for quantification of the human specific marker are recommended so that sites that test positive can be prioritized as to the magnitude of the problem, e.g. sanitary sewage as a minor contributor or major contributor to fecal pollution detected at the site.

- Ultimately, pathogens need to be measured directly in order to determine the pathogen loads entering Lake Michigan. This initial work focused on *Giardia* and *Cryptosporidium* methods; however, viruses will be an important group of pathogens to monitor.

6.0 FUTURE RESEARCH DIRECTIONS

Based on the results of this research, we recommend the following future research priorities:

- Quantify and understand the impacts of stormwater runoff on water quality. While it is imperative to continue efforts to reduce and/or prevent CSOs and SSOs from occurring, it is of equal importance to determine the proportion of bacterial contamination in the nearshore waters of Lake Michigan that originated from stormwater as opposed to CSOs.

- Identify stormwater outfalls within the Milwaukee River Drainage Basin that are discharging elevated levels of bacterial contamination into the stormwater system, and subsequently determine if the origin of the pollution is from primarily human or non-human sources.

- Assess stormwater outfalls for human viruses. Since stormwater is assumed to be derived from non-point sources (e.g. non-human sources) of fecal pollution, stormwater is assumed to be less of a health risk than sanitary sewage contamination. However, our findings demonstrate that human sources, and therefore human pathogens, may be present in urban stormwater.

- Develop more sensitive methods for detecting sources of sewage contamination because the traditional indicator organisms such as *E. coli* most likely have a poor relationship to protozoan and viral pathogens in Lake Michigan. Integration of source detection data into hydrodynamic modeling will be crucial in characterizing the ultimate fate of CSOs, stormwater, and pathogens that are carried with this pollution. Also, it is essential to expand upon and refine the methods for direct pathogen detection in environmental water samples.

7.0 PUBLIC COMMUNICATIONS

A list of speaking engagements, interactions, and interviews which presented this research to the public is listed in Appendix VI. In addition, current research and up-to-date results from these studies may be found http://www.uwm.edu/Dept/GLWI/ecoli/.

8.0 ACKNOWLEDGMENTS

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Patricia Bower, Research Specialist
Ola Olapade, Postdoctoral Scientist
Alissa Salmore, Assistant Researcher
Annette Daniels, Assistant Researcher
Marcia da Silva, Graduate Student
Caitlin Carlson, Project Assistant
Morgan Depas, Project Assistant
Beth Harris, Project Assistant
Josh Harris, Project Assistant
Caitlin Scopel, Project Assistant
Magnolia Tulod, Project Assistant
Meredith Van Dyke, Project Assistant

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APPENDIX I

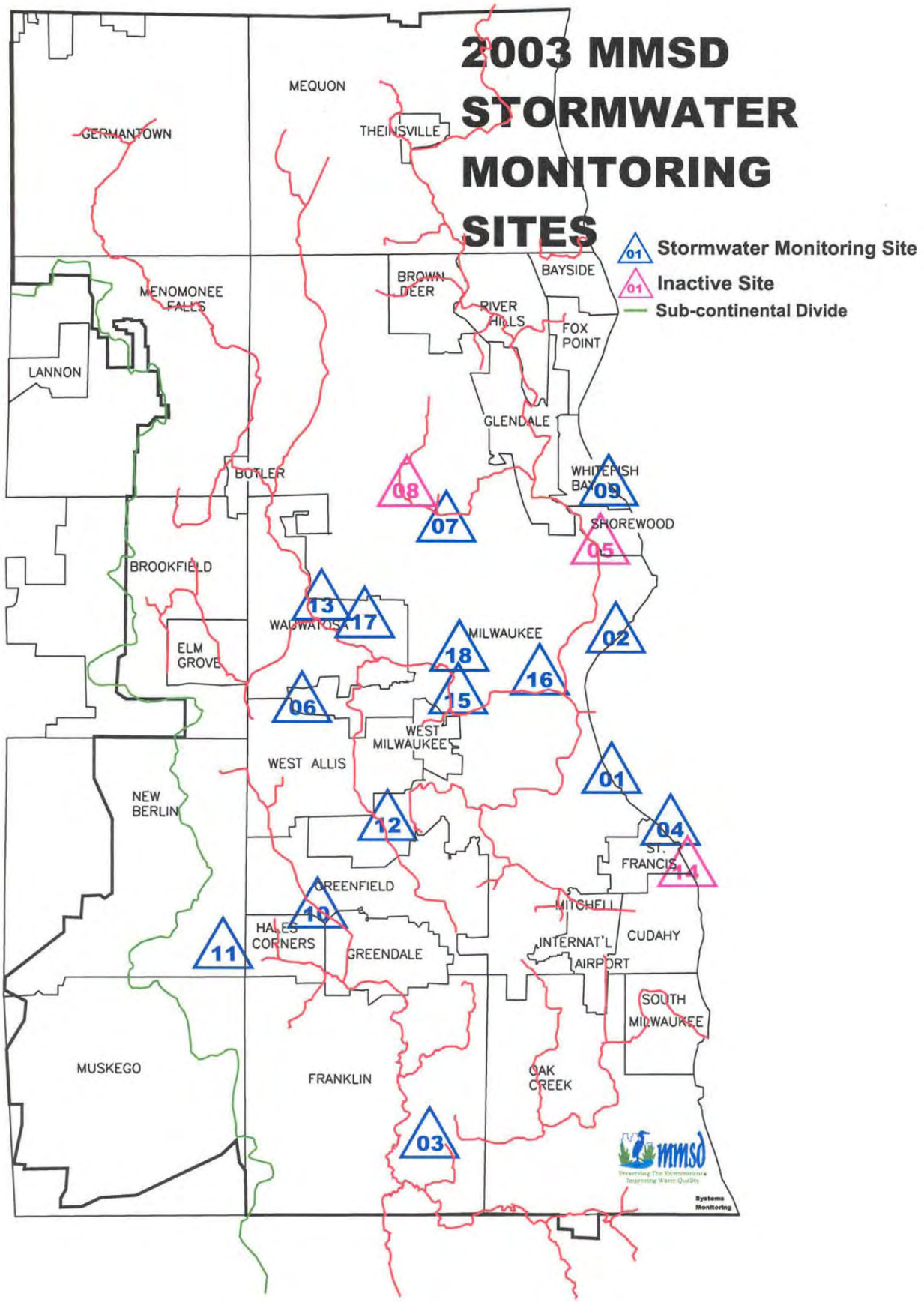
Stormwater Sites and Locations

(2003 – 2005)

2003 MMSD STORMWATER MONITORING SITES*

MAP ID	SITE ID	LOCATION	COMMUNITY	Receiving Water
01	SWMI01	LINCOLN MEMORIAL DR. AND CARFERRY DR. (Stormwater discharge to Lake Michigan)	Milwaukee	Lake Michigan
02	SWMI02	1700 N. LINCOLN MEMORIAL DR. @ LAFAYETTE HILL RD. (Stormwater to Lake @ McKinley Marina)	Milwaukee	Lake Michigan
03	SWFR03	54TH AND ASHLAND (Stormwater to Franklin Park to detention pond)	Franklin	Root River
04	SWMI04	3500 S. LAKE DR. @ BAY VIEW PARK (Stormwater to Lake across from St. Francis Seminary)	Milwaukee	Lake Michigan
05	SWMI05	1200 E. SINGER CIR. (Stormwater to Milw. River @ Kern Park) **INACTIVE IN 2003**	Milwaukee	Milwaukee River
06	SWMI06	MILW CNTY. ZOO (Stormwater to Underwood Creek across from Moose Encl.)	Milwaukee	Underwood Creek
07	SWMI07	4345 N. 47TH ST. (Stormwater to Lincoln Creek)	Milwaukee	Lincoln Creek
08	SWMI08	HAMPTON AND LINCOLN CR. PARKWAY (Stormwater to Lincoln Creek under bridge) **INACTIVE SINCE 2002**	Milwaukee	Lincoln Creek
09	SWWB09	4939 N. NEWHALL (Stormwater to Lake @ Big Bay Park)	Whitefish Bay	Lake Michigan
10	SWGF10	BOERNER BOTANICAL GARDENS FORMERLY 10007 W. MEADOW DR. (Stormwater to Root River)	Greenfield	Detention Pond
11	SWNB11	13380 EAGLE TRACE AND TIMBER RIDGE (Stormwater to wetland residential site)	New Berlin	Detention Pond
12	SWMI12	3275 S. 72ND ST. (Stormwater to Honey Creek)	Milwaukee	Honey Creek
13	SWWA13	RIDGE BLVD. AND HARDING BLVD. (Stormwater to Menomonee River Parkway)	Wauwatosa	Menomonee River
14	SWSF14	LAKE DR. AND TESCH AVE. (Stormwater to Lake Michigan) **INACTIVE IN 2003**	St. Francis	Lake Michigan
15	SWMI15	42ND AND MT. VERNON (I-94 x-way Stormwater to Menomonee River)	Milwaukee	Menomonee River
16	SWMI16	MARQUETTE INTERCHANGE	Milwaukee	Menomonee River
17	SWWA17	71ST AND CHESTNUT ST. (Stormwater to Menomonee River)	Wauwatosa	Menomonee River
18	SWMI18	MILLER PARK EAST PARKING LOT AT THE SAUSAGE HOUSE (Stormwater to Menomonee River)	Milwaukee	Menomonee River

***The Stormwater Monitoring Program started in 2000. A maximum of fifteen sites are sampled annually.**


2003 MMSD STORMWATER MONITORING SITES
01 Stormwater Monitoring Site
01 Inactive Site
Sub-continental Divide
MEQUON
GERMANTOWN
THIENSVILLE
BAYSIDE
BROWN DEER
MENOMONEE FALLS
RIVER HILLS
FOX POINT
LANNON
GLENDALE
BUTLER
WHITEFISH BAY
SHOREWOOD
BROOKFIELD
WAUWATOSA
MILWAUKEE
ELM GROVE
WEST MILWAUKEE
WEST ALLIS
NEW BERLIN
ST. FRANCIS
GREENFIELD
MITCHELL
HALES CORNERS
GREENDALE
INTERNAT'L AIRPORT
CUDAHY
SOUTH MILWAUKEE
MUSKEGO
FRANKLIN
OAK CREEK
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MMSD
Systems Monitoring

2004 MMSD STORMWATER MONITORING SITES*

MAP ID	SITE ID	LOCATION	COMMUNITY	Receiving Water
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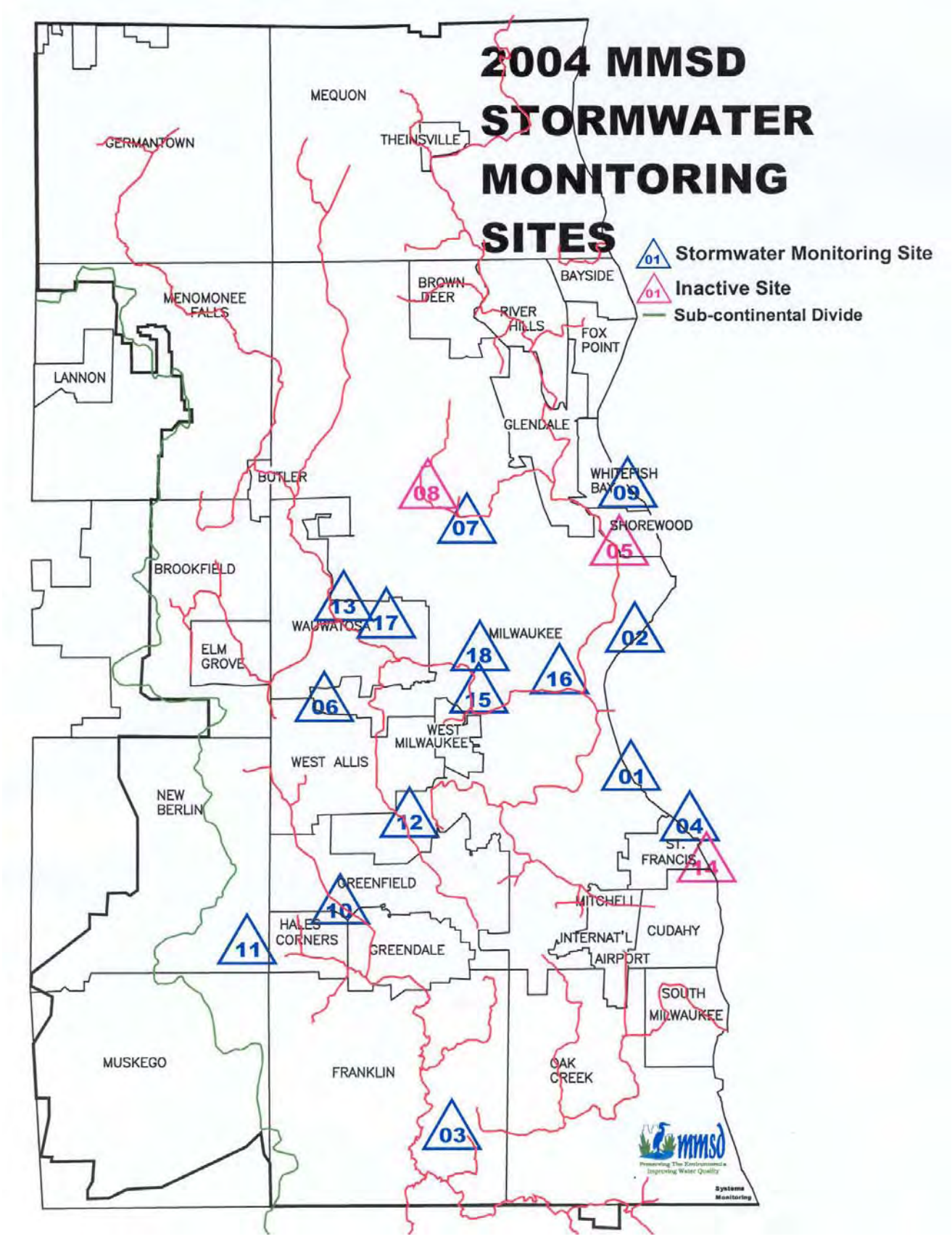
18	SWMI18	MILLER PARK EAST PARKING LOT AT THE SAUSAGE HOUSE (Stormwater to Menomonee River)	Milwaukee	Menomonee River

***The Stormwater Monitoring Program started in 2000. A maximum of fifteen sites are sampled annually.**


2004 MMSD STORMWATER MONITORING SITES
Stormwater Monitoring Site
Inactive Site
Sub-continental Divide
MEQUON
GERMANTOWN
THEINSVILLE
BAYSIDE
BROWN DEER
MENOMONEE FALLS
RIVER HILLS
FOX POINT
LANNON
GLENDALE
BUTLER
WHITEFISH BAY
SHOREWOOD
BROOKFIELD
WAUWATOSA
MILWAUKEE
ELM GROVE
WEST MILWAUKEE
WEST ALLIS
NEW BERLIN
ST. FRANCIS
GREENFIELD
MITCHELL
HALES CORNERS
GREENDALE
INTERNAT'L AIRPORT
CUDAHY
SOUTH MILWAUKEE
MUSKEGO
FRANKLIN
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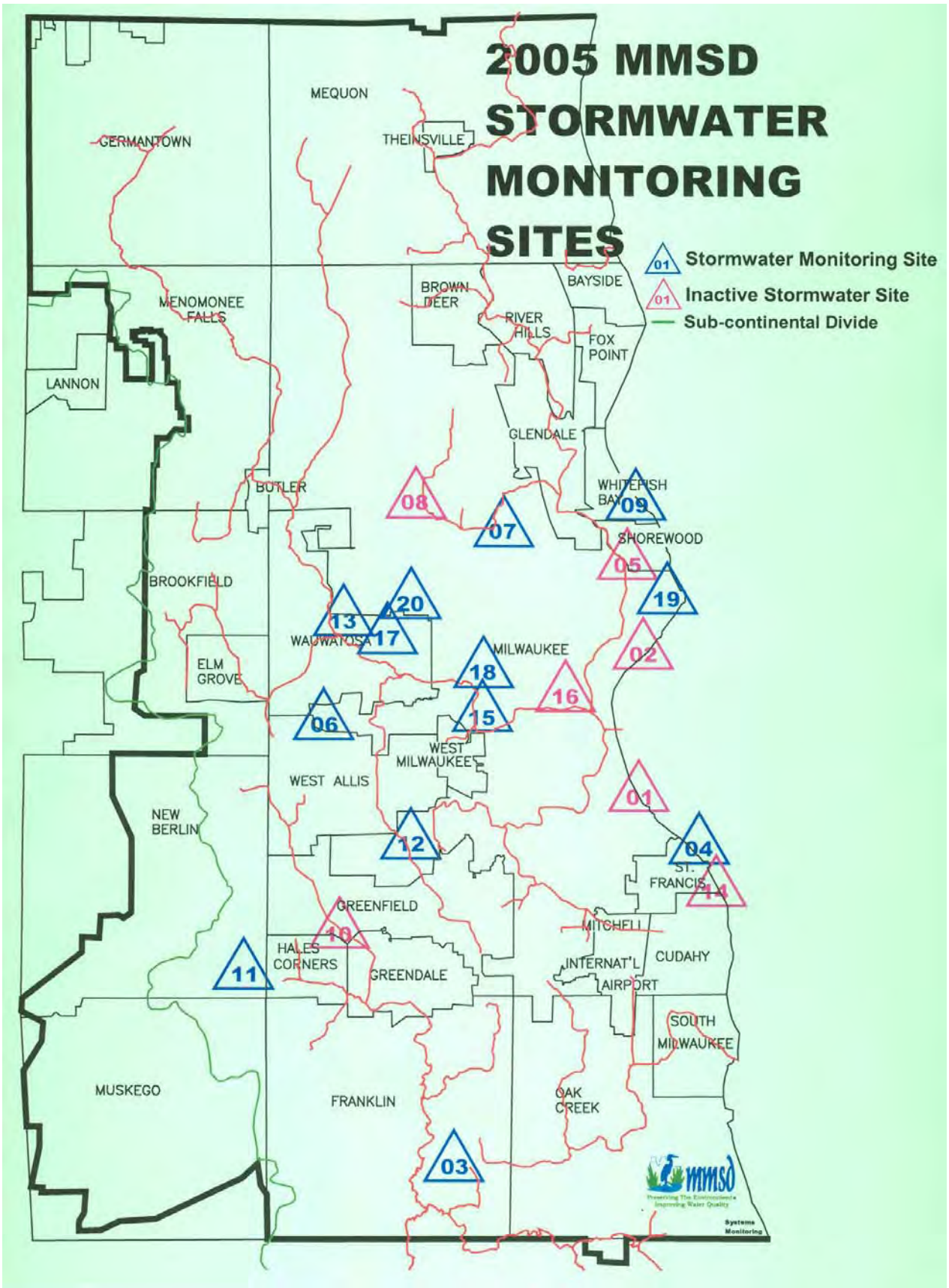
Systems Monitoring

2005 MMSD STORMWATER MONITORING SITES*

MAP ID	SITE ID	LOCATION	COMMUNITY	Receiving Water
01	SWMI01	LINCOLN MEMORIAL DR. AND CARFERRY DR. (Stormwater discharge to Lake Michigan) **INACTIVE IN 2005**	Milwaukee	Lake Michigan
02	SWMI02	1700 N. LINCOLN MEMORIAL DR. @ LAFAYETTE HILL RD. (Stormwater to Lake @ McKinley Marina) **INACTIVE IN 2005**	Milwaukee	Lake Michigan
03	SWFR03	54TH AND ASHLAND (Stormwater to Franklin Park to detention pond)	Franklin	Root River
04	SWMI04	3500 S. LAKE DR. @ BAY VIEW PARK (Stormwater to Lake across from St. Francis Seminary)	Milwaukee	Lake Michigan
05	SWMI05	1200 E. SINGER CIR. (Stormwater to Milw. River @ Kern Park) **INACTIVE IN 2003**	Milwaukee	Milwuakee River
06	SWMI06	MILW CNTY. ZOO (Stormwater to Underwood Creek across from Moose Encl.)	Milwaukee	Underwood Creek
07	SWMI07	4345 N. 47TH ST. (Stormwater to Lincoln Creek)	Milwaukee	Lincoln Creek
08	SWMI08	HAMPTON AND LINCOLN CR. PARKWAY (Stormwater to Lincoln Creek under bridge) **INACTIVE SINCE 2002**	Milwaukee	Lincoln Creek
09	SWWB09	4939 N. NEWHALL (Stormwater to Lake @ Big Bay Park)	Whitefish Bay	Lake Michigan
10	SWGF10	BOERNER BOTANICAL GARDENS FORMERLY 10007 W. MEADOW DR. (Stormwater to Root River) **INACTIVE IN 2005**	Greenfield	Detention Pond
11	SWNB11	13380 EAGLE TRACE AND TIMBER RIDGE (Stormwater to wetland residential site)	New Berlin	Detention Pond
12	SWMI12	3275 S. 72ND ST. (Stormwater to Honey Creek)	Milwaukee	Honey Creek
13	SWWA13	RIDGE BLVD. AND HARDING BLVD. (Stormwater to Menomonee River Parkway)	Wauwatosa	Menomonee River
14	SWSF14	LAKE DR. AND TESCH AVE. (Stormwater to Lake Michigan) **INACTIVE IN 2003**	St. Francis	Lake Michigan
15	SWMI15	42ND AND MT. VERNON (I-94 x-way Stormwater to Menomonee River)	Milwaukee	Menomonee River
16	SWMI16	MARQUETTE INTERCHANGE **INACTIVE IN 2005**	Milwaukee	Menomonee River
17	SWWA17	71ST AND CHESTNUT ST. (Stormwater to Menomonee River) **INACTIVE IN 2006**	Wauwatosa	Menomonee River
18	SWMI18	MILLER PARK EAST PARKING LOT AT THE SAUSAGE HOUSE (Stormwater to Menomonee River)	Milwaukee	Menomonee River
19	SWMI19	LINCOLN MEMORIAL DR. AND PICNIC POINT (Stormwater discharge to Lake Michigan)	Milwaukee	Lake Michigan
20	SWWA20	DANA CT. AND 83RD ST. EXT'D (Stormwater to Honey Creek)	Wauwatosa	Honey Creek

***The Stormwater Monitoring Program started in 2000. A maximum of fifteen sites are sampled annually.**


2005 MMSD STORMWATER MONITORING SITES
01 Stormwater Monitoring Site
01 Inactive Stormwater Site
Sub-continental Divide
MEQUON
GERMANTOWN
THEINSVILLE
BAYSIDE
BROWN DEER
MENOMONEE FALLS
RIVER HILLS
FOX POINT
LANNON
GLENDALE
BUTLER
WHITEFISH BAY
SHOREWOOD
BROOKFIELD
WAUWATOSA
MILWAUKEE
ELM GROVE
WEST MILWAUKEE
WEST ALLIS
NEW BERLIN
ST. FRANCIS
GREENFIELD
MITCHELL
HALES CORNERS
GREENDALE
INTERNAT'L AIRPORT
CUDAHY
SOUTH MILWAUKEE
MUSKEGO
FRANKLIN
OAK CREEK
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APPENDIX II

Stormwater Data
2003-2005

Stormwater Data 2003

E. coli data (CFU/100 ml) from stormwater samples collected in 2003 from MMSD automated inline stormwater samplers located throughout the MMSD service region. Precipitation data acquired from NOAA Milwaukee/Sullivan weather station (http://www.crh.noaa.gov/mkx/climate) and is listed in total inches accumulated within the 24 hour time period prior to sampling.

Date	Precipitation	Site	Flush	*E. coli*
07/15/03	0.37	SWWA17	1st	870000
07/15/03	0.37	SWWA17	2nd	2570000
07/15/03	0.37	SWM115	1st	4092
07/15/03	0.37	SWM115	2nd	2667
07/15/03	0.37	SWWA13	1st	405072
07/15/03	0.37	SWWA13	2nd	11826090
07/15/03	0.37	SWM104	1st	26480
07/15/03	0.37	SWM104	2nd	12790
07/15/03	0.37	SWWB09	1st	622720
07/15/03	0.37	SWWB09	2nd	37780
07/15/03	0.37	SWM106	1st	138540
07/15/03	0.37	SWM106	2nd	1011180
07/15/03	0.37	SWM118	1st	500000
07/15/03	0.37	SWM118	2nd	12290
07/30/03	0.01	SWNB11		16670
08/01/03	0.05	SWNB11		256670
08/06/03	0.01	SWNB11		120000
09/12/03	0.35	SWMI01	1st	6500
09/12/03	0.35	SWMI01	2nd	1830
09/12/03	0.35	SWMI04	1st	259100
09/12/03	0.35	SWMI04	2nd	25000
09/12/03	0.35	SWMI15	1st	33340
09/12/03	0.35	SWWA17	1st	113750
09/12/03	0.35	SWFR03	1st	17750
09/12/03	0.35	SWFR03	2nd	7000
09/12/03	0.35	SWM118	1st	72870
09/12/03	0.35	SWM118	2nd	500000
09/13/03	0.15	SWBB09	1st	88460
09/13/03	0.15	SWWB09	2nd	42500
09/13/03	0.15	SWMI06	1st	104110
09/13/03	0.15	SWWA13	1st	575000
09/13/03	0.15	SWWA13	2nd	56100
09/13/03	0.15	SWMI01	1st	533300
09/26/03	0.24	SWFR03	1st	5670
09/26/03	0.24	SWFR03	2nd	1770
09/26/03	0.24	SWNB11	1st	2800
09/26/03	0.24	SWNB11	2nd	2500
10/03/03	0.14	SWNB11	1st	100
10/03/03	0.14	SWFR03	1st	1650
10/03/03	0.14	SWFR03	2nd	480
10/11/03	0.10	SWFR03	1st	40
10/11/03	0.10	SWFR03	2nd	840
10/14/03	0.54/0.55-24hrs	SWFR03	1st	6400

Date	**Precipitation**	**Site**	**Flush**	***E. coli***
10/14/03	0.54/0.55-24hrs	SWFR03	2nd	350
10/14/03	0.54/0.55-24hrs	SWNB11	1st	1230
10/14/03	0.54/0.55-24hrs	SWNB11	2nd	400
10/24/03	0.59	SWMI01	1st	1000
10/24/03	0.59	SWMI01	2nd	300
10/24/03	0.59	SWMI18	1st	18560
10/24/03	0.59	SWMI18	2nd	840
10/25/03	Trace	SWNB11	1st	100
10/25/03	Trace	SWNB11	2nd	290
10/25/03	Trace	SWMI06	1st	8840
10/25/03	Trace	SWMI06	2nd	3170
10/25/03	Trace	SWWB09	1st	179170
10/25/03	Trace	SWWB09	2nd	28750
10/25/03	Trace	SWWA17	1st	153330
10/25/03	Trace	SWWA17	2nd	21080
10/25/03	Trace	SWMI12	1st	14000
10/25/03	Trace	SWMI12	2nd	15440

Stormwater Data 2004

E. coli data (CFU/100 ml) from stormwater samples collected and analyzed in year two of study (2004) from MMSD automated inline stormwater samplers and stormwater discharge points along the Milwaukee, Menomonee, Honey Creek, Underwood, and Kinnickinnic Rivers. Precipitation data acquired from NOAA Milwaukee/Sullivan weather station (http://www.crh.noaa.gov/mkx/climate) and is listed in total inches accumulated within the 24 hour time period prior to sampling.

Date	Precipitation	Stormwater Sampling Site	Flush	*E. coli*
03/01/04	0.25	Honey Creek 1 (80 & Arthur)	1st	2028
03/01/04	0.25	Honey Creek 2 (80th & Greenfield)	1st	1204
03/01/04	0.25	Honey Creek 3 (O'Conner North of I94)	1st	1500
03/01/04	0.25	Honey Creek 4 (West of WI Luth. HS dam)	1st	1100
03/01/04	0.25	Honey Creek 4 outfall (West of Wis. Lutheran H.S)	1st	810
03/01/04	0.25	Honey Creek 4a (Glenview Dr. & Hill St.)	1st	2137
03/01/04	0.25	Honey Creek 4b (Manhole on Hill Street)	1st	998
03/01/04	0.25	Honey Creek & Bluemound Ave A	1st	1170
03/01/04	0.25	Honey Creek & Bluemound Ave B	1st	823
03/01/04	0.25	Honey Creek at Ped Bridge in Hart Park	1st	1187
03/01/04	0.25	Wauwatosa Chancery 1 (Railroad Bridge)	1st	1188
03/01/04	0.25	Wauwatosa Chancery 2 (Railroad Bridge)	1st	695
03/01/04	0.25	Hoyt Park (Pedestrian Bridge) A	1st	708
03/01/04	0.25	Hoyt Park (Pedestrian Bridge) B	1st	681
03/01/04	0.25	MN River at Hart Park Ped. Bridge	1st	802
03/01/04	0.25	SWWA13	1st	356
03/01/04	0.25	Confluence of MN & HC (near 73rd)	1st	1063
03/01/04	0.25	North Ave. Bridge over Menomonee River	1st	415
04/21/04	0.31/1.21-24hrs	SWMI101	1st	2250
04/21/04	0.31/1.21-24hrs	SWMI101	2nd	127
04/21/04	0.31/1.21-24hrs	SWGF10	1st	13
04/21/04	0.31/1.21-24hrs	SWGF10	2nd	12
04/21/04	0.31/1.21-24hrs	SWWA13	1st	9167
04/21/04	0.31/1.21-24hrs	SWWA13	2nd	1971
04/21/04	0.31/1.21-24hrs	SWMI12	1st	25289
04/21/04	0.31/1.21-24hrs	SWMI12	2nd	411
04/21/04	0.31/1.21-24hrs	SWWA17	1st	45833
04/21/04	0.31/1.21-24hrs	SWWA17	2nd	23544
04/21/04	0.31/1.21-24hrs	SWFR03	1st	200
04/21/04	0.31/1.21-24hrs	SWFR03	2nd	7
04/21/04	0.31/1.21-24hrs	SWM102	1st	25
04/21/04	0.31/1.21-24hrs	SWM102	2nd	3
04/21/04	0.31/1.21-24hrs	SWM107	1st	6554
04/21/04	0.31/1.21-24hrs	SWM107	2nd	1300
04/21/04	0.31/1.21-24hrs	SWWB09	1st	28336
04/21/04	0.31/1.21-24hrs	SWWB09	2nd	3167
04/21/04	0.31/1.21-24hrs	SWM106	1st	9308
04/21/04	0.31/1.21-24hrs	SWM106	2nd	4210
04/21/04	0.31/1.21-24hrs	SWNB11	1st	8833
04/21/04	0.31/1.21-24hrs	SWNB11	2nd	1000
04/21/04	0.31/1.21-24hrs	SWM104	1st	13000

Date	Precipitation	Stormwater Sampling Site	Flush	*E. coli*
04/21/04	0.31/1.21-24hrs	SWM104	2nd	1491
04/21/04	0.31/1.21-24hrs	SWMI15	1st	813
04/21/04	0.31/1.21-24hrs	SWMI15	2nd	69
04/21/04	0.31/1.21-24hrs	SWMI16	1st	66125
04/21/04	0.31/1.21-24hrs	SWMI16	2nd	5383
06/22/04	0.00/0.63-24hrs	SWRF03	1st	8800
06/22/04	0.00/0.63-24hrs	SWFR03	2nd	66000
06/22/04	0.00/0.63-24hrs	SWGF10	1st	8733
06/22/04	0.00/0.63-24hrs	SWGF10	2nd	350
06/22/04	0.00/0.63-24hrs	SWSF14	1st	380000
06/22/04	0.00/0.63-24hrs	SWSF14	2nd	8600
06/22/04	0.00/0.63-24hrs	SWM106	1st	180
06/22/04	0.00/0.63-24hrs	SWM106	2nd	26500
06/22/04	0.00/0.63-24hrs	SWM107	1st	163125
06/22/04	0.00/0.63-24hrs	SWM107	2nd	10000
06/22/04	0.00/0.63-24hrs	SWNB11	1st	21200
06/22/04	0.00/0.63-24hrs	SWNB11	2nd	7160
06/22/04	0.00/0.63-24hrs	SWMI12	1st	50740
06/22/04	0.00/0.63-24hrs	SWMI12	2nd	8680
06/22/04	0.00/0.63-24hrs	SWWA13	1st	543750
06/22/04	0.00/0.63-24hrs	SWWA13	2nd	8200
06/22/04	0.00/0.63-24hrs	SWMI15	1st	1000
06/22/04	0.00/0.63-24hrs	SWMI15	2nd	934
06/22/04	0.00/0.63-24hrs	SWMI16	1st	10500
06/22/04	0.00/0.63-24hrs	SWMI16	2nd	20000
06/22/04	0.00/0.63-24hrs	SWWA17	1st	57500
06/22/04	0.00/0.63-24hrs	SWWA17	2nd	37500
06/22/04	0.00/0.63-24hrs	SWWA18	1st	12500
06/22/04	0.00/0.63-24hrs	SWWA18	2nd	26120
06/24/04	0.10/0.46-24hrs	KK 2403 A	1st	3600
06/24/04	0.10/0.46-24hrs	KK 2403 B	1st	4900
06/24/04	0.10/0.46-24hrs	KK 2402 A	1st	2700
06/24/04	0.10/0.46-24hrs	KK 2402 B	1st	2300
06/24/04	0.10/0.46-24hrs	KK 2401 A	1st	7500
06/24/04	0.10/0.46-24hrs	KK 2401 B	1st	7200
06/24/04	0.10/0.46-24hrs	Broken Pipe A	1st	12300
06/24/04	0.10/0.46-24hrs	Broken Pipe B	1st	16700
06/24/04	0.10/0.46-24hrs	City of Milwaukee Outfall 2392 A	1st	4700
06/24/04	0.10/0.46-24hrs	City of Milwaukee Outfall 2392 B	1st	3800
06/24/04	0.10/0.46-24hrs	City of Milwaukee Outfall 2393 A	1st	2800
06/24/04	0.10/0.46-24hrs	City of Milwaukee Outfall 2393 B	1st	3000
06/24/04	0.10/0.46-24hrs	KK river at Chase Ave. A	1st	3600
06/24/04	0.10/0.46-24hrs	KK river at Chase Ave. B	1st	5300
06/24/04	0.10/0.46-24hrs	City of Milwaukee Outfall 2408 A	1st	5200
06/24/04	0.10/0.46-24hrs	City of Milwaukee Outfall 2408 B	1st	5100
06/24/04	0.10/0.46-24hrs	KK River at Lincoln Ave. A	1st	1100
06/24/04	0.10/0.46-24hrs	KK River at Lincoln Ave. B	1st	900
06/24/04	0.10/0.46-24hrs	RR Outfall (SS#1 A)	1st	1200
06/24/04	0.10/0.46-24hrs	SS#1 B	1st	1700
06/24/04	0.10/0.46-24hrs	Waterfall at A	1st	3600

Date	Precipitation	Stormwater Sampling Site	Flush	*E. coli*
06/24/04	0.10/0.46-24hrs	Waterfall at B	1st	2800
07/22/04	0.00/0.28-24 hrs	SWM101	1st	5000
07/22/04	0.00/0.28-24 hrs	SWM101	2nd	30000
07/22/04	0.00/0.28-24 hrs	SWM102	1st	7000
07/22/04	0.00/0.28-24 hrs	SWM102	2nd	200
07/22/04	0.00/0.28-24 hrs	SWM104	1st	360000
07/22/04	0.00/0.28-24 hrs	SWFR03	1st	5400
07/22/04	0.00/0.28-24 hrs	SWMI16	1st	18900
07/22/04	0.00/0.28-24 hrs	SWGF10	1st	21000
07/22/04	0.00/0.28-24 hrs	SWGF10	2nd	23000
07/22/04	0.00/0.28-24 hrs	SWMI06	1st	TNTC
07/22/04	0.00/0.28-24 hrs	SWMI06	2nd	400
07/22/04	0.00/0.28-24 hrs	SWMI18	1st	3600
07/22/04	0.00/0.28-24 hrs	SWNB11	1st	92000
07/22/04	0.00/0.28-24 hrs	SWM112	1st	TNTC
07/22/04	0.00/0.28-24 hrs	SWMI15	1st	3100
10/04/04	0.00	SWMI01	1st	16500
10/04/04	0.00	SWWA13	1st	301000
10/04/04	0.00	SWM107	1st	203000
10/04/04	0.00	SWWA13	2nd	500000
10/04/04	0.00	SWMI04	1st	500000
10/04/04	0.00	SWMI07	2nd	500000
10/08/04	0.17	Honey Creek & WI Ave.	1st	14500
10/08/04	0.17	120th & Diane Drive	1st	6550
10/08/04	0.17	115th & Honey Creek Bike Trail	1st	55750
10/08/04	0.17	Watertown Plank Rd & Hwy 100	1st	100
10/08/04	0.17	103rd & W Fisher Parkway	1st	6625
11/01/04	0.58	State Street & 62nd Main Flow A	1st	60000
11/01/04	0.58	State Street & 62nd Main Flow B	1st	80000
11/01/04	0.58	State Street & 62nd West Outfall A	1st	16000
11/01/04	0.58	State Street & 62nd West Outfall B	1st	10600
11/01/04	0.58	Men. Parkway & Concordia A	1st	21100
11/01/04	0.58	Men. Parkway & Concordia B	1st	25300
11/01/04	0.58	Men. Parkway & Keefe A	1st	60000
11/01/04	0.58	Men. Parkway & Keefe B	1st	60000
11/01/04	0.58	Madison Park A	1st	21200
11/01/04	0.58	Madison Park B	1st	20600
11/01/04	0.58	Grand Tosa Blvd. & 100th A	1st	29700
11/01/04	0.58	Grand Tosa Blvd. & 100th B	1st	37200
11/01/04	0.58	Jacobus Park A	1st	50000
11/01/04	0.58	Jacobus Park B	1st	60000
11/01/04	0.58	WI Luther High School South A	1st	23900
11/01/04	0.58	WI Luther High School South B	1st	22900
11/01/04	0.58	WI Luther High School North A	1st	30000
11/01/04	0.58	WI Luther High School North B	1st	25400

Stormwater Data 2005

E. coli data (CFU/100 ml) from stormwater samples collected and analyzed in year three of study (2005) from MMSD automated inline stormwater samplers and stormwater discharge points along the Milwaukee, Menomonee, Honey Creek, Underwood, and Kinnickinnic Rivers. Precipitation data acquired from NOAA Milwaukee/Sullivan weather station (http://www.crh.noaa.gov/mkx/climate) and is listed in total inches accumulated within the 24 hour time period prior to sampling.

Date	Precipitation	Stormwater Sampling Site	Flush	*E. coli*
01/12/05	0.75/0.76-24hrs	Main Channel off Summerfest	1st	303
01/12/05	0.75/0.76-24hrs	Broadway Bridge	1st	1126
01/12/05	0.75/0.76-24hrs	KK river off WATER Inst.	1st	170
01/12/05	0.75/0.76-24hrs	Main Channel off Summerfest	1st	1023
01/12/05	0.75/0.76-24hrs	Broadway Bridge	1st	1294
01/12/05	0.75/0.76-24hrs	KK river off WATER Inst.	1st	223
01/12/05	0.75/0.76-24hrs	Main Channel off Summerfest	1st	250
01/12/05	0.75/0.76-24hrs	Broadway Bridge	1st	790
01/12/05	0.75/0.76-24hrs	KK river off WATER Inst.	1st	30
01/13/05	0.04	Main Channel off Summerfest	1st	1070
01/13/05	0.04	Broadway Bridge	1st	1150
01/13/05	0.04	KK river off WATER Inst.	1st	207
03/16/05	0.01	SWWB09		10733
03/16/05	0.01	SWMI07		2000
03/16/05	0.01	SWMI04		1200
03/16/05	0.01	SWMI16		<10
03/16/05	0.01	SWMI18		<10
03/16/05	0.01	SWWA13		27667
03/16/05	0.01	SWWA17		11667
03/16/05	0.01	SWMI12		100
03/16/05	0.01	Big Bay Park Outfall		6767
04/07/05	0.00/0.42-24 hrs	SWFR03	1st	100
04/07/05	0.00/0.42-24 hrs	SWFR03	2nd	200
04/07/05	0.00/0.42-24 hrs	SWMI12	1st	43333
04/07/05	0.00/0.42-24 hrs	SWMI12	2nd	700
04/07/05	0.00/0.42-24 hrs	SWMI07	1st	1400
04/07/05	0.00/0.42-24 hrs	SWMI07	2nd	3433
04/07/05	0.00/0.42-24 hrs	SWNB11	1st	100
04/07/05	0.00/0.42-24 hrs	SWNB11	2nd	200
04/07/05	0.00/0.42-24 hrs	SWMI06	1st	1200
04/07/05	0.00/0.42-24 hrs	SWWB09	1st	17400
04/07/05	0.00/0.42-24 hrs	SWMI15	1st	100
04/07/05	0.00/0.42-24 hrs	SWMI15	2nd	<10
04/07/05	0.00/0.42-24 hrs	SWWA17	1st	160000
04/07/05	0.00/0.42-24 hrs	SWWA17	2nd	1100
04/07/05	0.00/0.42-24 hrs	SWMI18	1st	9400
04/07/05	0.00/0.42-24 hrs	SWMI18	2nd	733
04/07/05	0.00/0.42-24 hrs	SWWA20	1st	1050
04/07/05	0.00/0.42-24 hrs	SWWA20	2nd	950

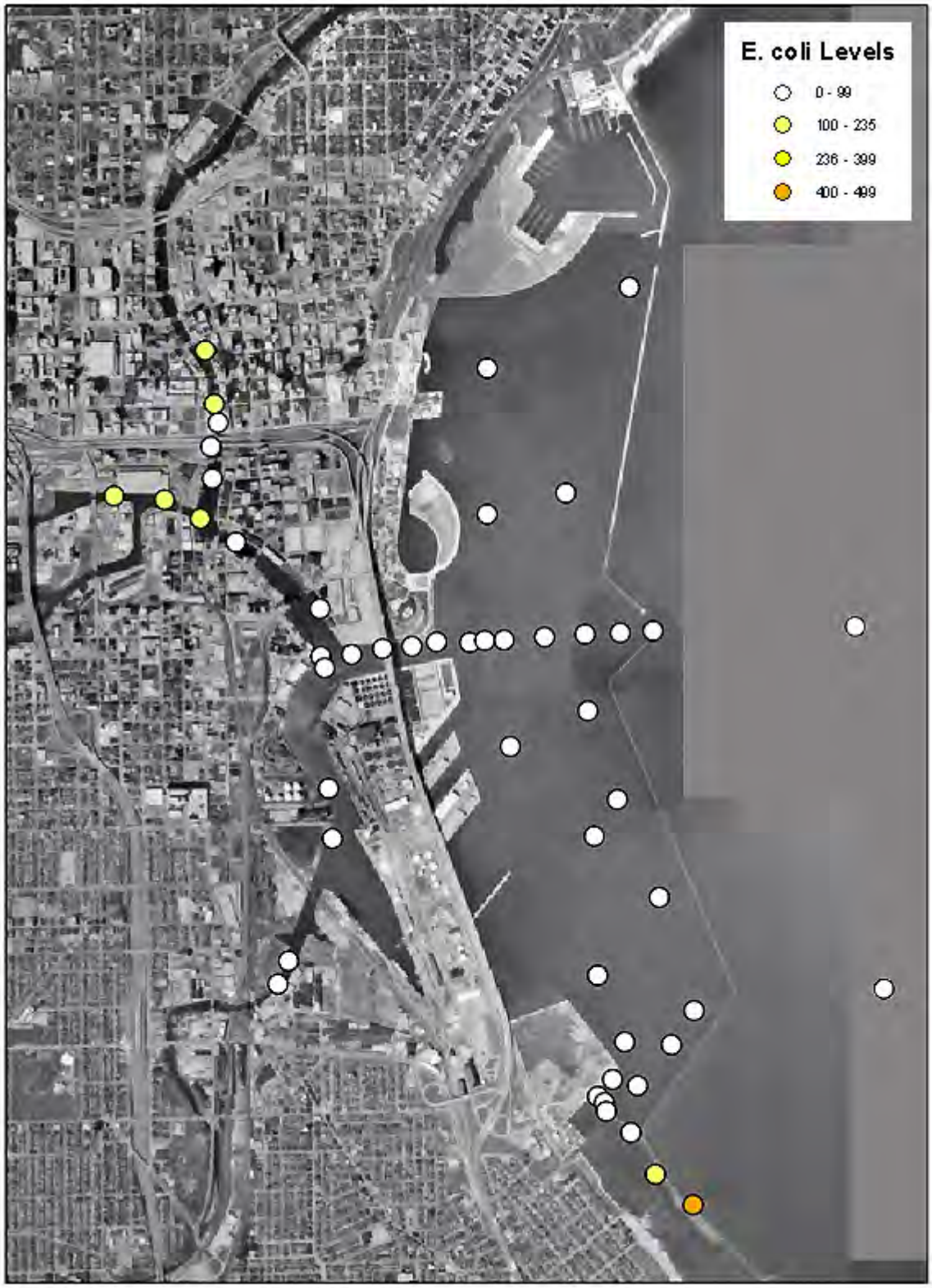
Date	Precipitation	Stormwater Sampling Site	Flush	*E. coli*
05/06/05	0.36	SWFR03	1st	13700
05/06/05	0.36	SWFR03	2nd	900
05/06/05	0.36	SWMI04	1st	800
05/06/05	0.36	SWMI04	2nd	4200
05/06/05	0.36	SWWB09	1st	12200
05/06/05	0.36	SWWB09	2nd	5300
05/06/05	0.36	SWMI07	1st	1500
05/06/05	0.36	SWMI07	2nd	700
05/06/05	0.36	SWMI06	1st	<10
05/06/05	0.36	SWMI06	2nd	1000
05/06/05	0.36	SWNB11	1st	2550
05/06/05	0.36	SWNB11	2nd	1600
05/06/05	0.36	SWMI12	1st	8600
05/06/05	0.36	SWMI12	2nd	500
05/06/05	0.36	SWWA13	1st	787000
05/06/05	0.36	SWWA13	2nd	12500
05/06/05	0.36	SWMI15	1st	2200
05/06/05	0.36	SWMI15	2nd	300
05/06/05	0.36	SWWA17	1st	179500
05/06/05	0.36	SWWA17	2nd	19500
05/06/05	0.36	SWMI18	1st	215000
05/06/05	0.36	SWMI18	2nd	7900
05/06/05	0.36	SWMI19	1st	<10
05/06/05	0.36	SWMI19	2nd	46000
06/13/05	0.02	SWWB11	1st	3100
06/13/05	0.02	SWMI12	1st	336000
06/13/05	0.02	SWWA20	1st	339000
06/13/05	0.02	SWMI06	1st	60000
06/13/05	0.02	SWMI07	1st	210000
06/13/05	0.02	SWMI07	2nd	4250
06/13/05	0.02	SWWA13	1st	5343000
06/13/05	0.02	SWMI06	2nd	3450
06/13/05	0.02	SWMI15	1st	9050
06/13/05	0.02	SWWA17	1st	123000
06/13/05	0.02	SWMI18	1st	352750
06/13/05	0.02	SWMI18	2nd	32600
06/13/05	0.02	51st St & KK River	1st	1350
06/13/05	0.02	53rd St. & KK River	1st	9300
07/13/05	1.29/1.42-24hrs	SWMI04	2nd	17900
07/13/05	1.29/1.42-24hrs	SWMI18	1st	26900
07/13/05	1.29/1.42-24hrs	SWMI18	2nd	3850
07/13/05	1.29/1.42-24hrs	SWMI12	1st	34250
07/13/05	1.29/1.42-24hrs	SWNB11	1st	2100
07/20/05	0.11	SWMI07	1st	120000
07/20/05	0.11	SWWA20	1st	18000
07/20/05	0.11	SWWA20	2nd	121500
07/20/05	0.11	SWNB11	1st	225000
07/20/05	0.11	SWFR03	1st	50000

Date	Precipitation	Stormwater Sampling Site	Flush	*E. coli*
07/21/05	0.29	UPS station on Bluemound Ave.	1st	100
07/21/05	0.29	Underwood Pkwy at MMSD Pumping Station	1st	2050
07/21/05	0.29	North Mayfair Rd	1st	29500
07/21/05	0.29	Runoff from bridge under Hwy 100	1st	1000
07/21/05	0.29	Underwood Pkwy at WI Luth.Stadium	1st	500
07/21/05	0.29	Discharge from Hwy 100 to wetland	1st	4850
07/21/05	0.29	River outfall At Hart Park	1st	27500
07/21/05	0.29	Train Bridge at Chancery Rest.	1st	16000
07/21/05	0.29	SWMI12		500000
07/21/05	0.29	SWMI15		7000
07/21/05	0.29	SWMI18	1st	140000
07/21/05	0.29	SWWA13	1st	500000
07/21/05	0.29	SWWA17	1st	500000
07/21/05	0.29	SWWA17	2nd	180000
07/21/05	0.29	SWMI06	1st	96000
07/21/05	0.29	SWMI06	2nd	<10
07/26/05	0.19/0.27-24hrs	SWMI07	composite	8750
07/26/05	0.19/0.27-24hrs	SWWA13	composite	38000
07/26/05	0.19/0.27-24hrs	SWMI15	composite	1500

APPENDIX III

Harbor Spatial Surveys
2003

Harbor Survey 5-29-03



Harbor Survey 6-10-03

N

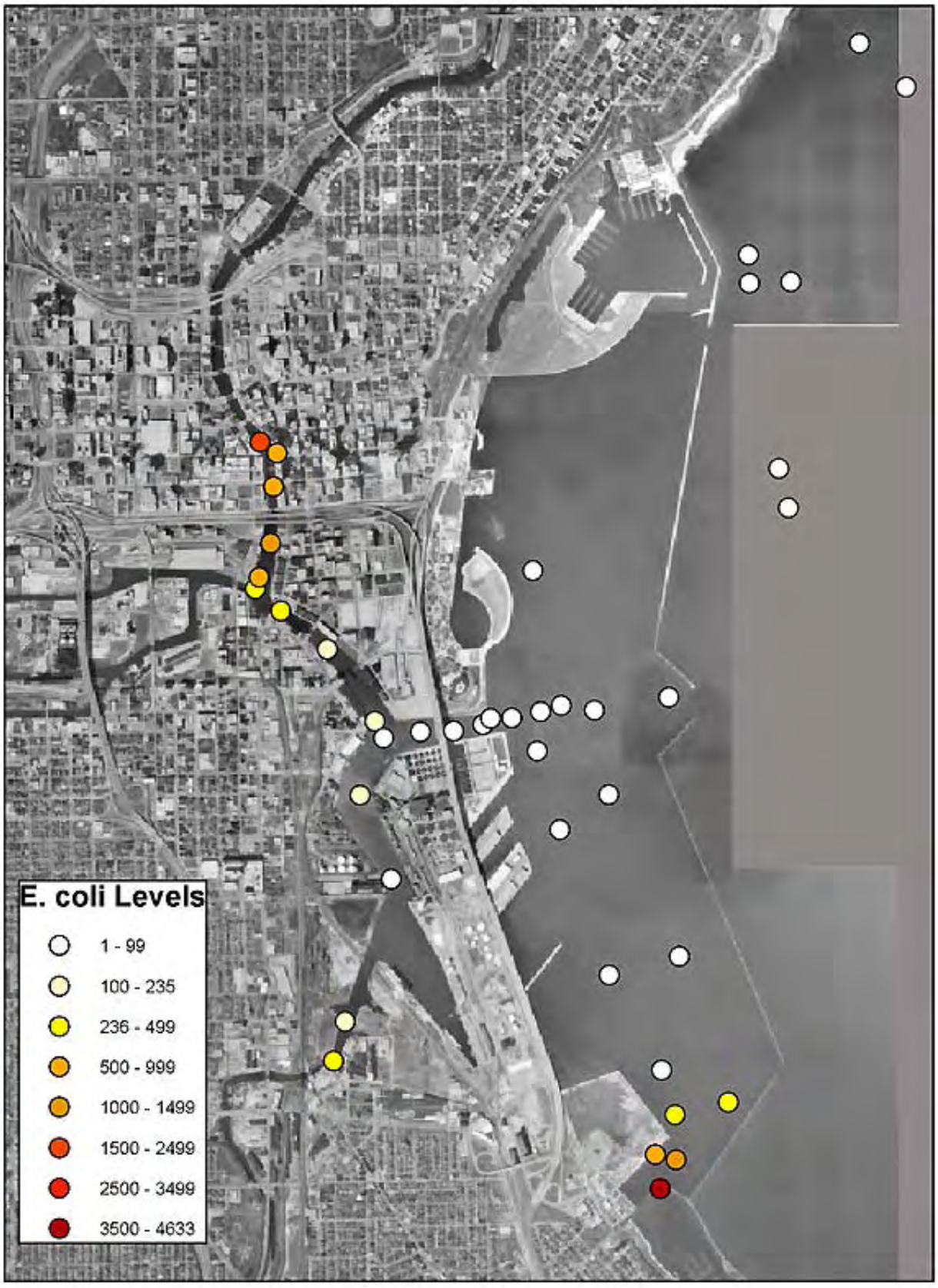
0 250 500 1,000

Meters

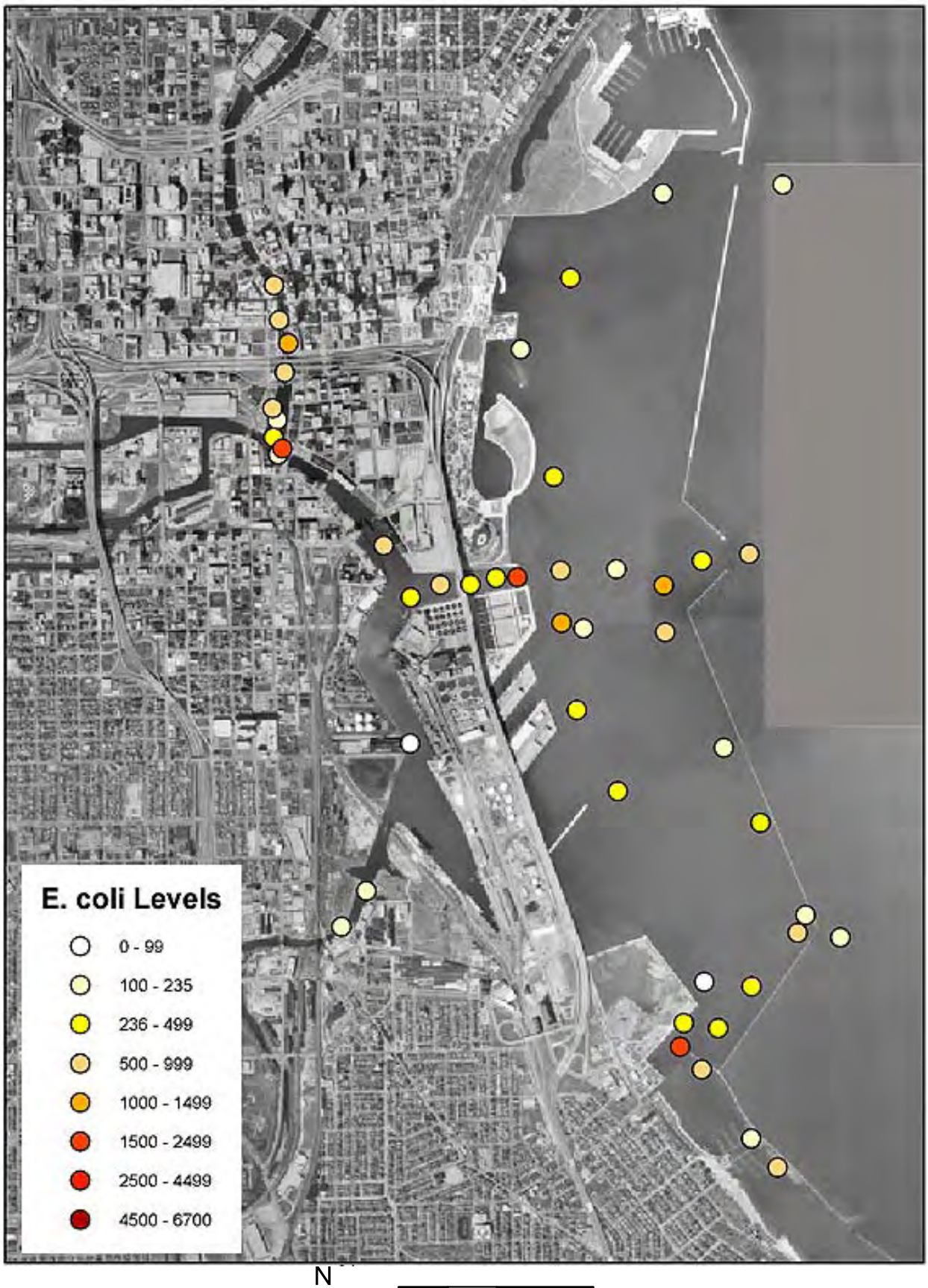
Harbor Survey 6-11-03

Harbor Survey 6-26-03

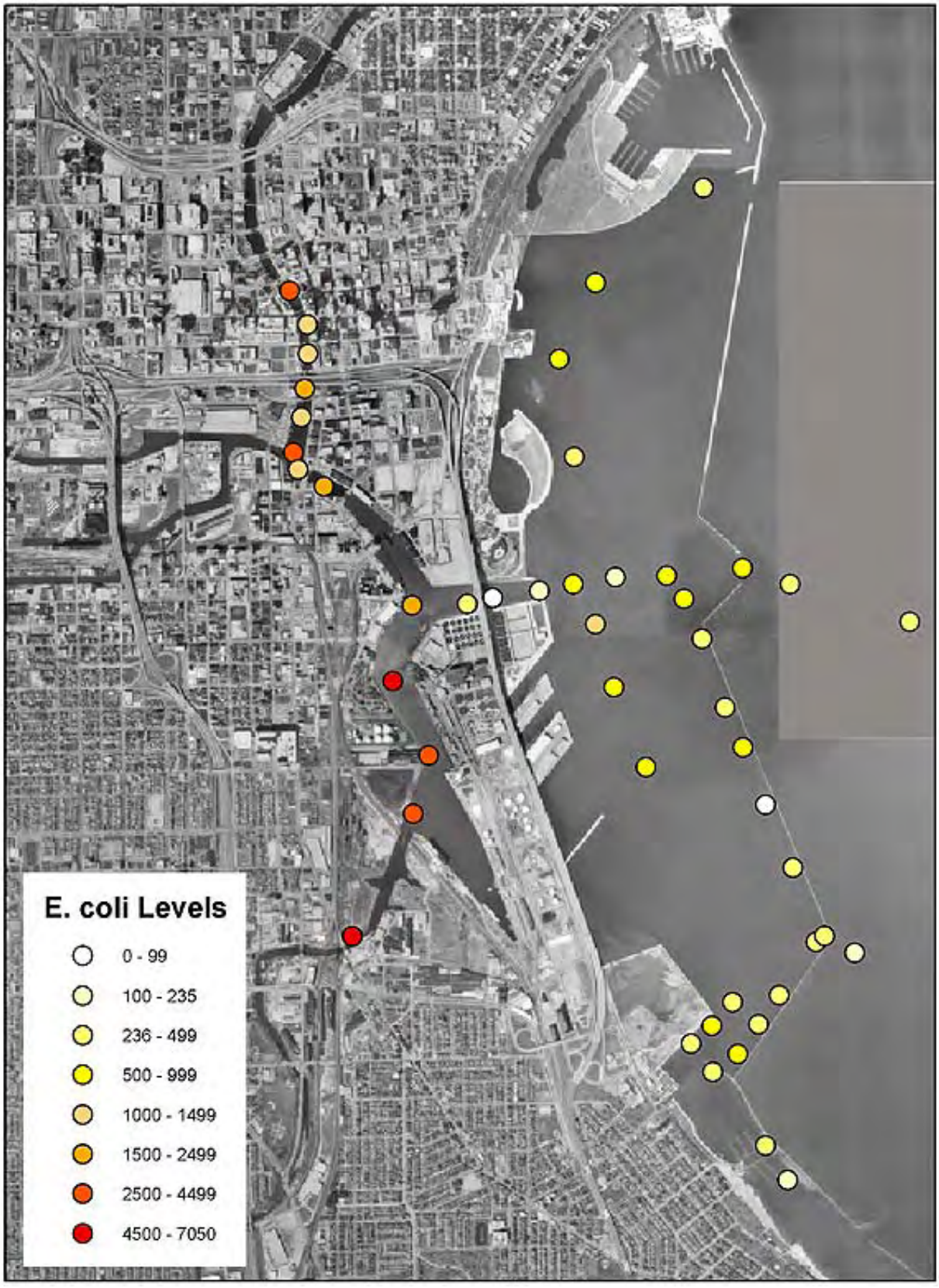
Harbor Survey 7-05-03



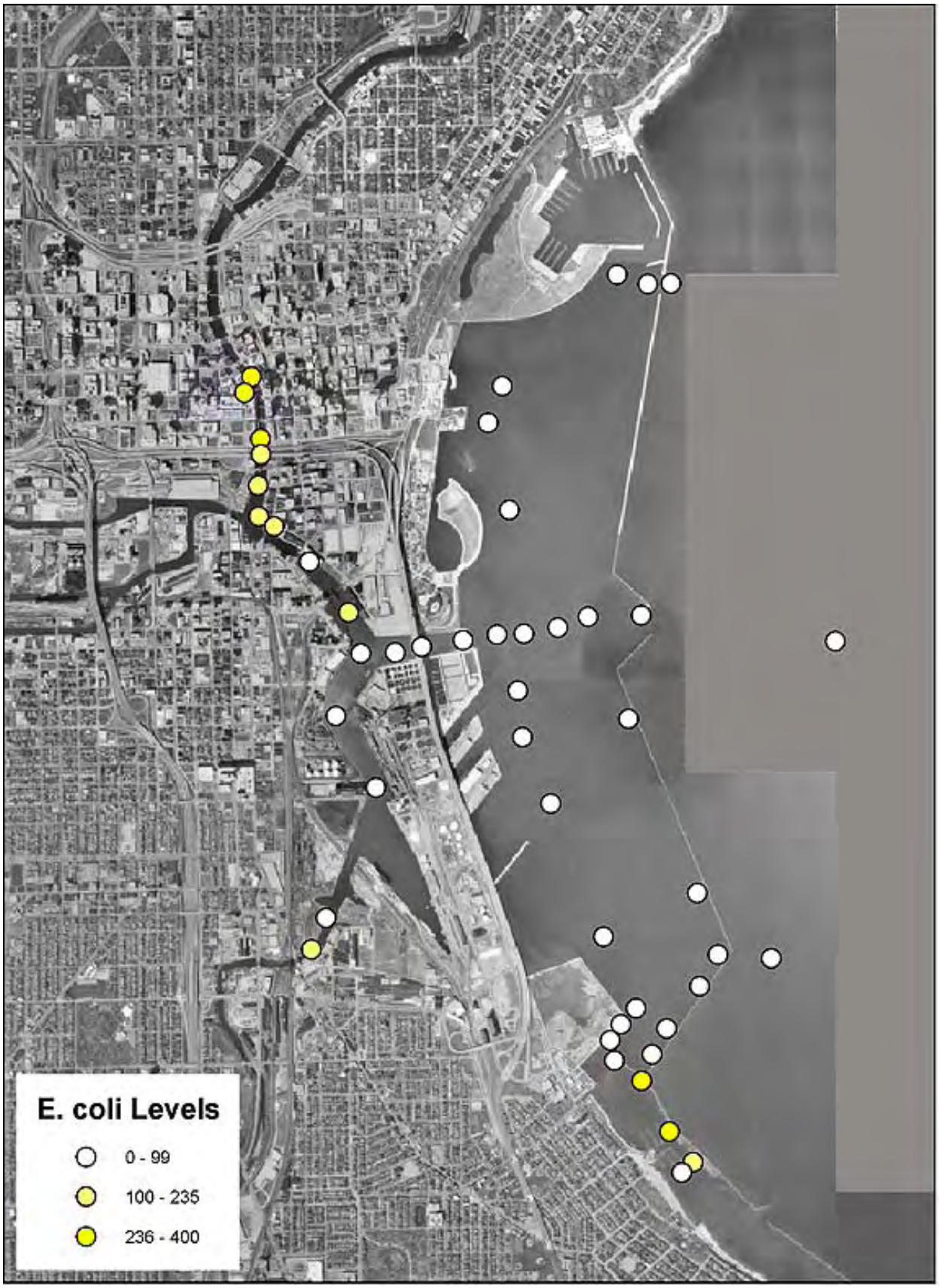
Harbor Survey 7-07-03



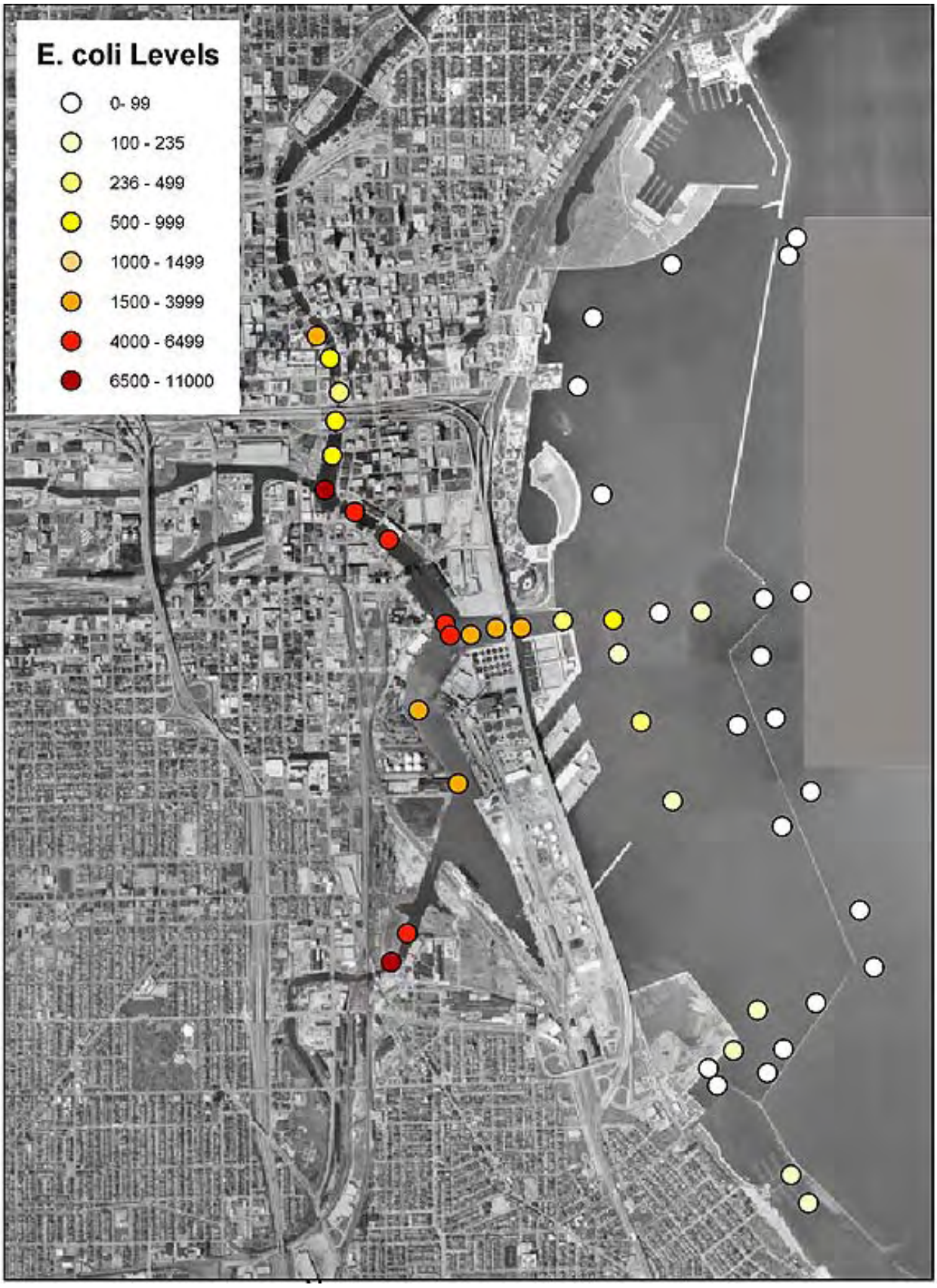
Harbor Survey 7-10-03



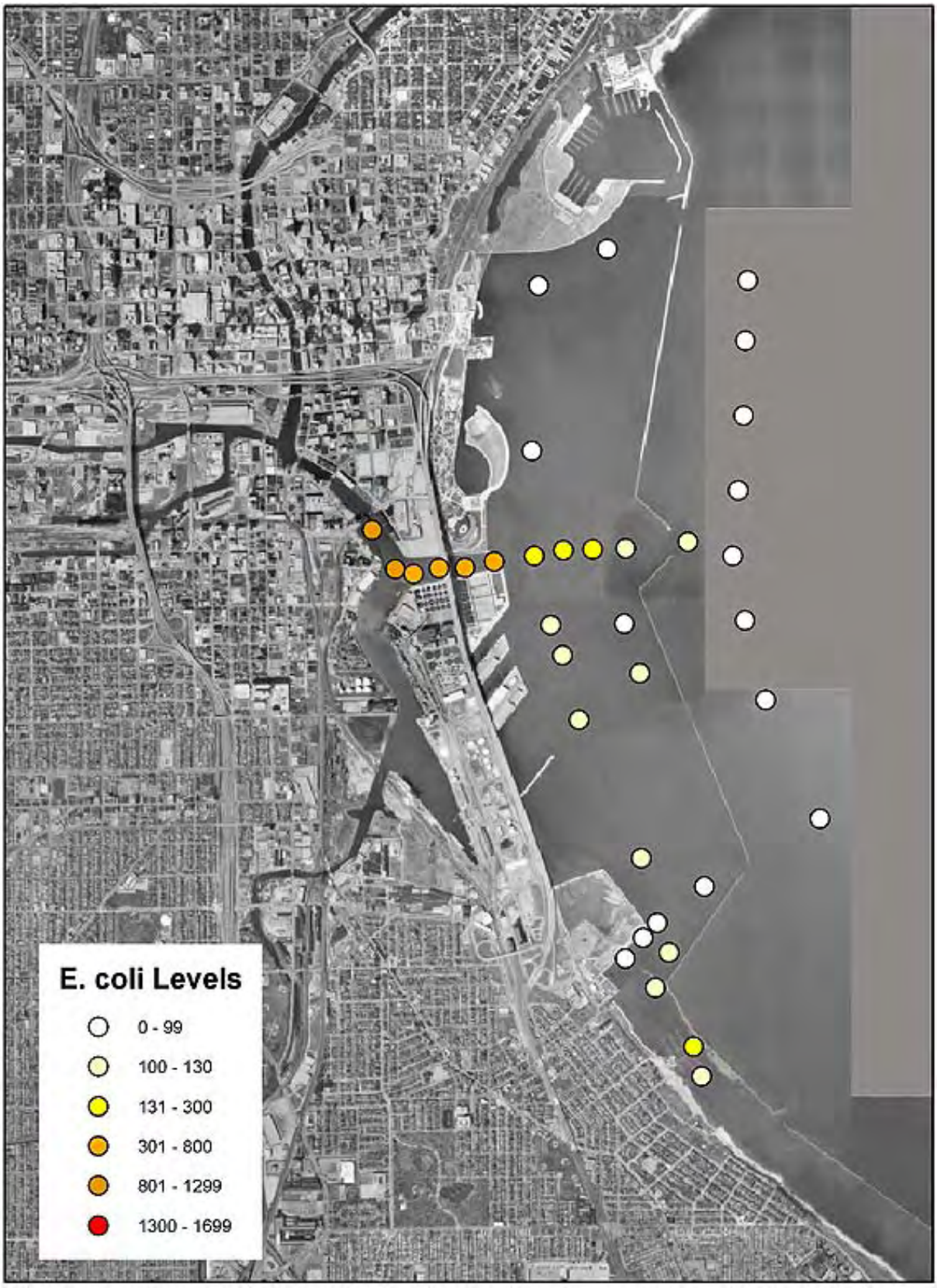
Harbor Survey 7-15-03



Harbor Survey 8-04-03



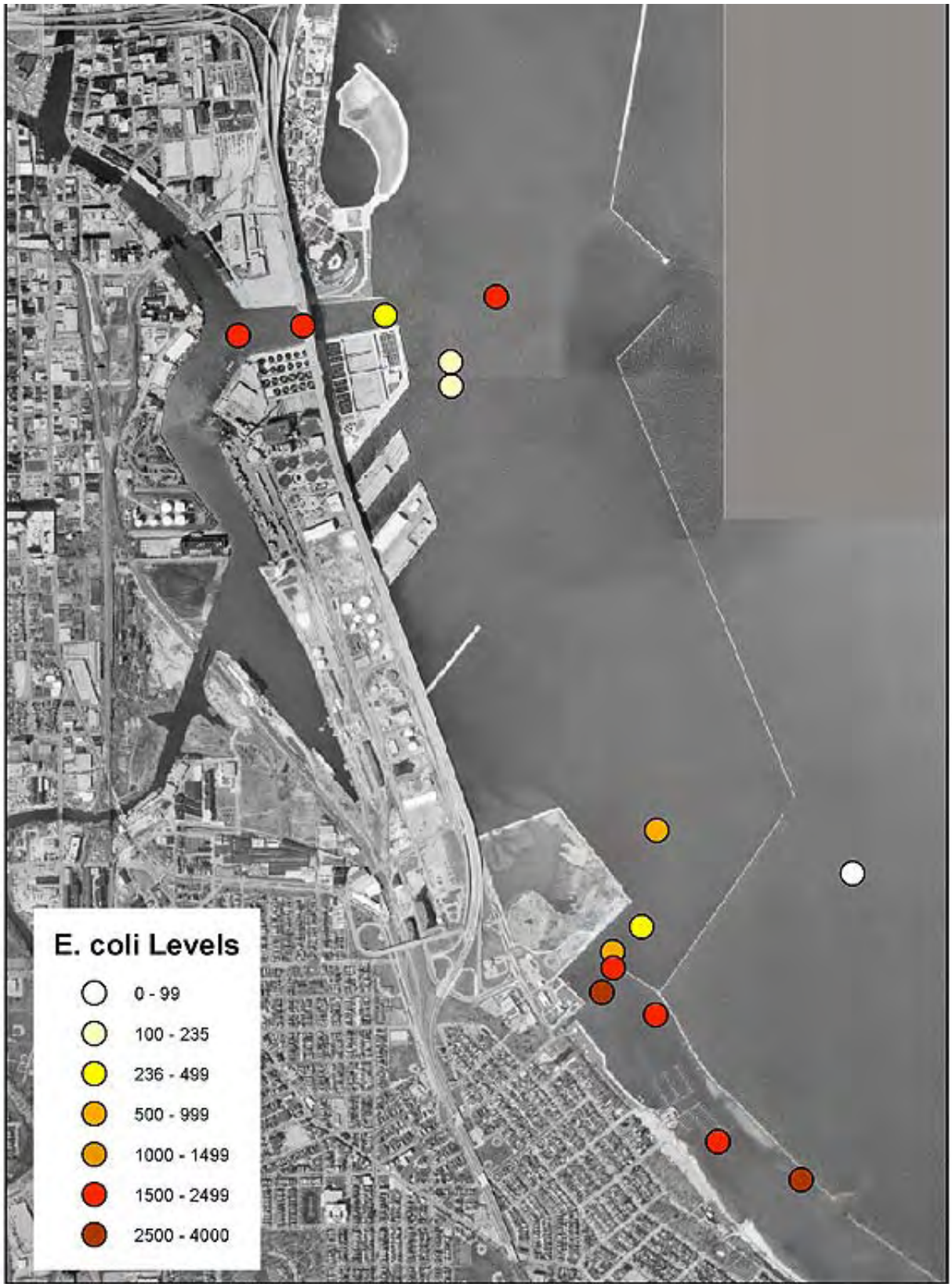
Harbor Survey 8-05-03

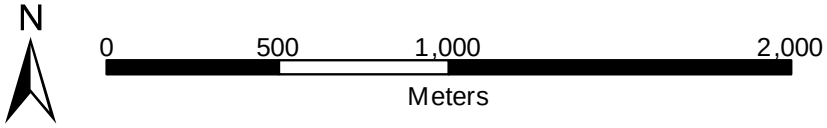


APPENDIX IV

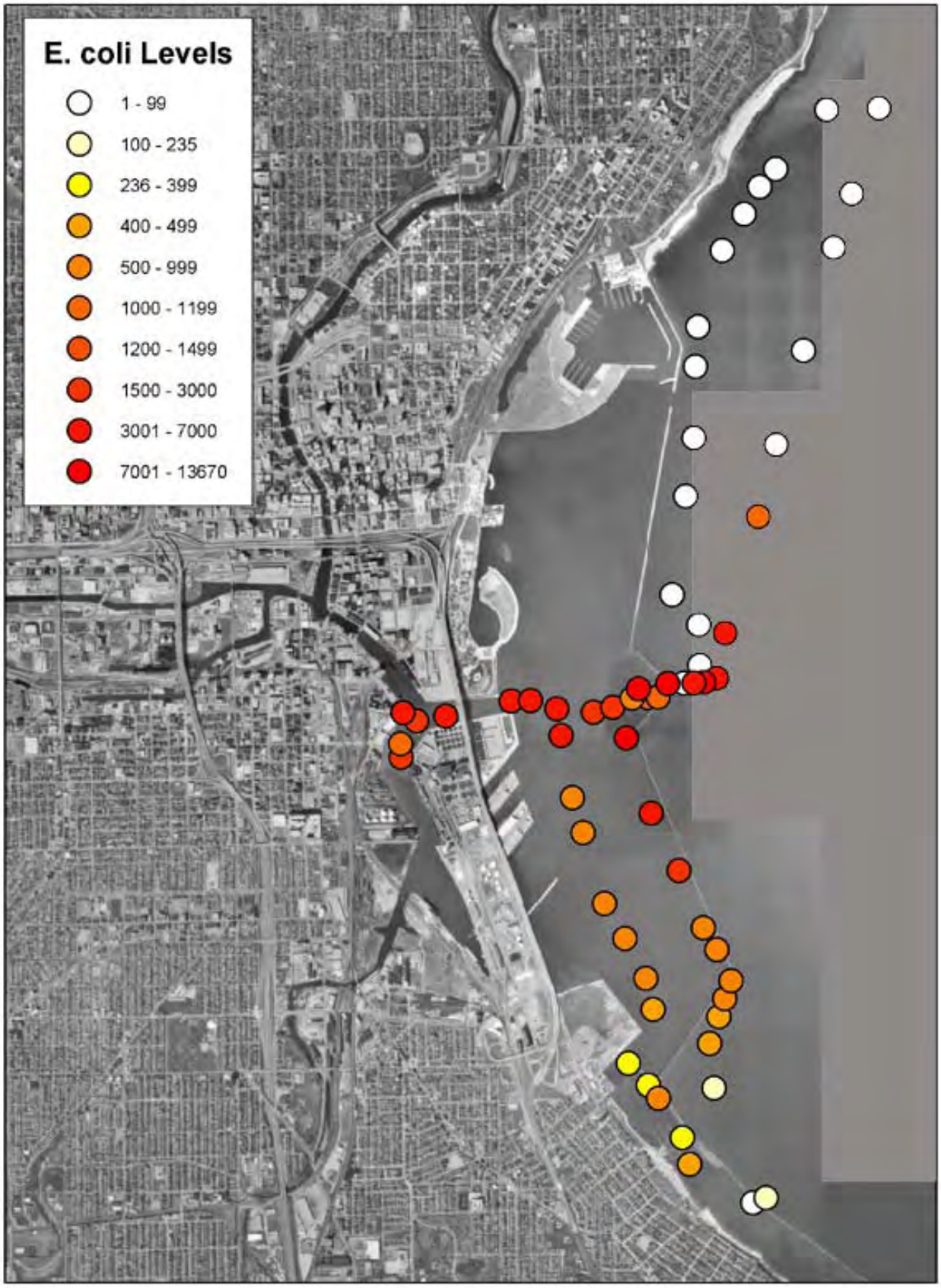
Harbor Spatial Surveys
2004

Harbor Survey 5-10-04

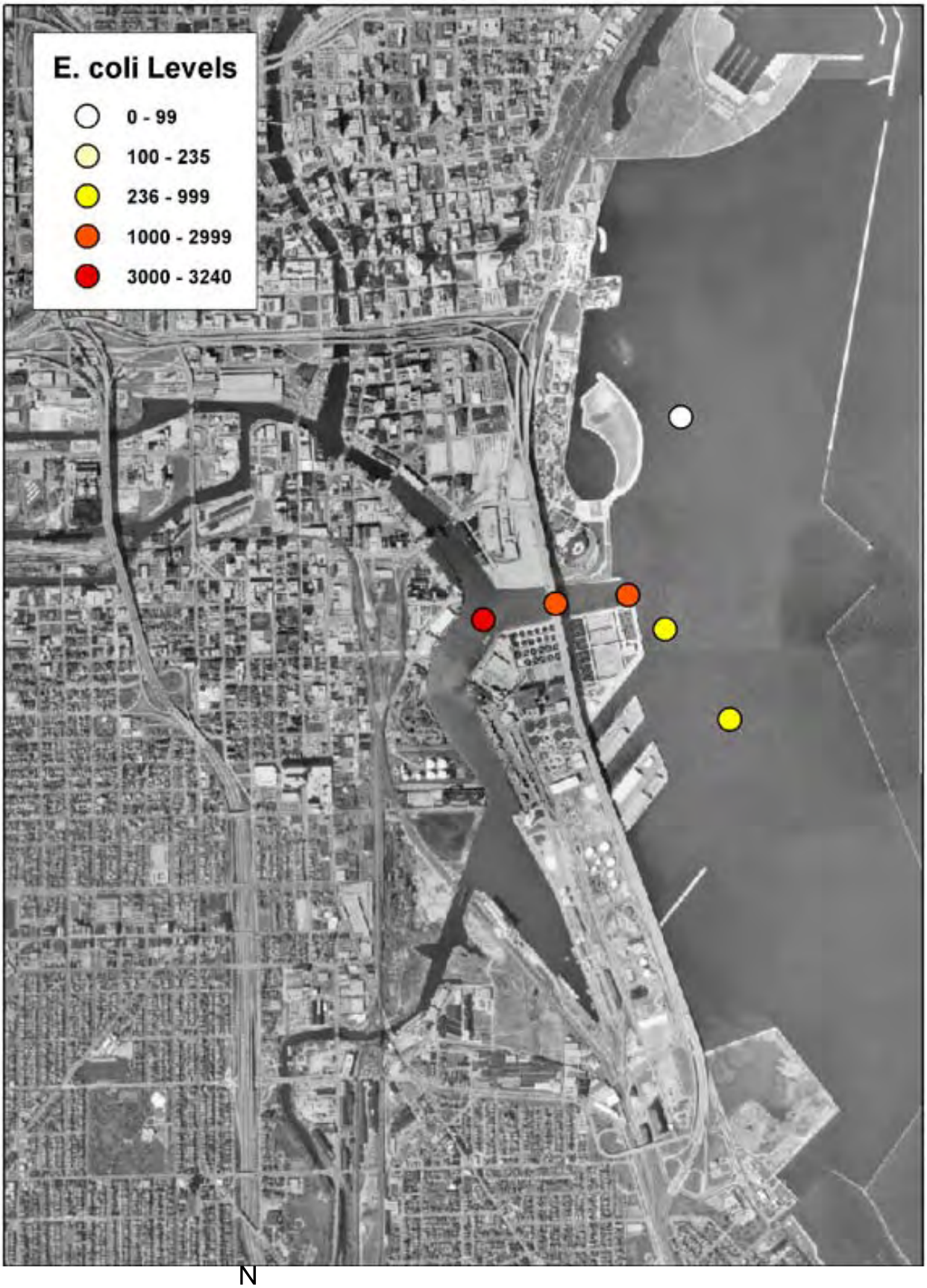




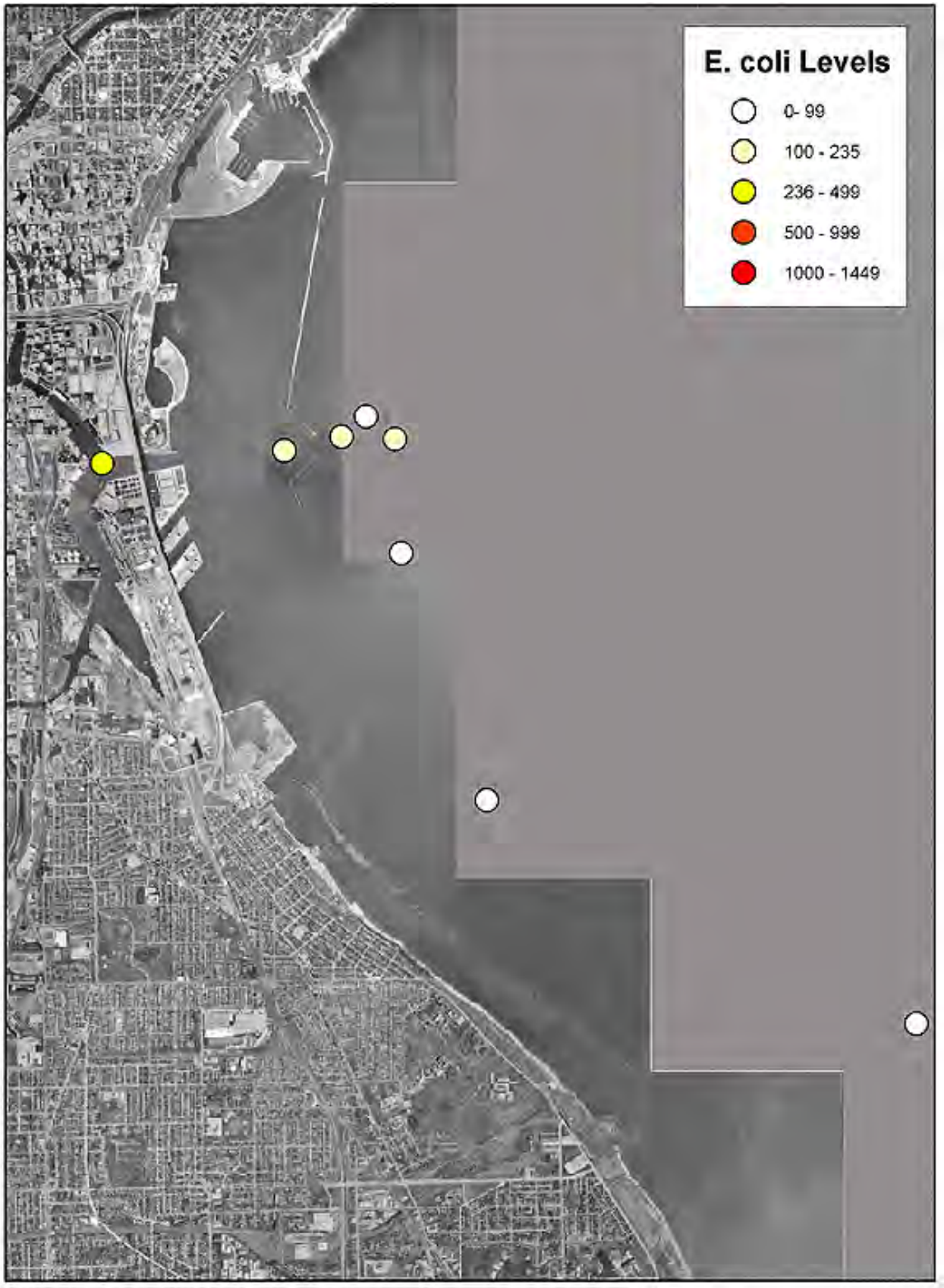
Harbor Survey 5-14-04



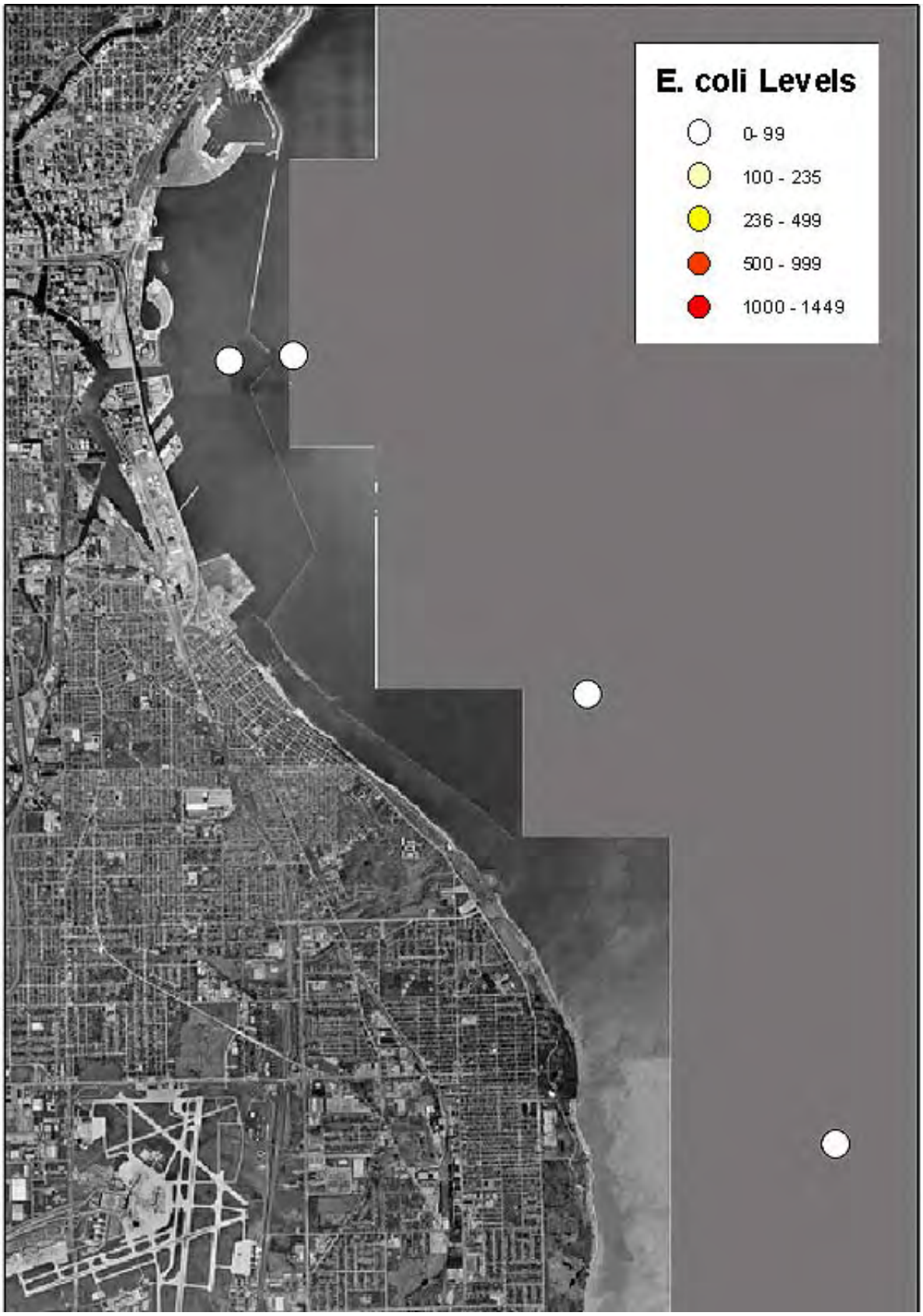
Harbor Survey 5-18-04



Harbor Survey 5-19-04

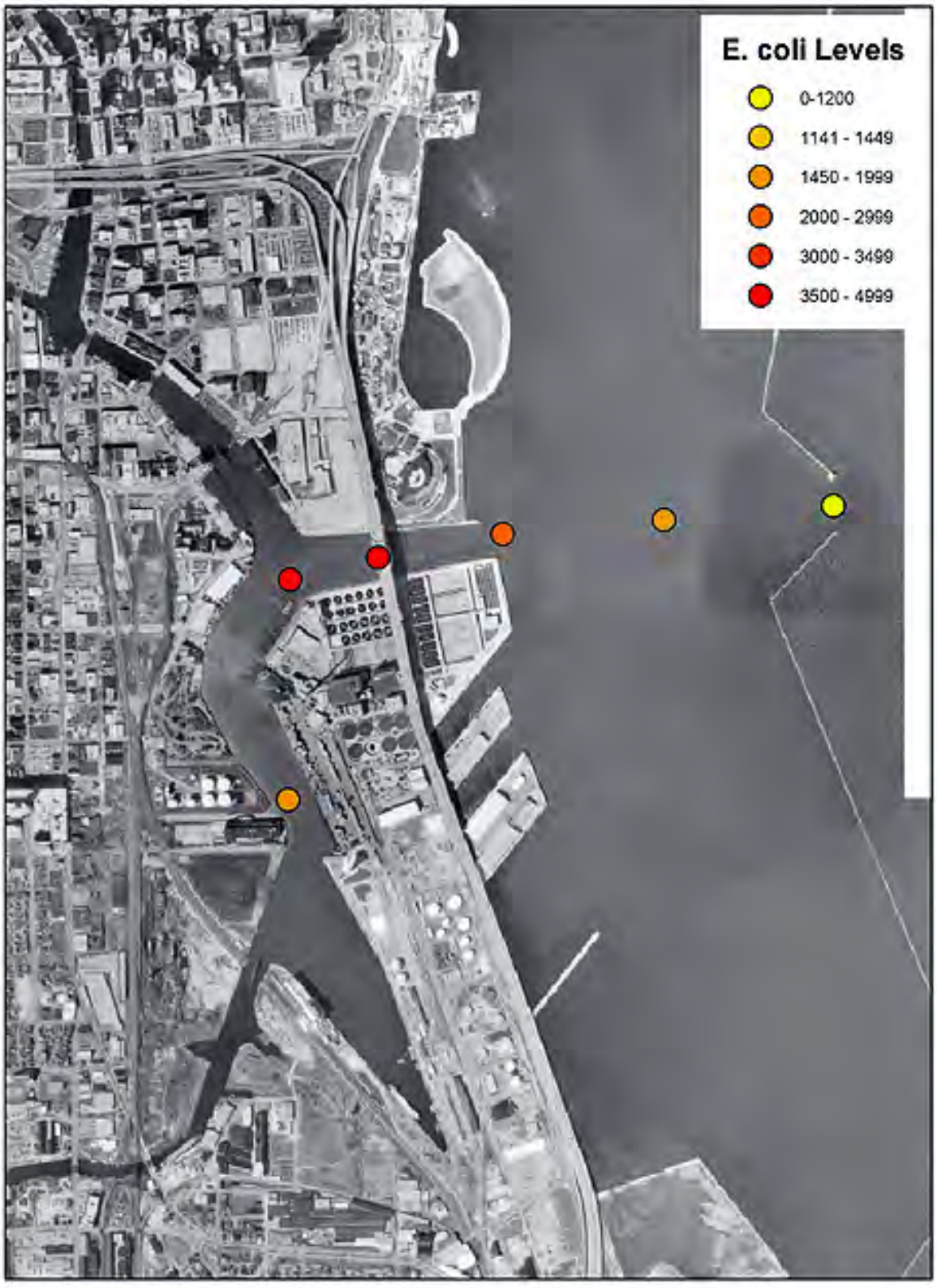


Harbor Survey 5-20-04

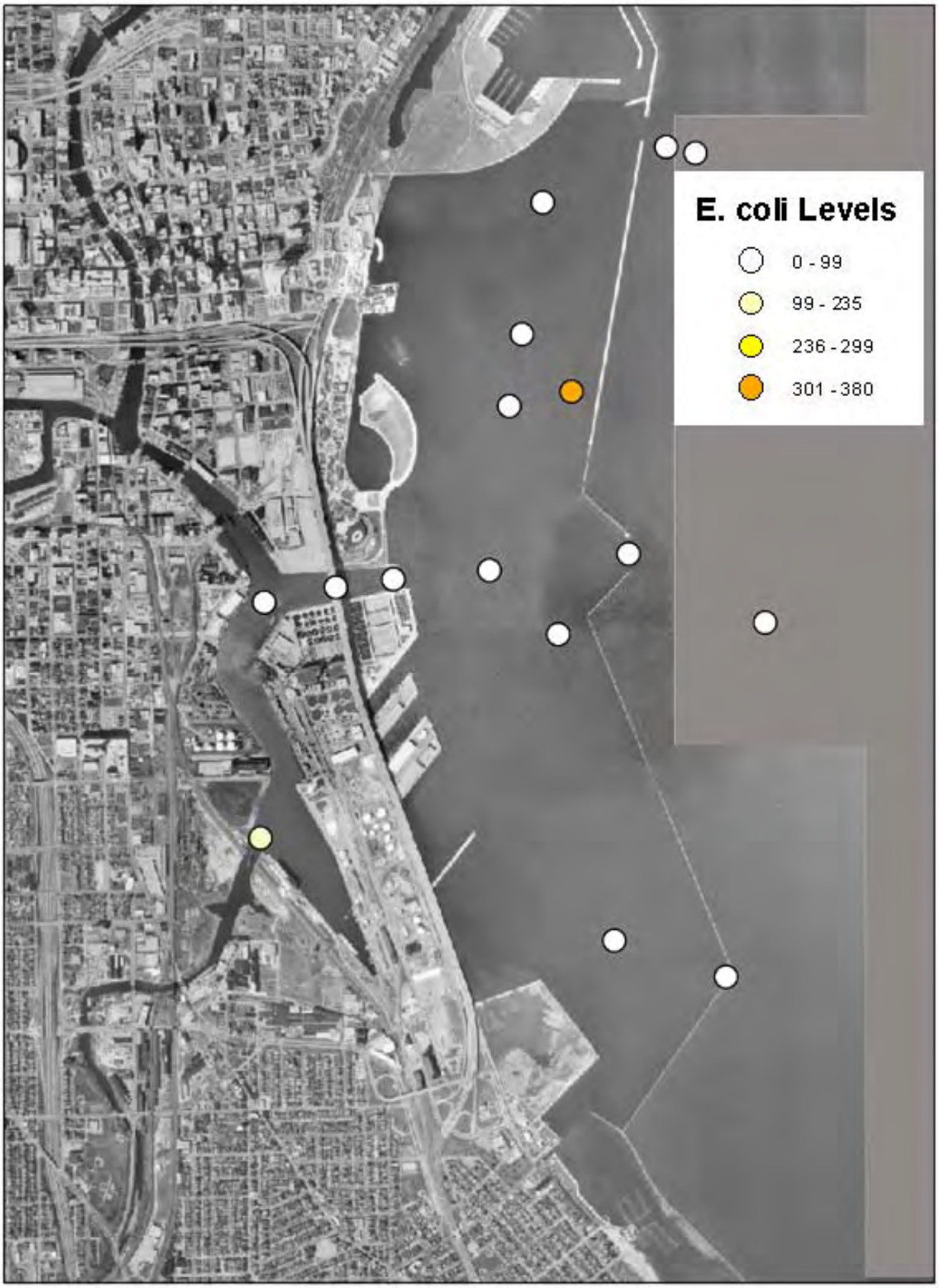


Harbor Survey 5-21-04

Harbor Survey 5-22-04

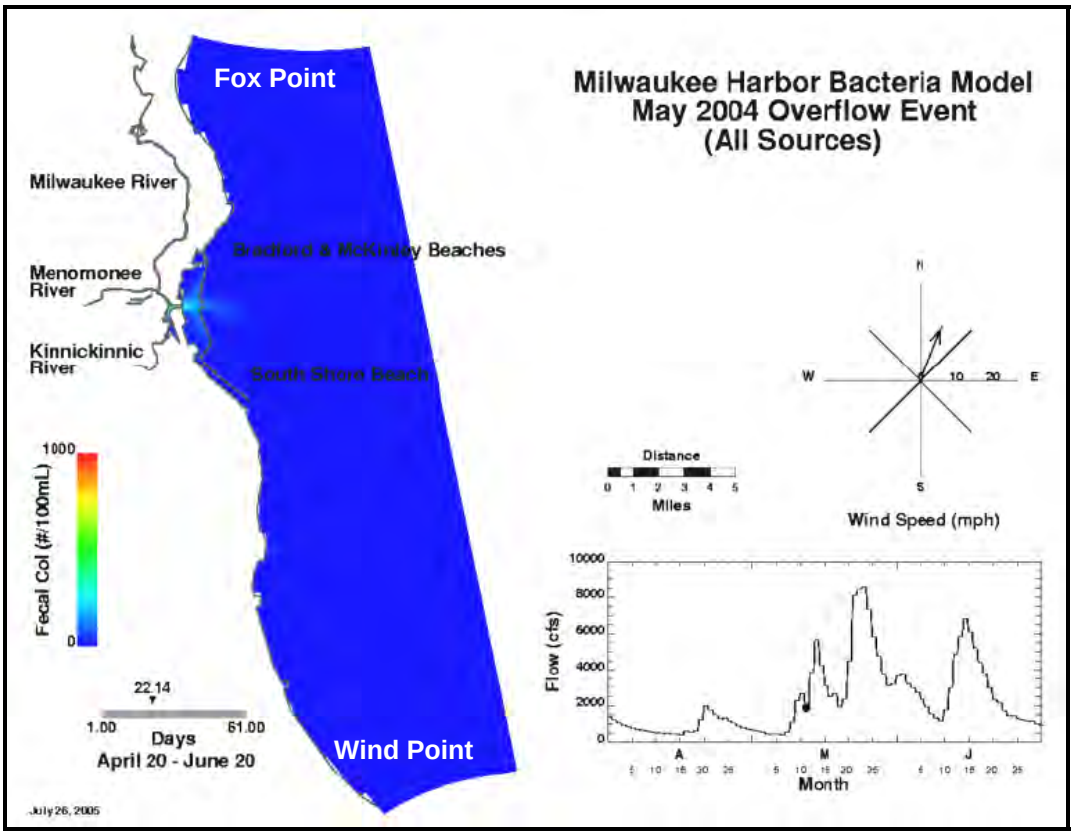


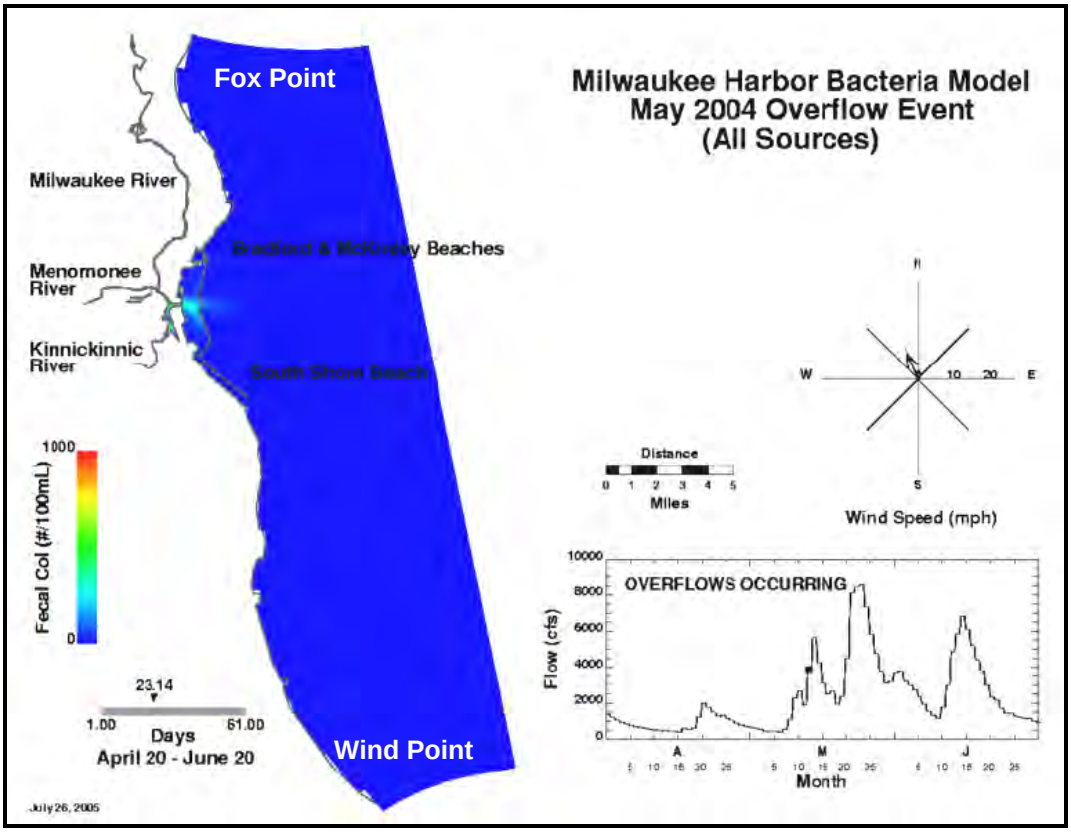
Harbor Survey 5-28-04



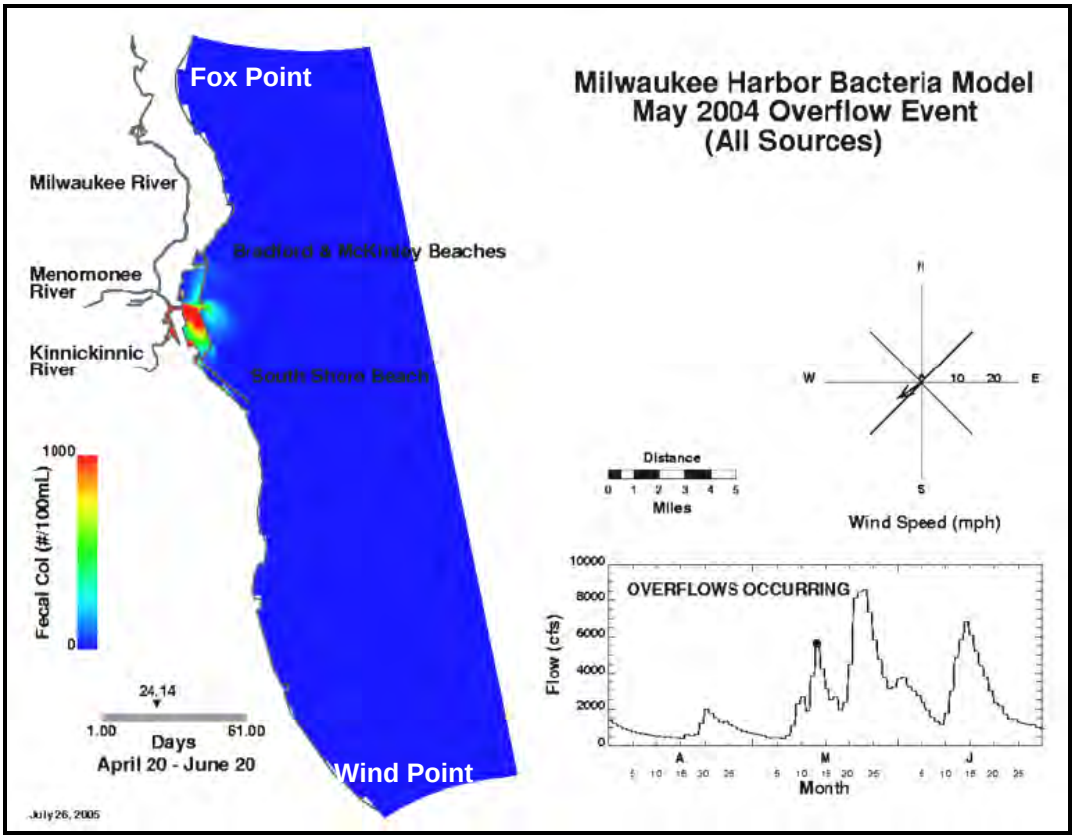
APPENDIX V

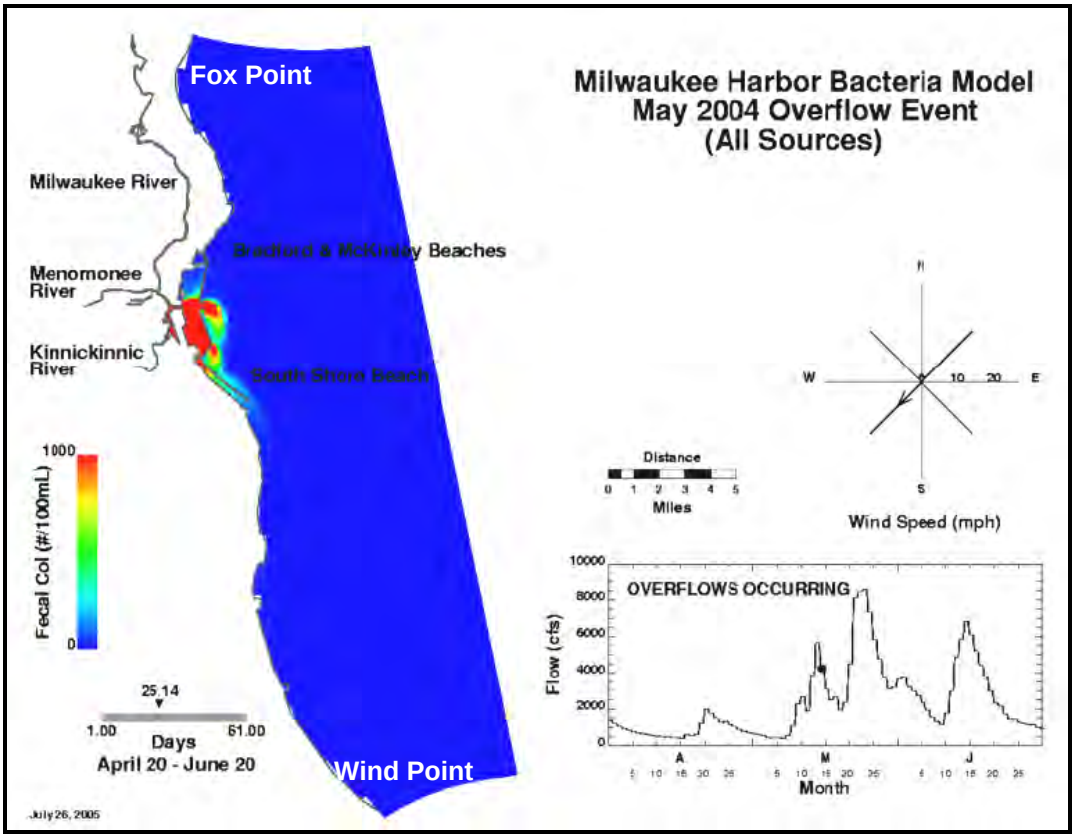
HYDRO-DYNAMIC MODEL SIMULATIONS



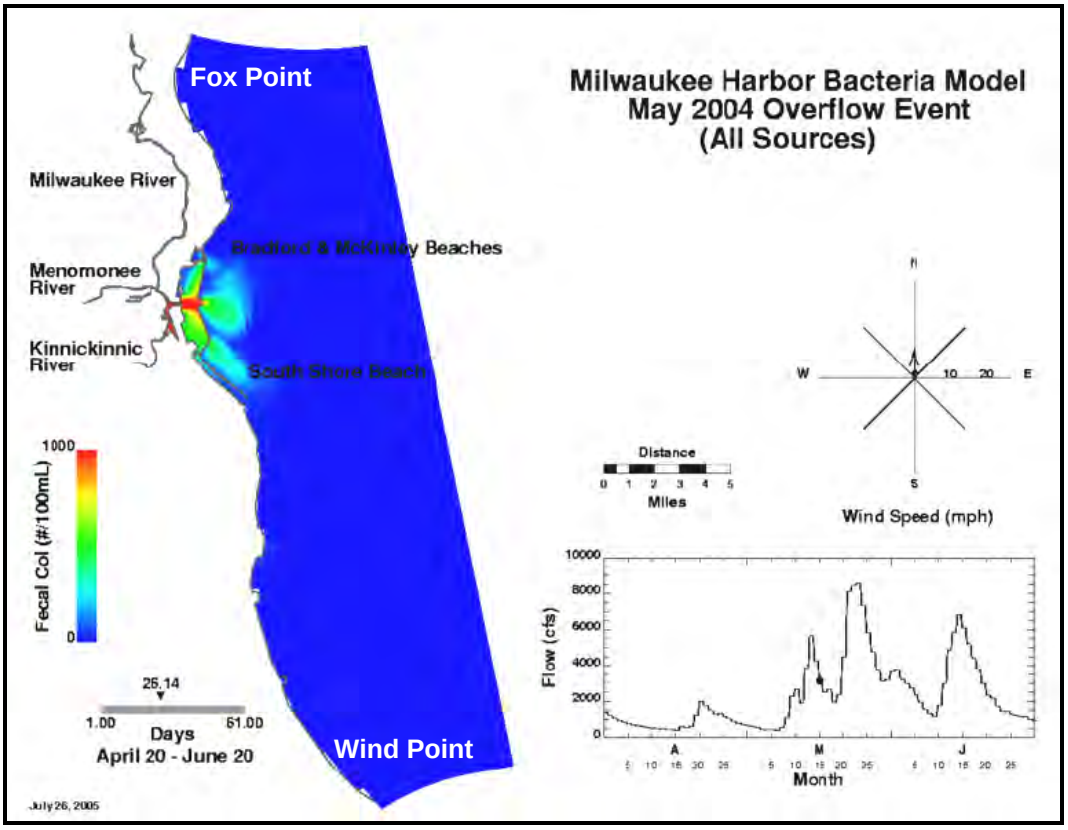


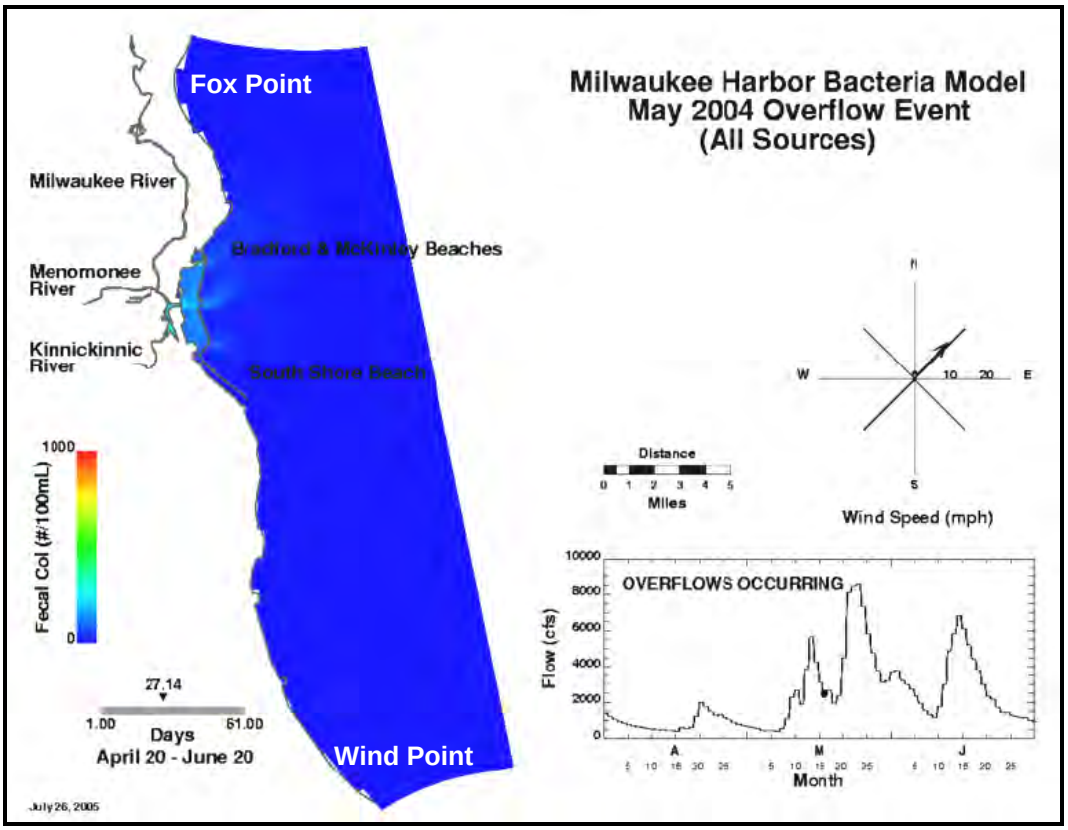
Hydrodynamic simulation/animation of Fecal coliform bacteria transport/dispersion in to Lake Michigan during extreme wet weather (May 11th & 12th, 2004). The aerial extend of the bacteria contamination and concentrations are limited to several kilometers even at peak river discharge. Bacteria levels represent the combined effect of CSO's, SSO's and stormwater runoff.



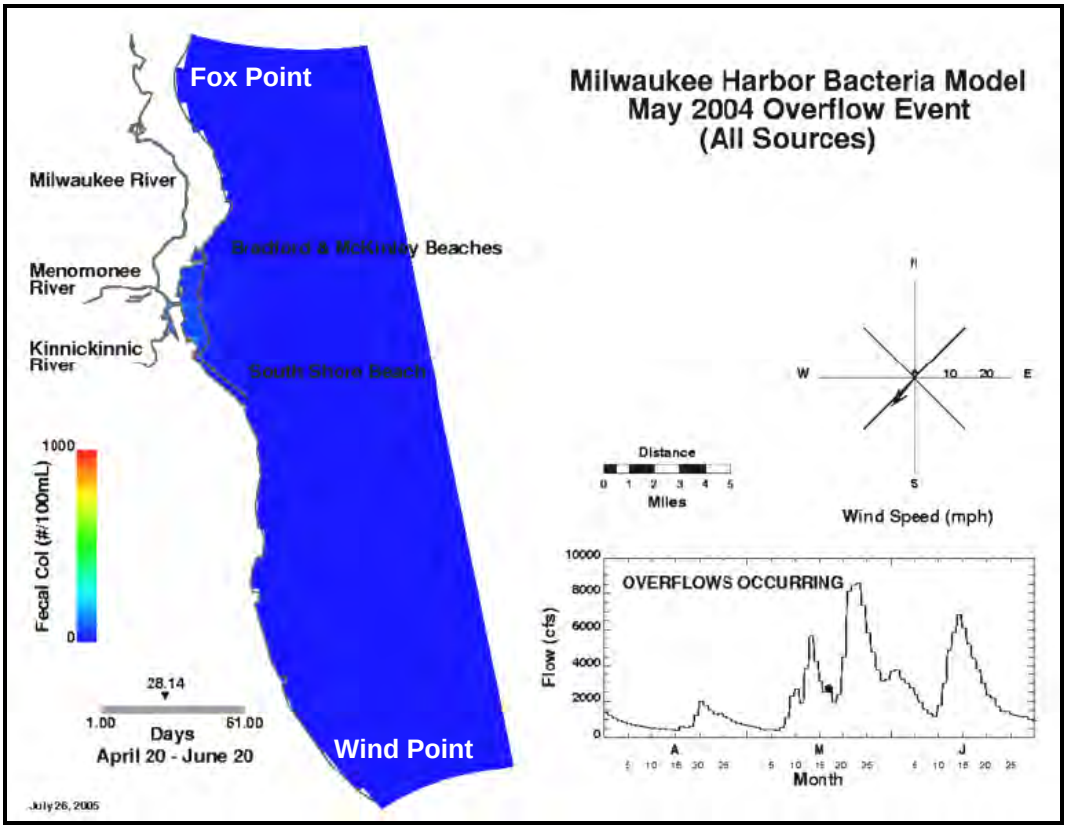


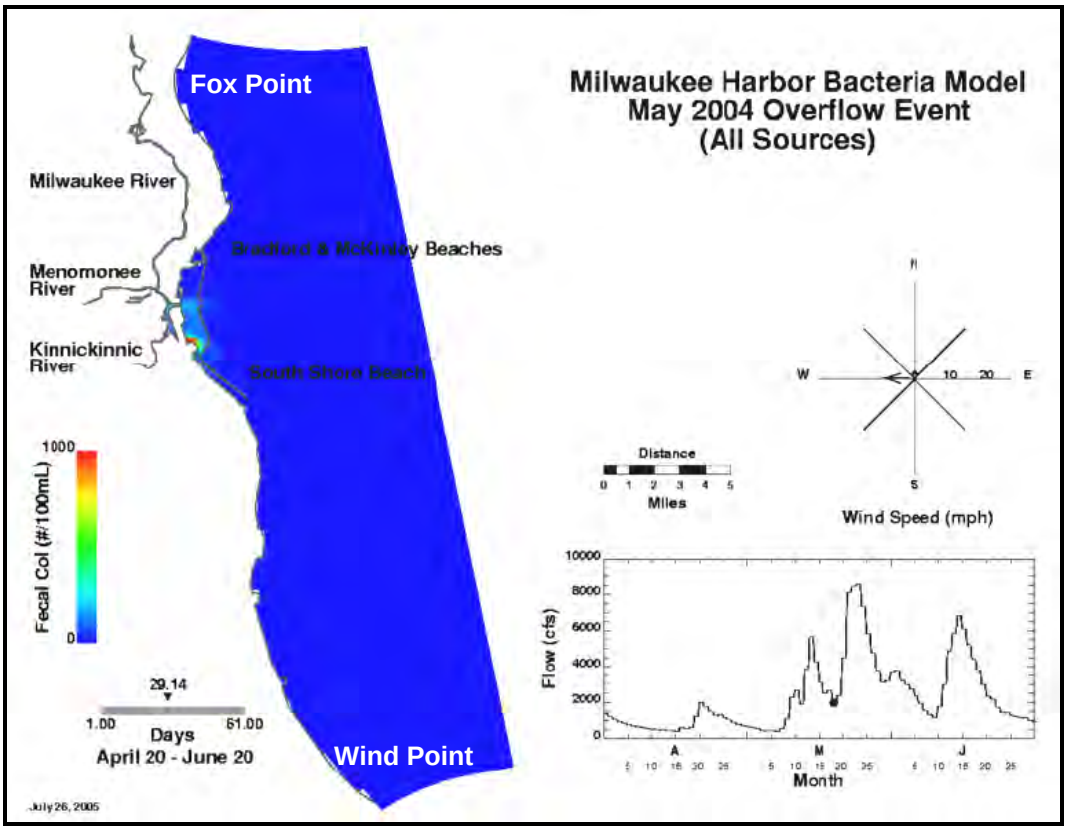
Hydrodynamic simulation/animation of Fecal coliform bacteria transport/dispersion in to Lake Michigan during extreme wet weather (May 13th & 14th, 2004). The aerial extend of the bacteria contamination and concentrations are limited to several kilometers even at peak river discharge. Bacteria levels represent the combined effect of CSO's, SSO's and stormwater runoff.



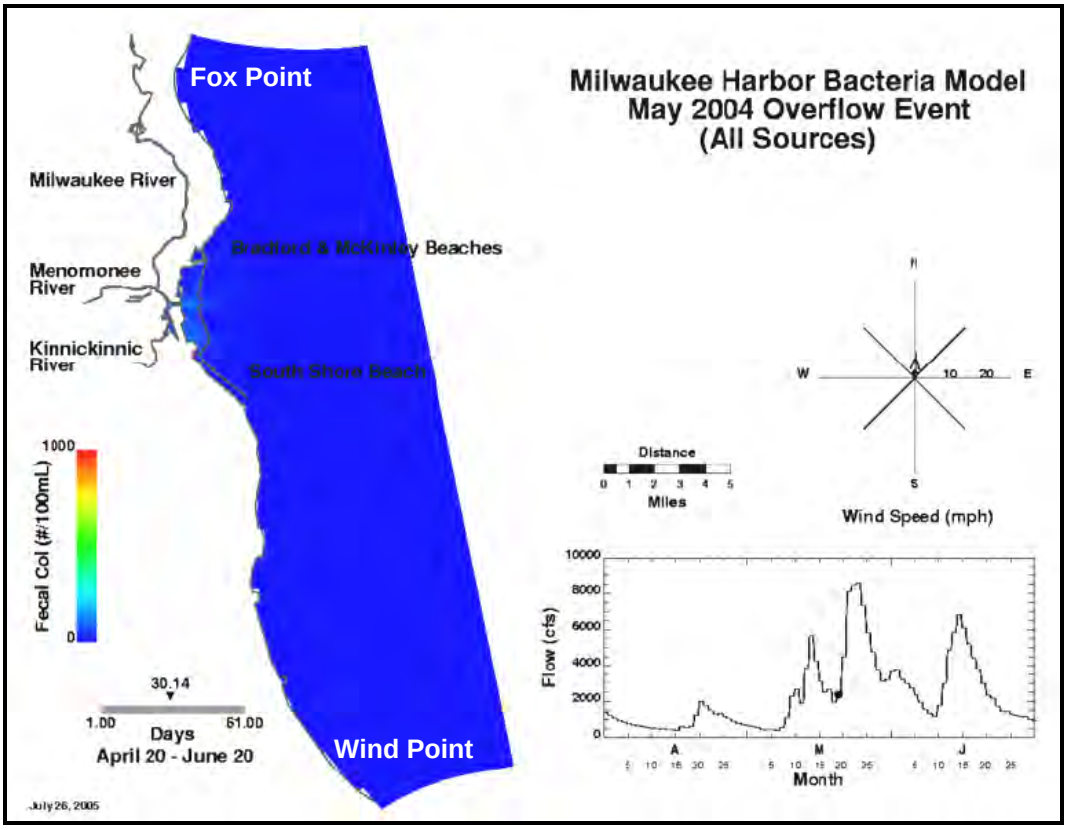


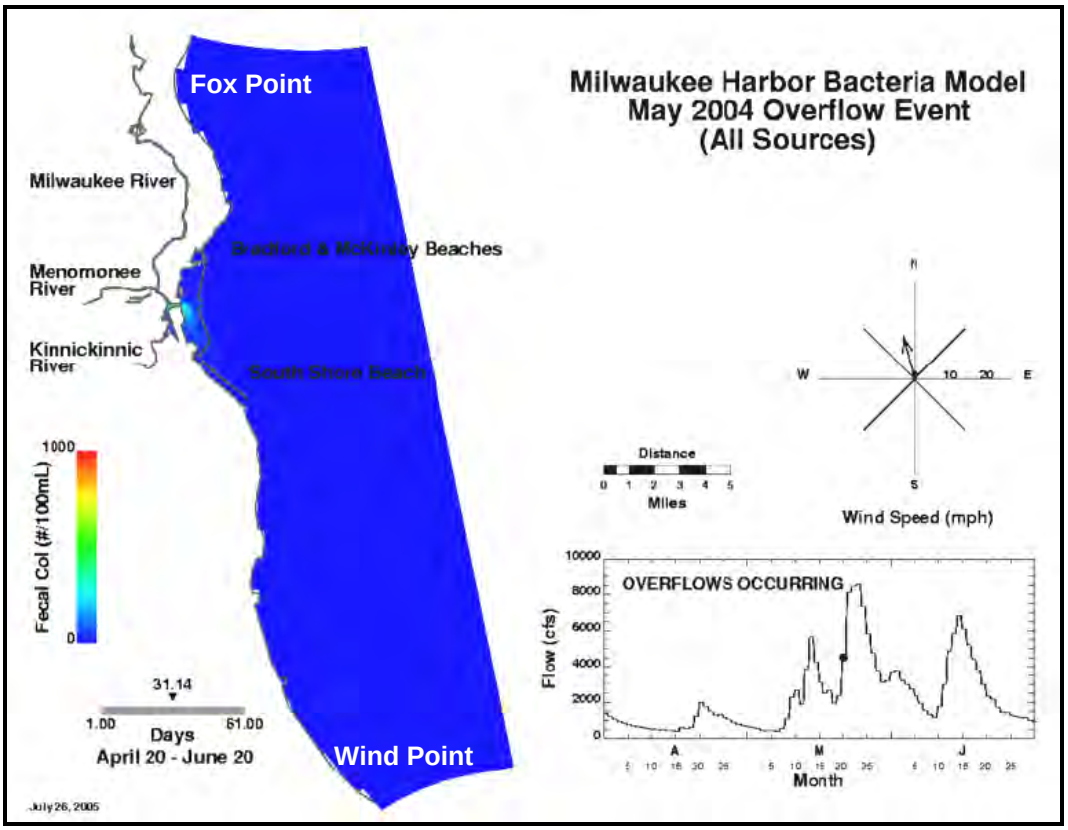
Hydrodynamic simulation/animation of Fecal coliform bacteria transport/dispersion in to Lake Michigan during extreme wet weather (May 15th & 16th, 2004). The aerial extend of the bacteria contamination and concentrations are limited to several kilometers even at peak river discharge. Bacteria levels represent the combined effect of CSO's, SSO's and stormwater runoff.



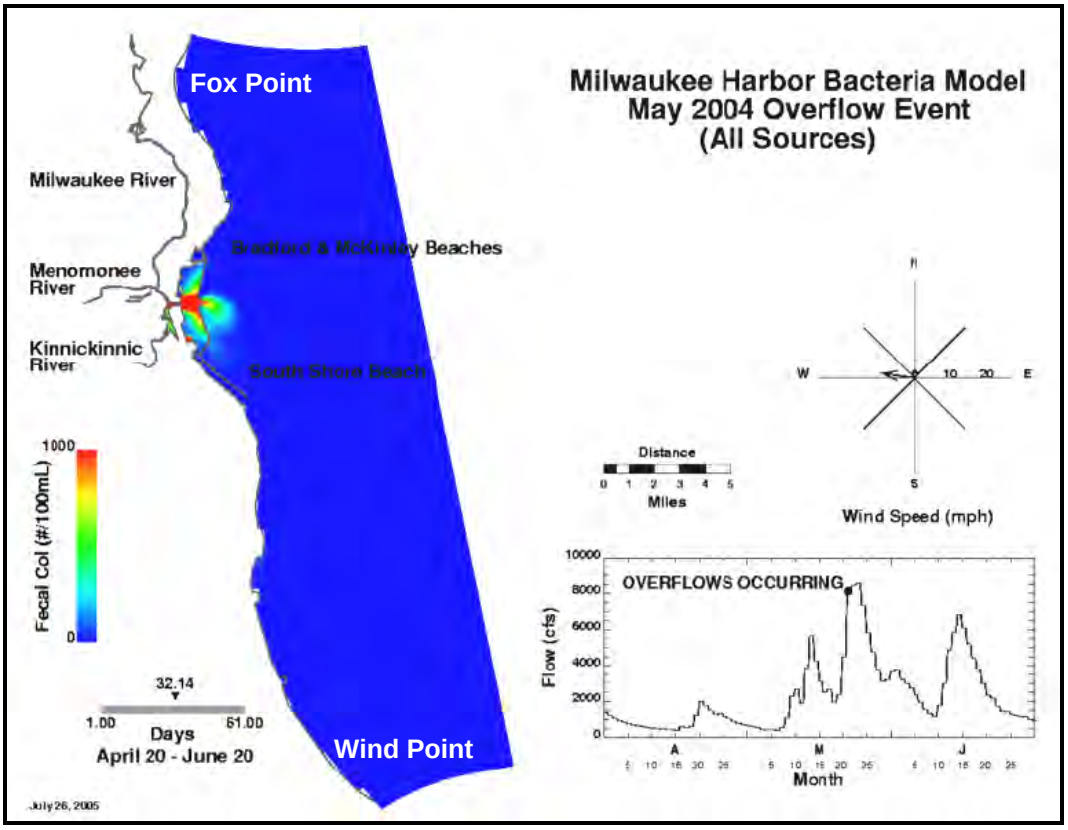


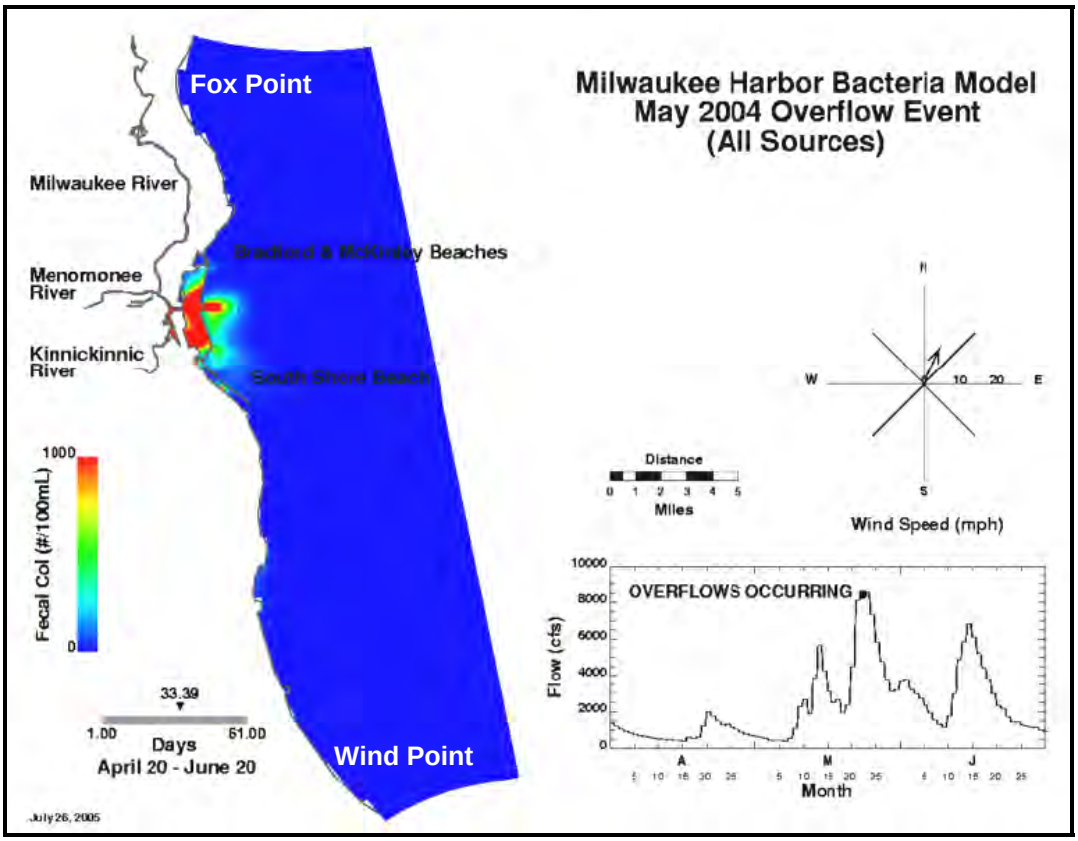
Hydrodynamic simulation/animation of Fecal coliform bacteria transport/dispersion in to Lake Michigan during extreme wet weather (May 17th & 18th, 2004). The aerial extend of the bacteria contamination and concentrations are limited to several kilometers even at peak river discharge. Bacteria levels represent the combined effect of CSO's, SSO's and stormwater runoff.



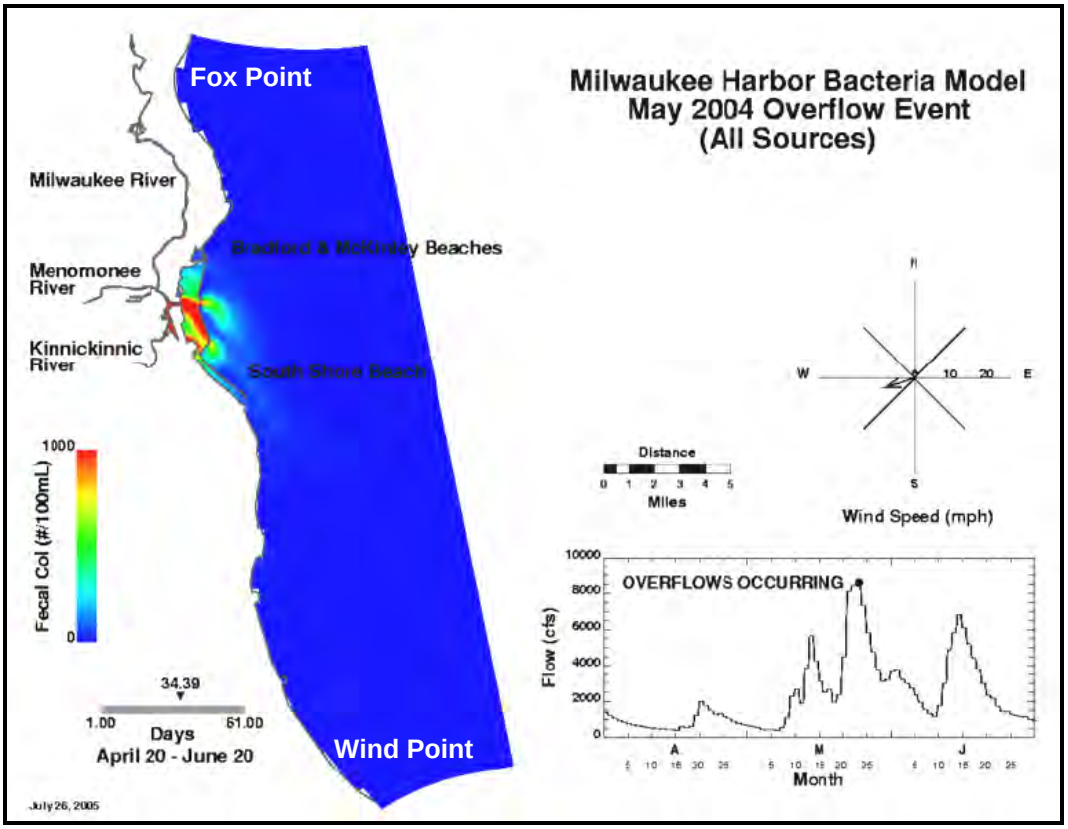


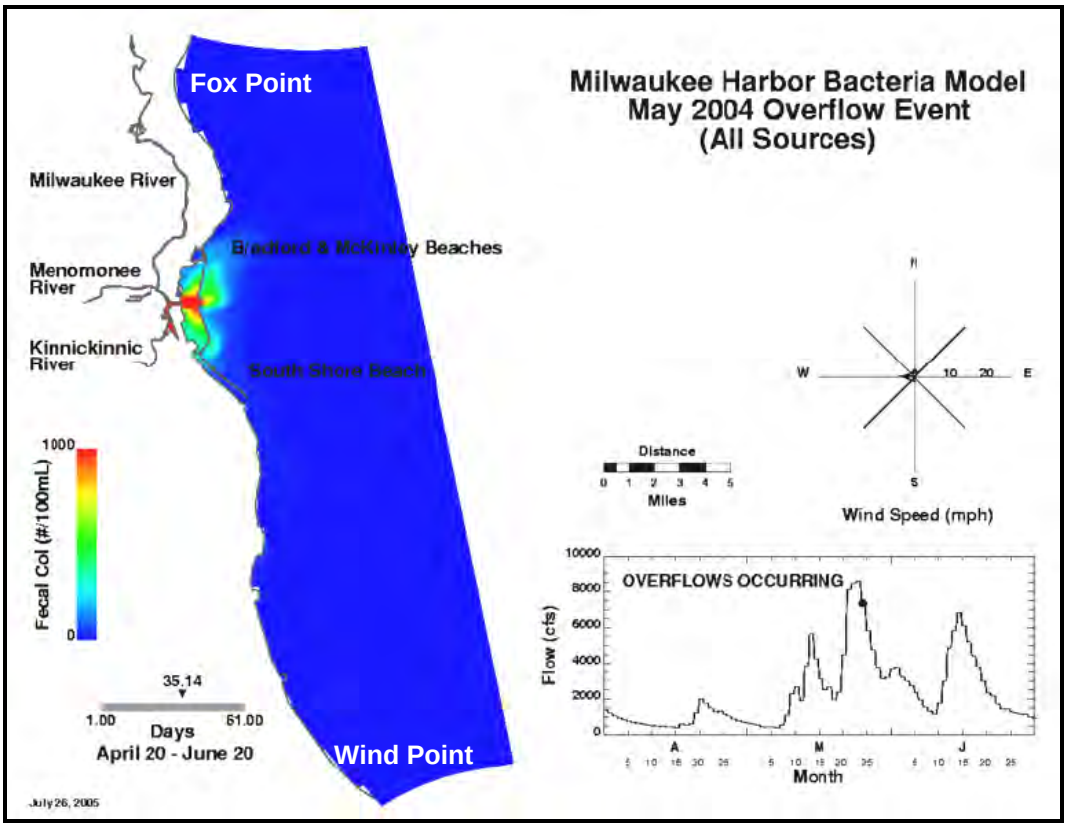
Hydrodynamic simulation/animation of Fecal coliform bacteria transport/dispersion in to Lake Michigan during extreme wet weather (May 19th & 20th, 2004). The aerial extend of the bacteria contamination and concentrations are limited to several kilometers even at peak river discharge. Bacteria levels represent the combined effect of CSO's, SSO's and stormwater runoff.



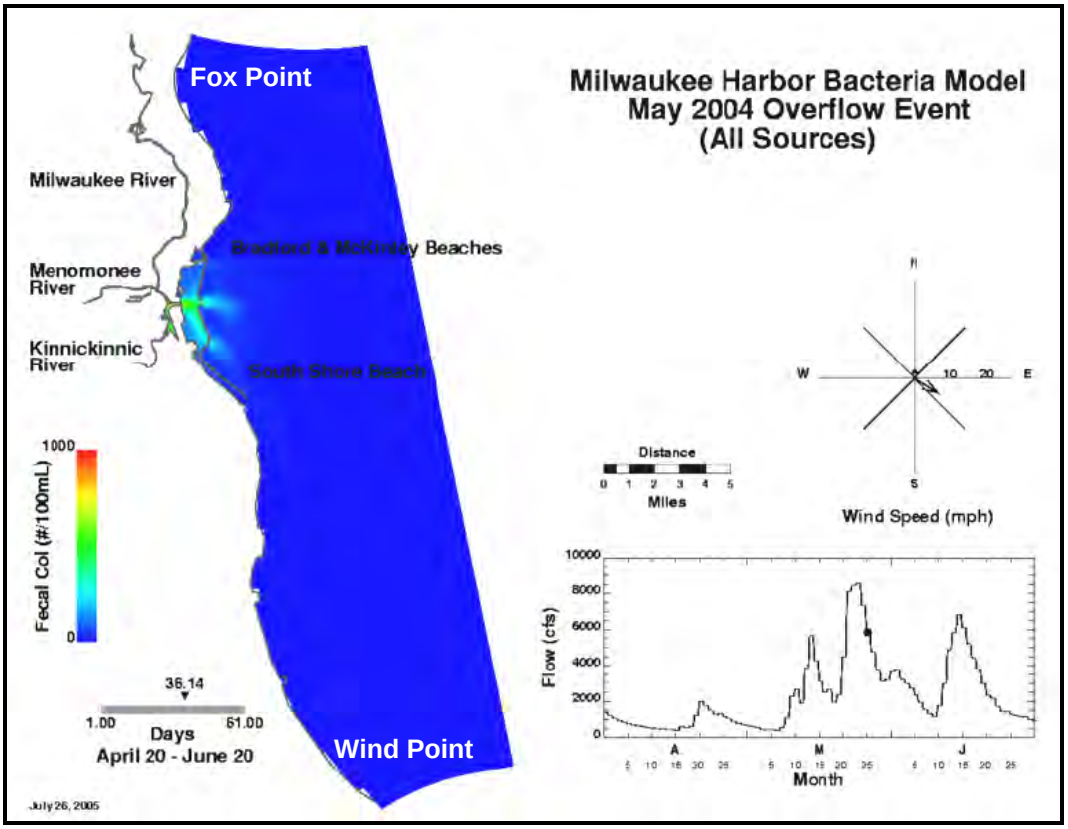


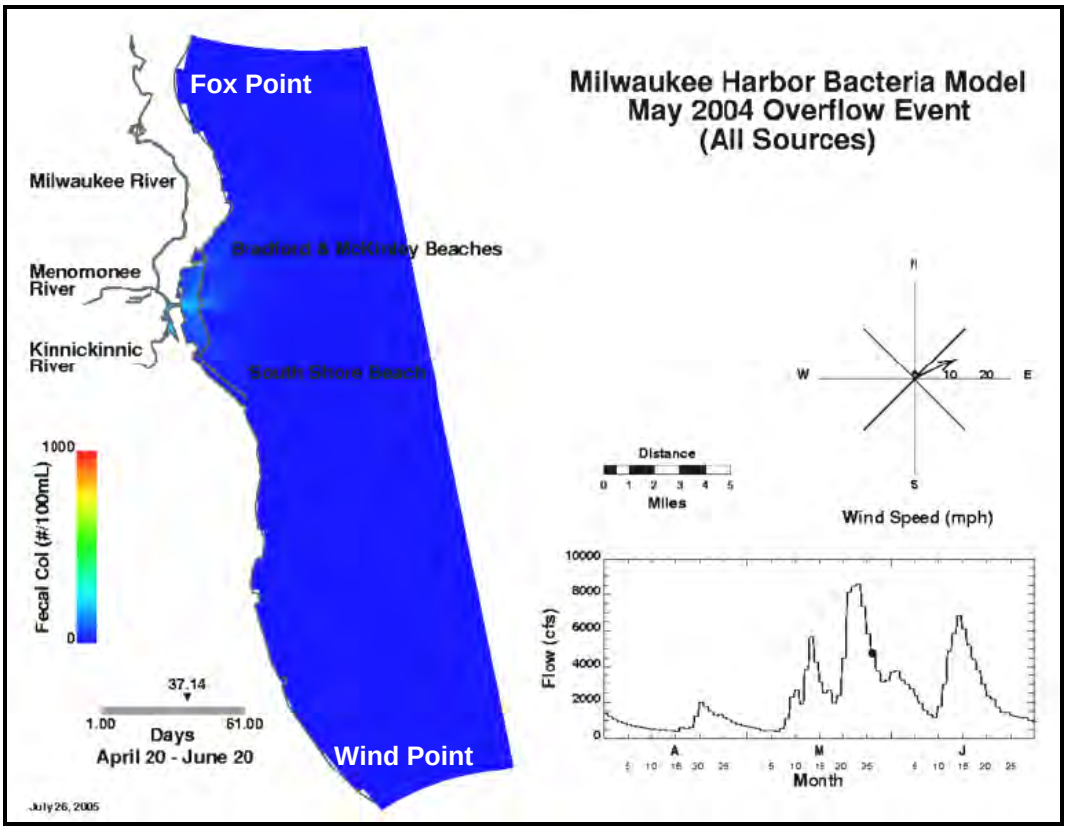
Hydrodynamic simulation/animation of Fecal coliform bacteria transport/dispersion in to Lake Michigan during extreme wet weather (May 21st & 22nd, 2004). The aerial extend of the bacteria contamination and concentrations are limited to several kilometers even at peak river discharge. Bacteria levels represent the combined effect of CSO's, SSO's and stormwater runoff.



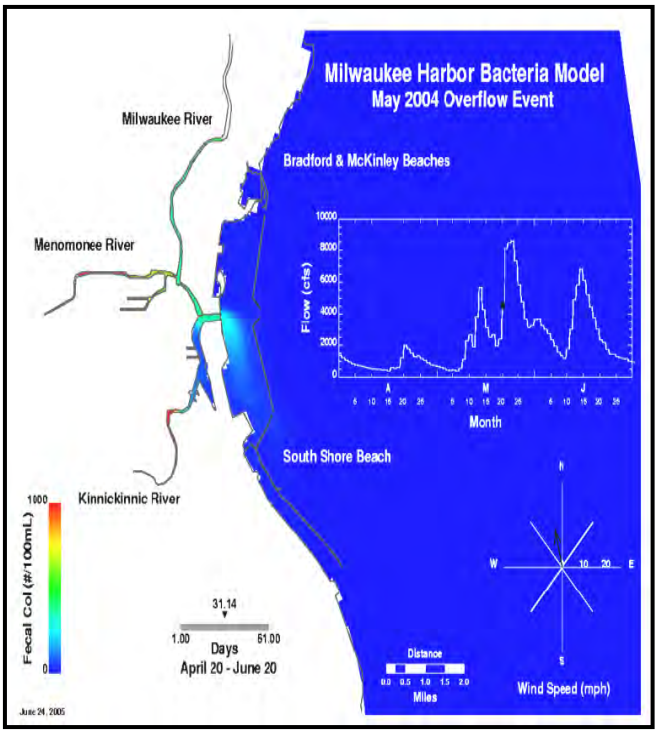


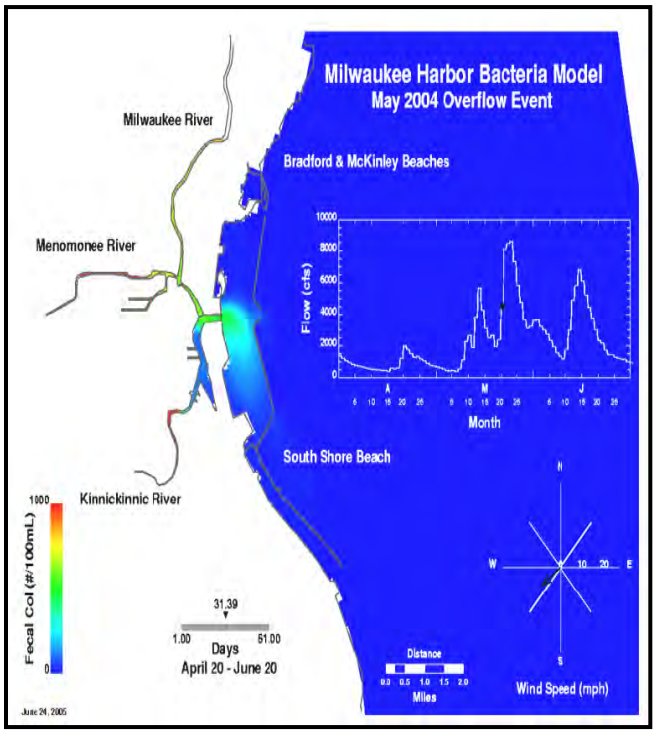
Hydrodynamic simulation/animation of Fecal coliform bacteria transport/dispersion in to Lake Michigan during extreme wet weather (May 23rd & 24th, 2004). The aerial extend of the bacteria contamination and concentrations are limited to several kilometers even at peak river discharge. Bacteria levels represent the combined effect of CSO's, SSO's and stormwater runoff.

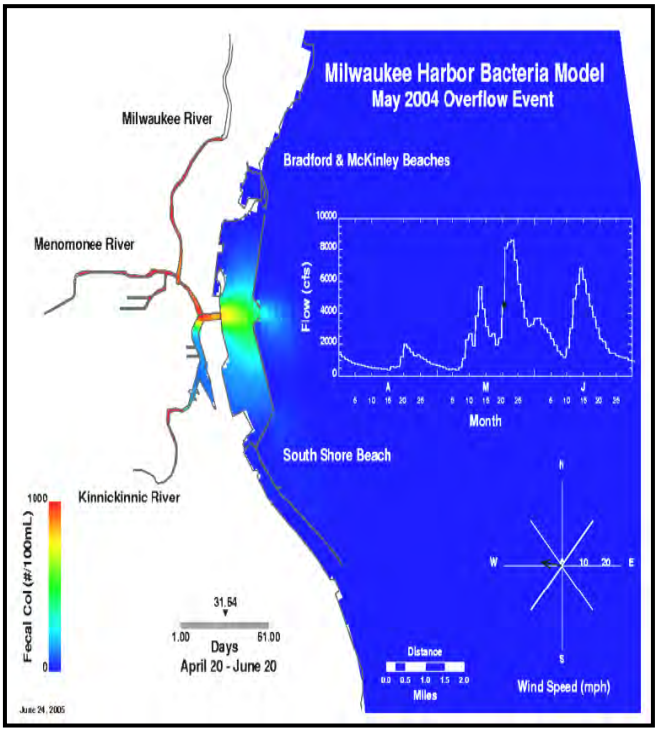


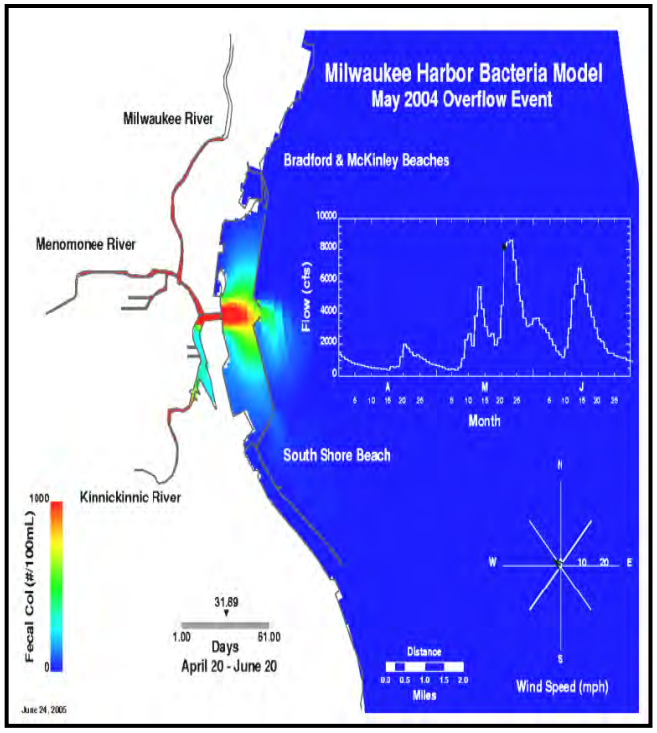


Hydrodynamic simulation/animation of Fecal coliform bacteria transport/dispersion in to Lake Michigan during extreme wet weather (May 25th & 26th, 2004). The aerial extend of the bacteria contamination and concentrations are limited to several kilometers even at peak river discharge. Bacteria levels represent the combined effect of CSO's, SSO's and stormwater runoff.

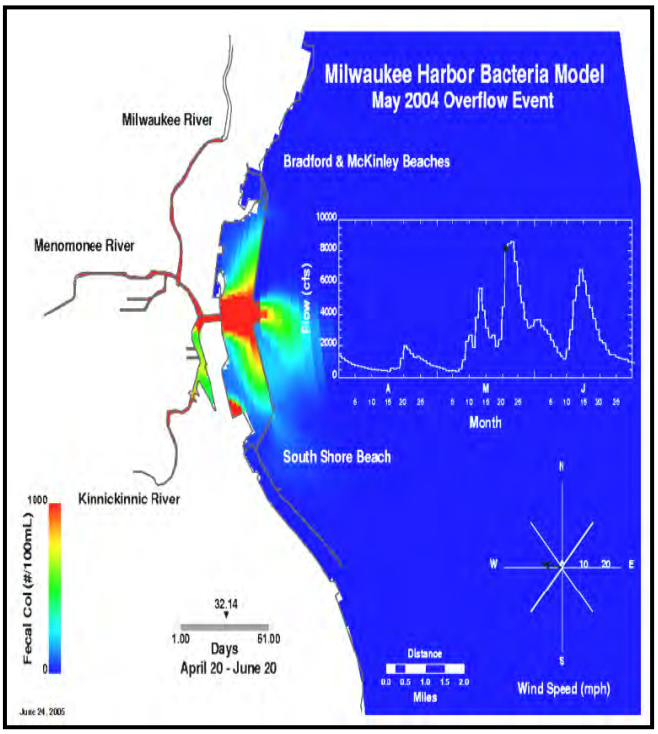


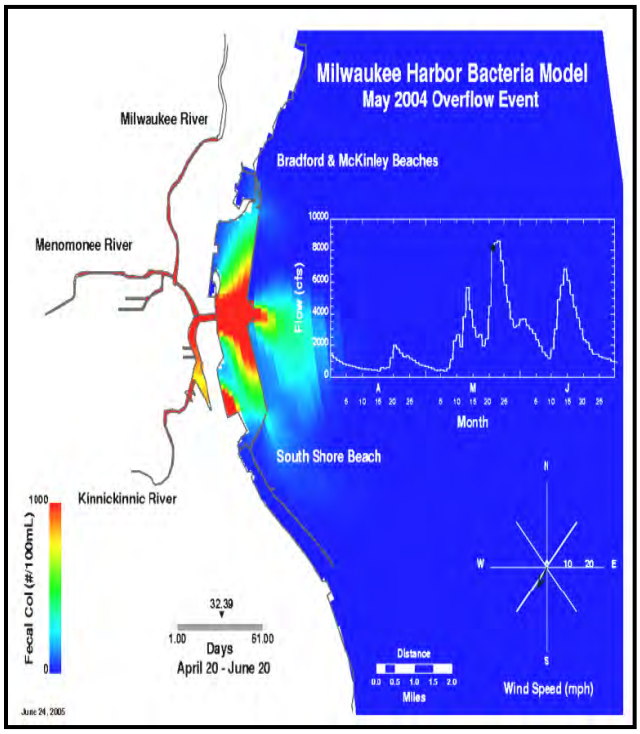


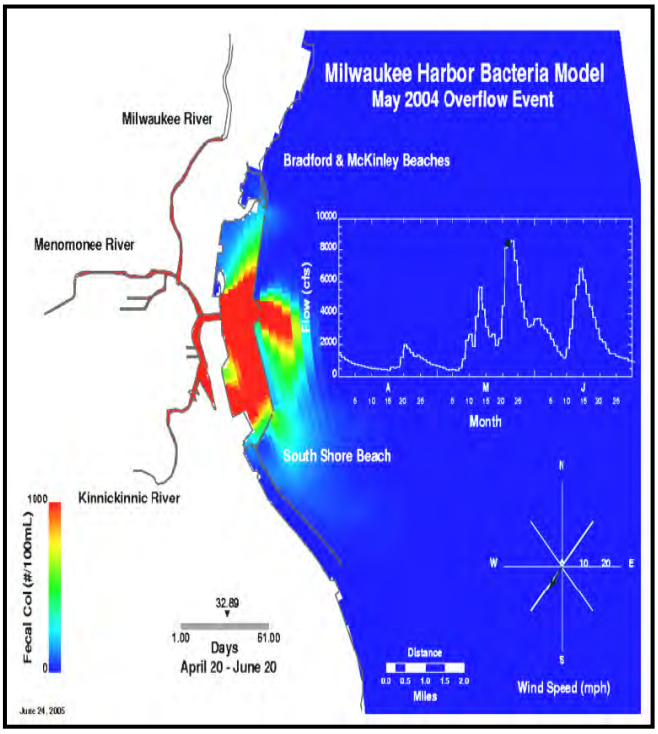


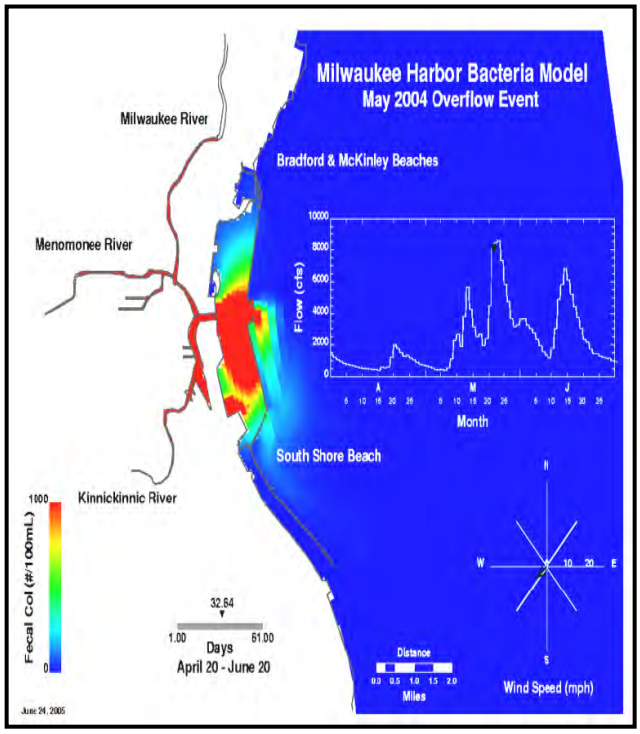


Hydrodynamic simulation/animation of Fecal coliform bacteria transport/dispersion in to Milwaukee's Outer Harbor and Lake Michigan during extreme wet weather on May 20, 2004. Each panel represents sequential 6 hour time intervals along the daily hydrograph.

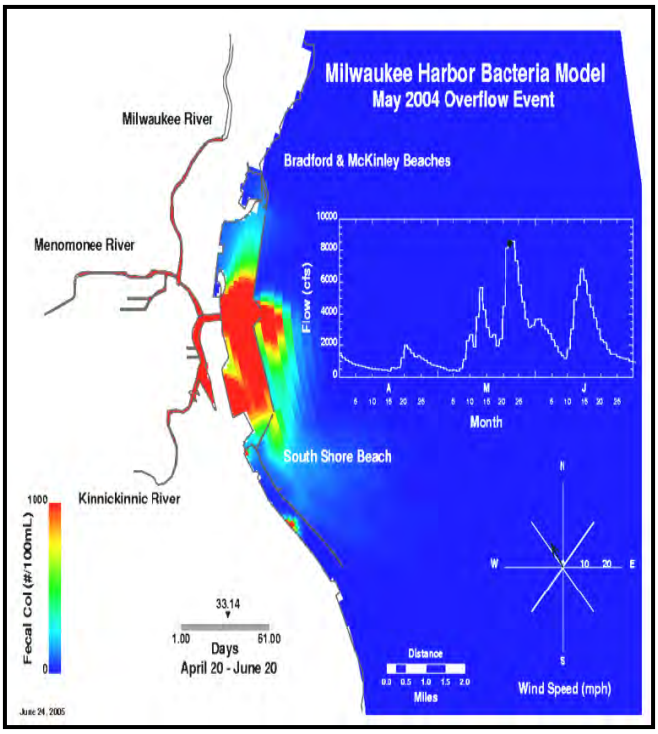


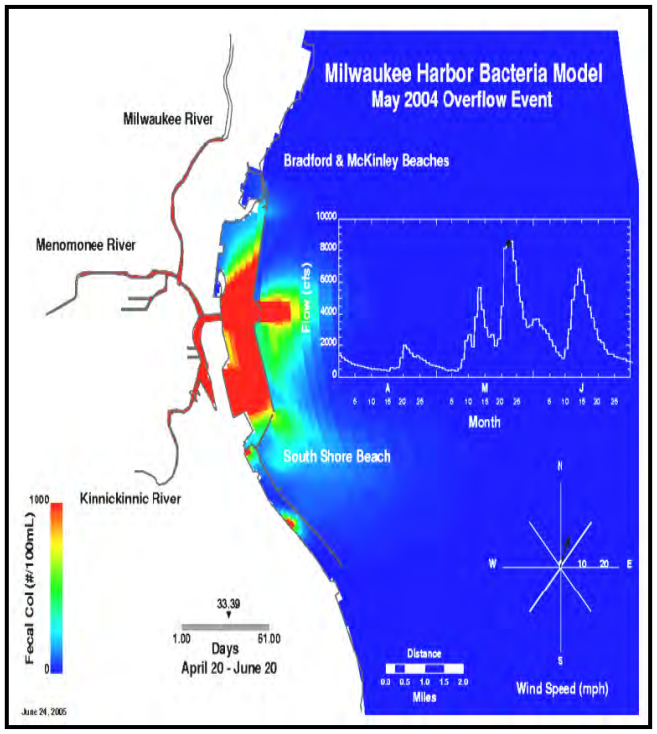


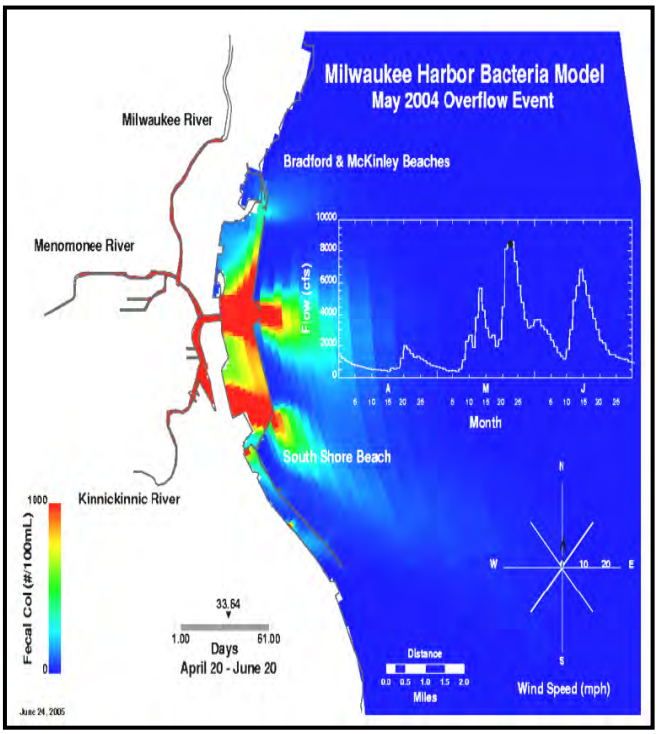


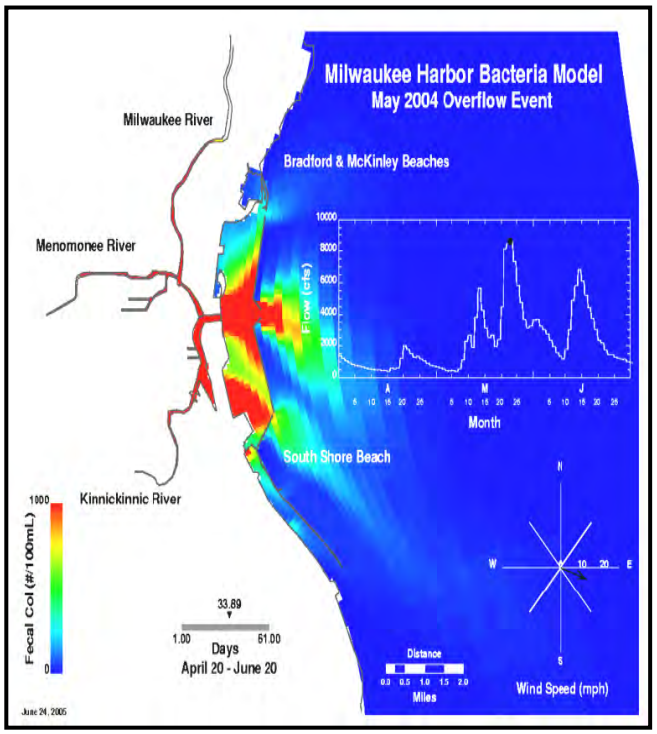


Hydrodynamic simulation/animation of Fecal coliform bacteria transport/dispersion in to Milwaukee's Outer Harbor and Lake Michigan during extreme wet weather on May 21, 2004. Each panel represents sequential 6 hour time intervals along the daily hydrograph.

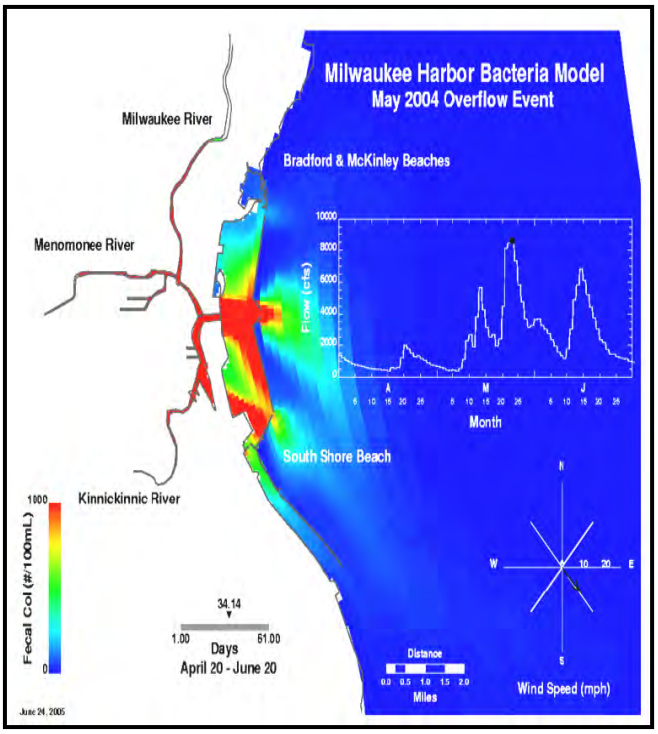


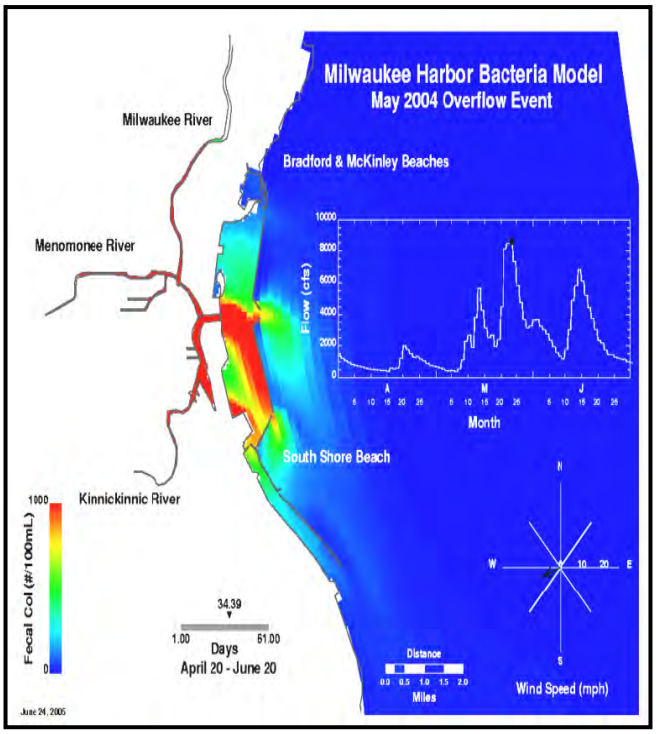


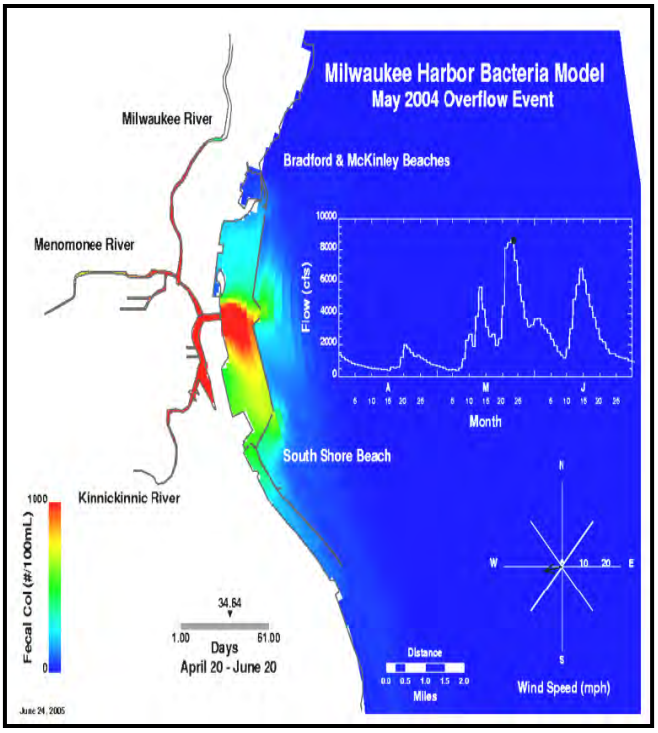


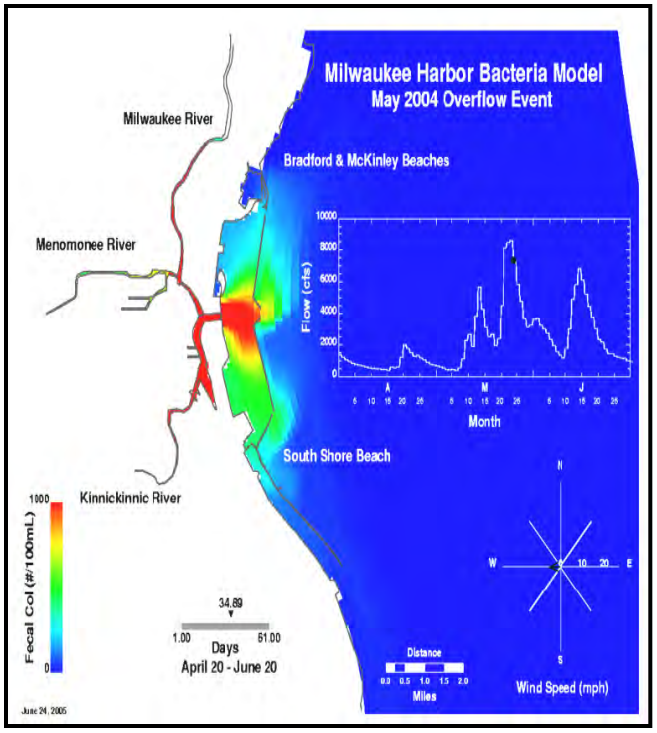


Hydrodynamic simulation/animation of Fecal coliform bacteria transport/dispersion in to Milwaukee's Outer Harbor and Lake Michigan during extreme wet weather on May 22, 2004. Each panel represents sequential 6 hour time intervals along the daily hydrograph.

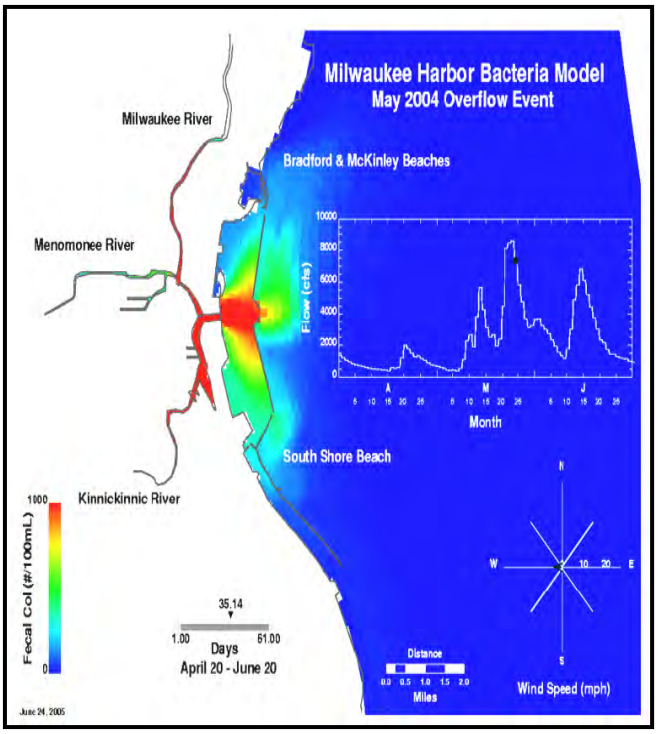


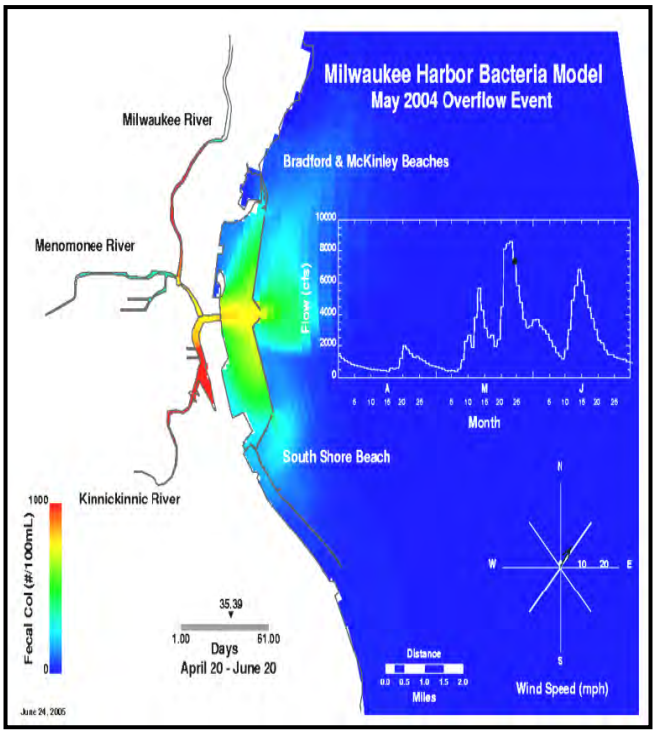


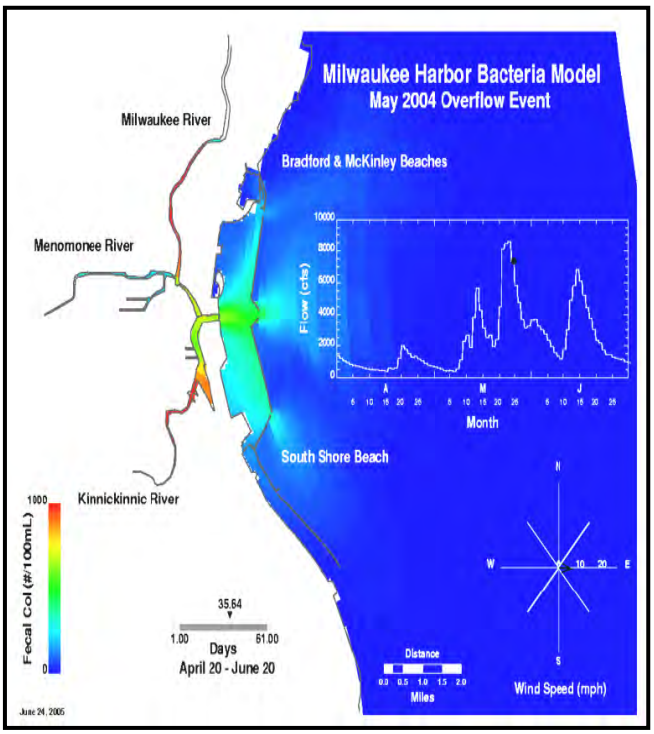


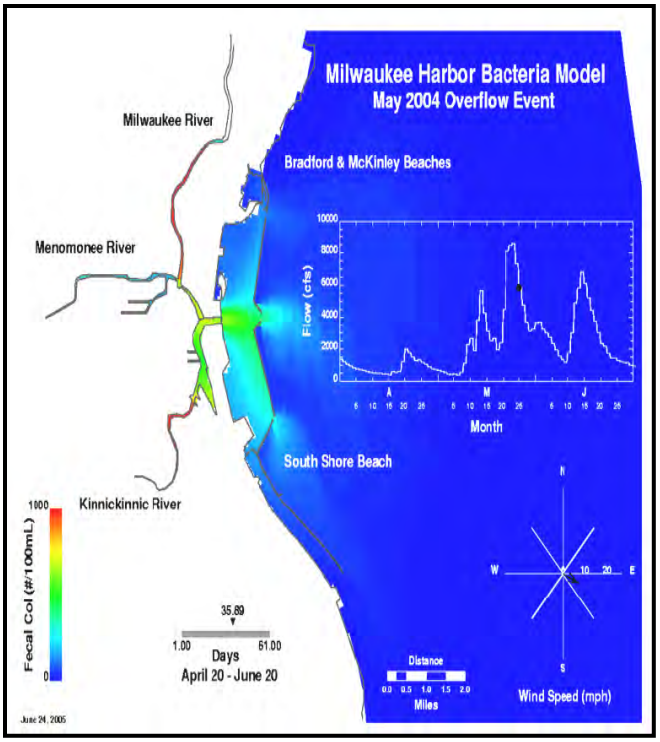


Hydrodynamic simulation/animation of Fecal coliform bacteria transport/dispersion in to Milwaukee's Outer Harbor and Lake Michigan during extreme wet weather on May 23, 2004. Each panel represents sequential 6 hour time intervals along the daily hydrograph.

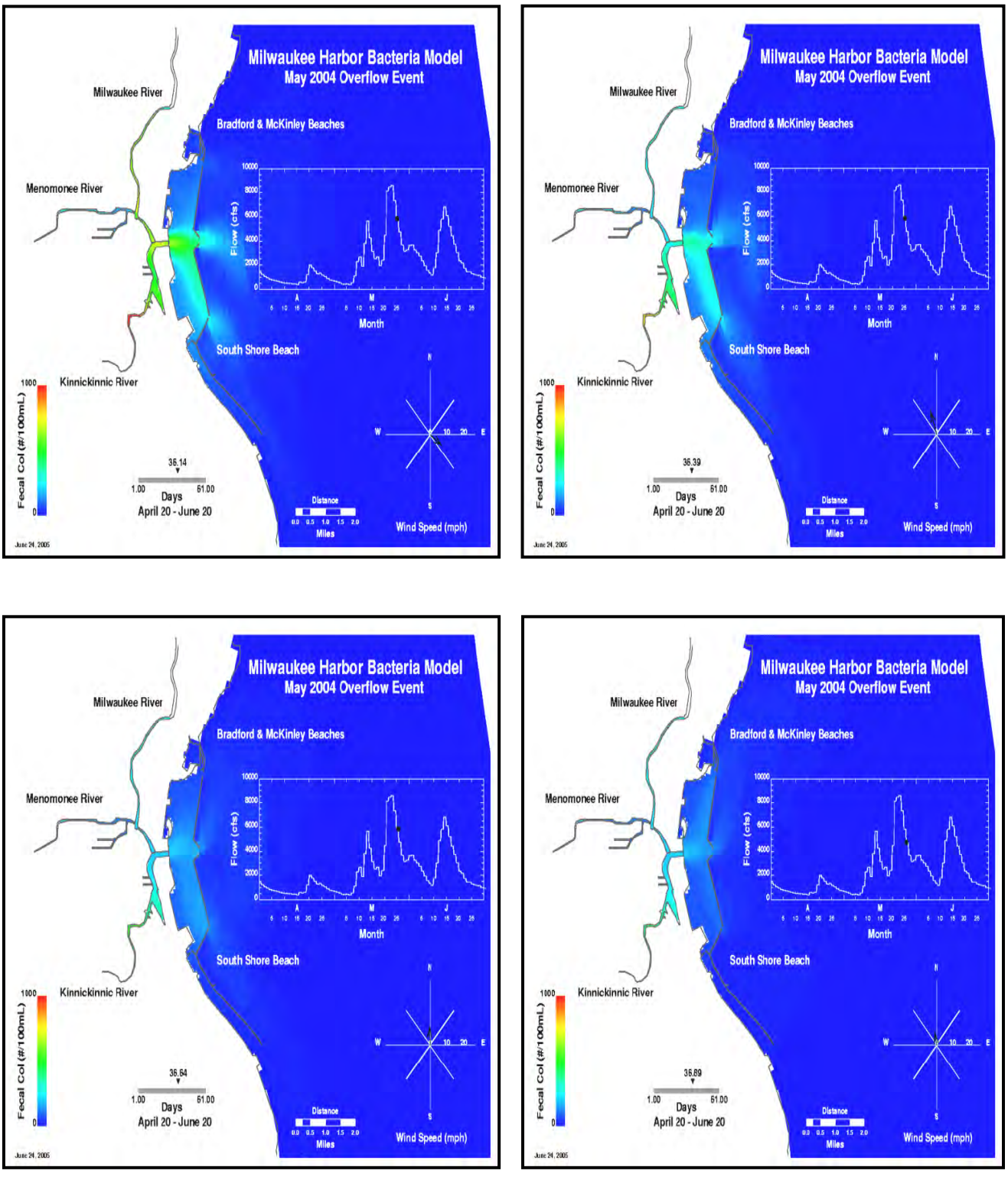




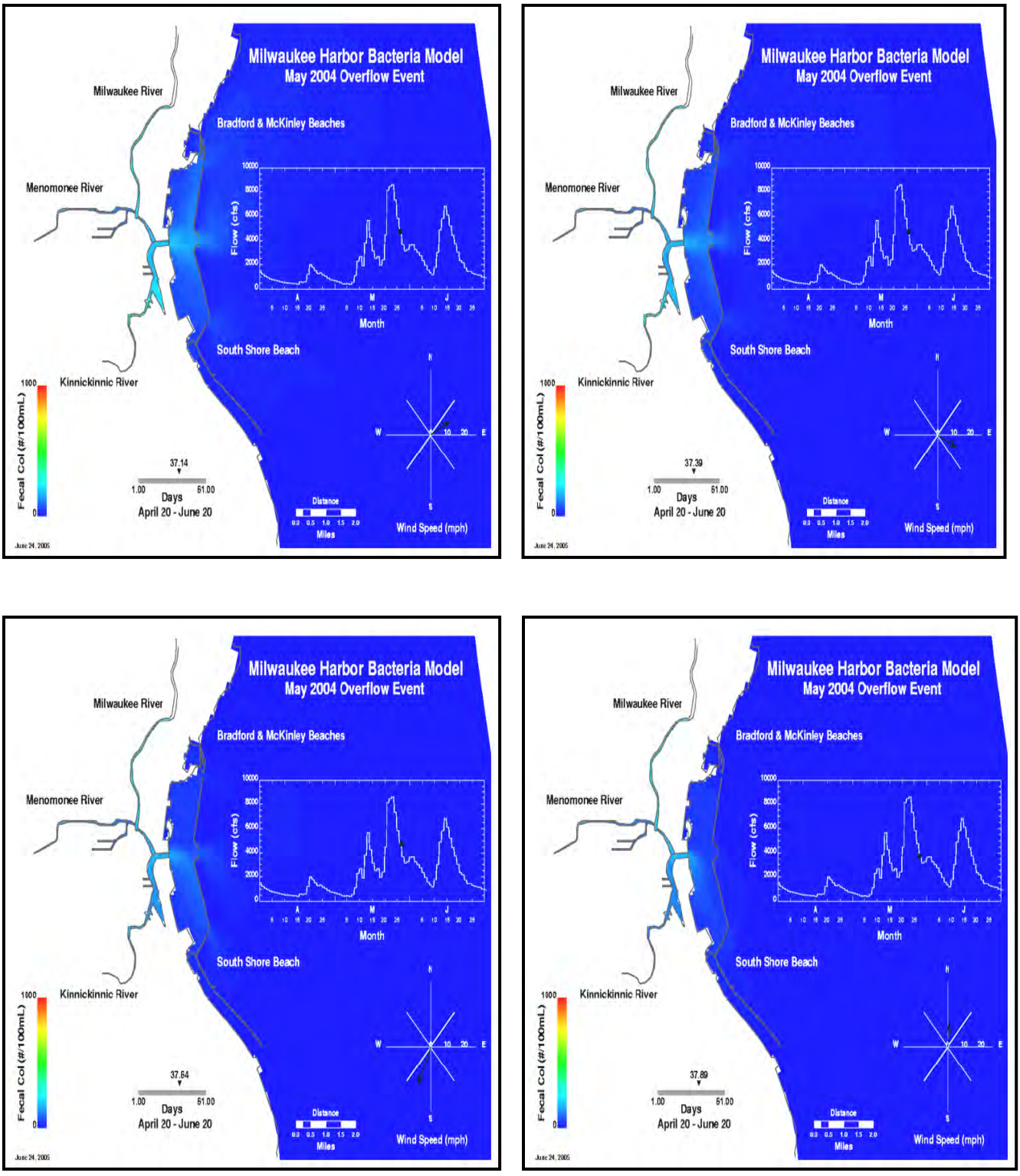




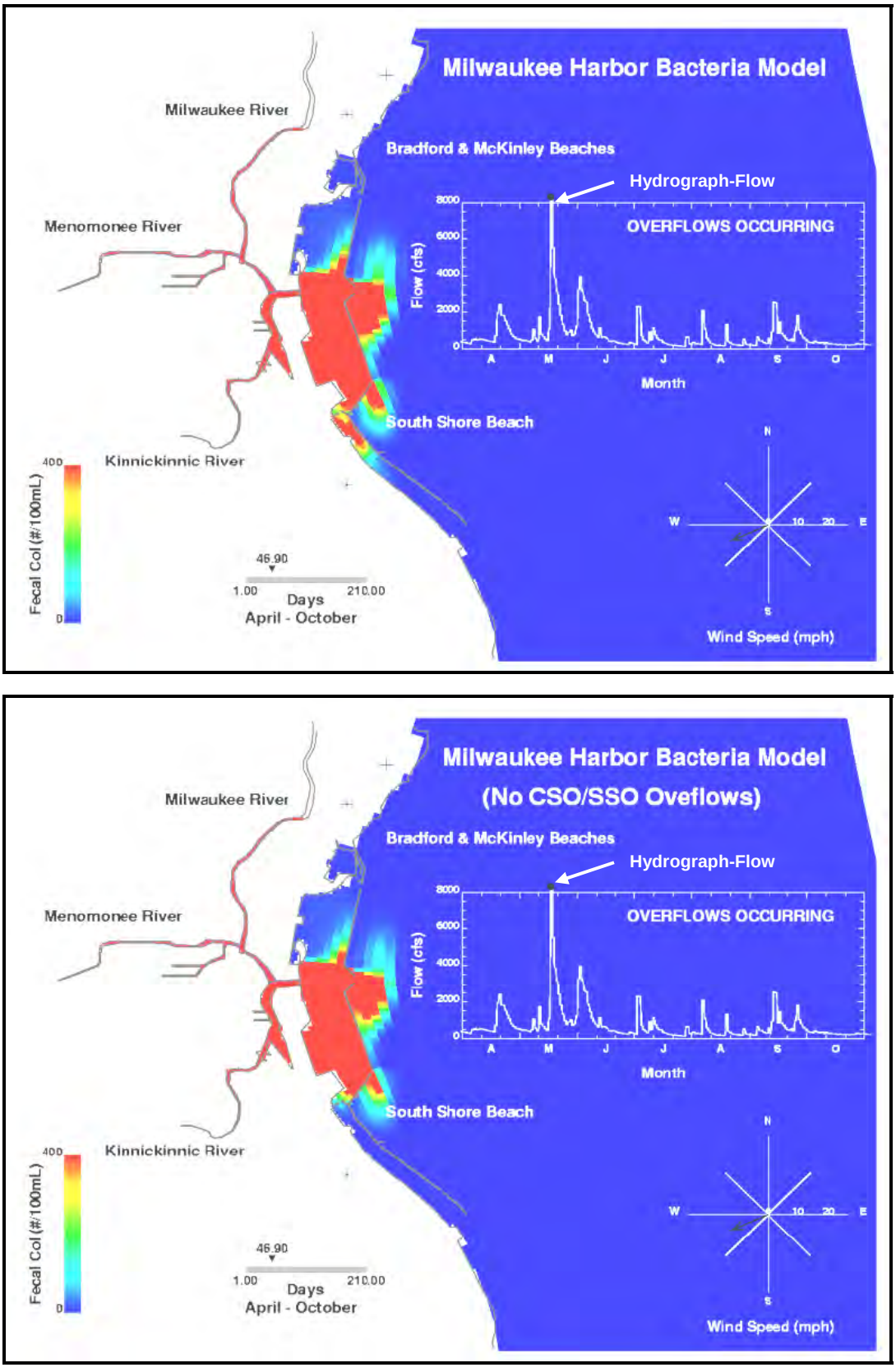
Hydrodynamic simulation/animation of Fecal coliform bacteria transport/dispersion in to Milwaukee's Outer Harbor and Lake Michigan during extreme wet weather on May 24, 2004. Each panel represents sequential 6 hour time intervals along the daily hydrograph.



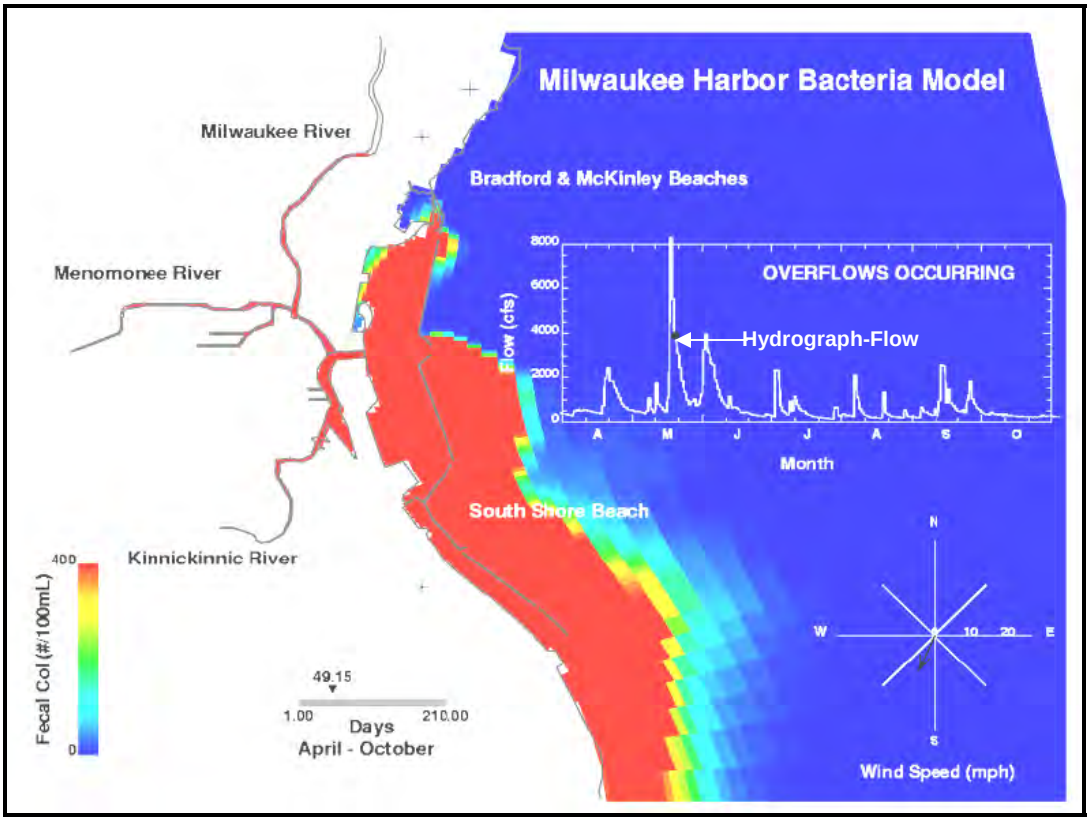
Hydrodynamic simulation/animation of Fecal coliform bacteria transport/dispersion in to Milwaukee's Outer Harbor and Lake Michigan during extreme wet weather on May 25, 2004. Each panel represents sequential 6 hour time intervals along the daily hydrograph.

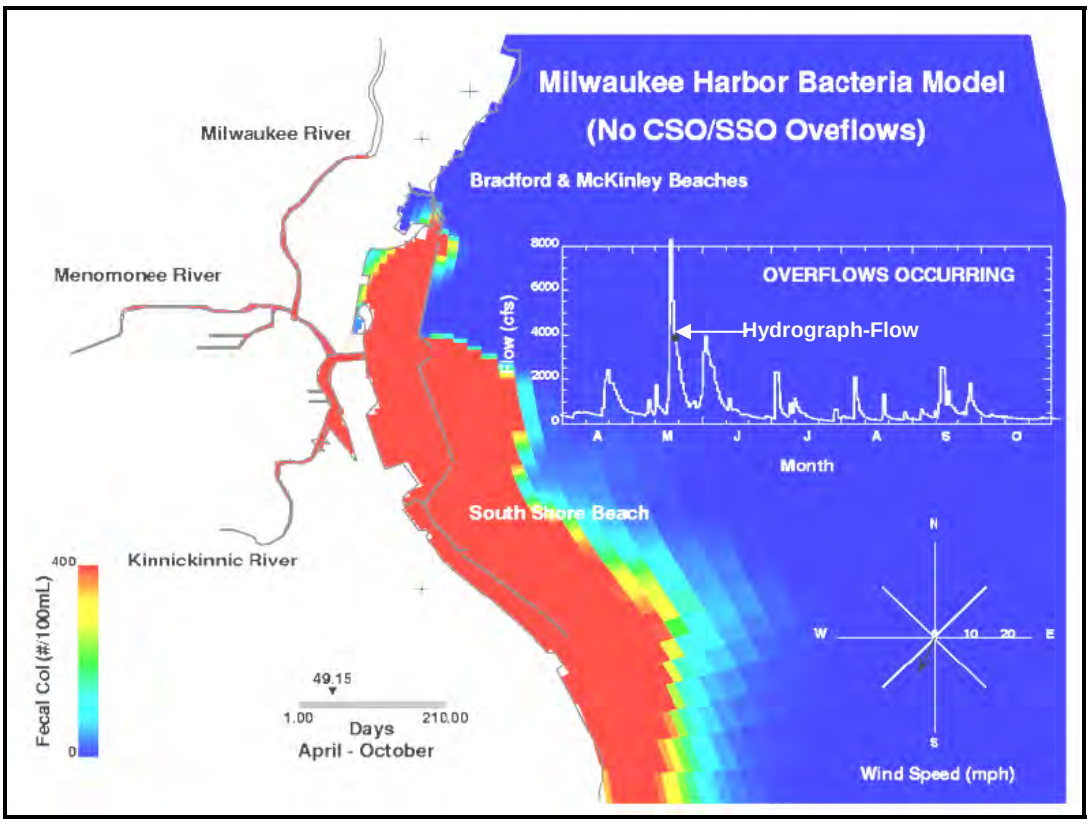


Hydrodynamic simulation/animation of Fecal coliform bacteria transport/dispersion in to Milwaukee's Outer Harbor and Lake Michigan during extreme wet weather on May 26, 2004. Each panel represents sequential 6 hour time intervals along the daily hydrograph.

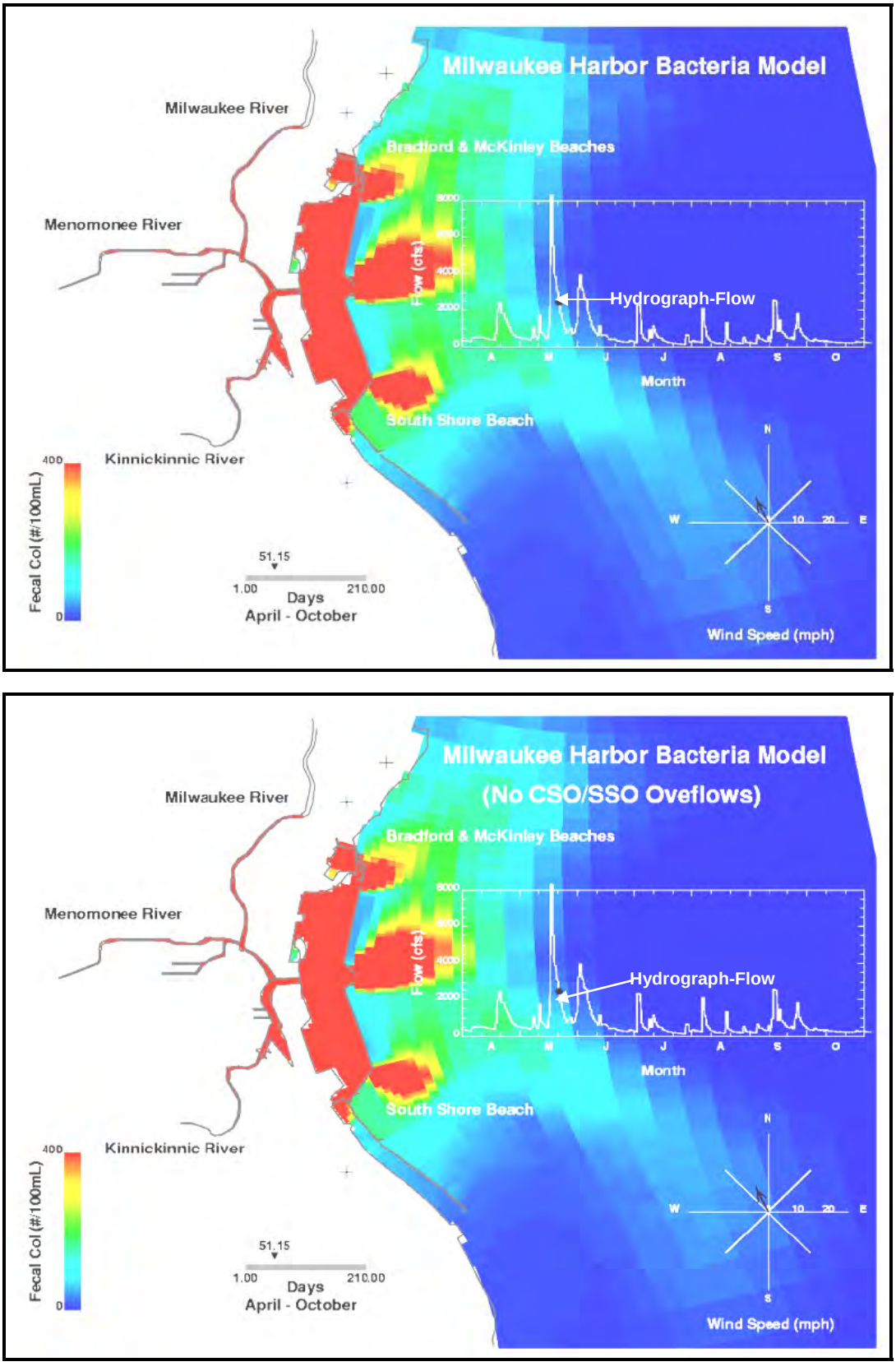


Hydrodynamic animation of Fecal coliform bacteria (FC) dispersion in to Milwaukee's Outer Harbor and Lake Michigan during extreme wet weather (May, 2000). The water quality standard for Fecal coliform bacteria (400 counts/ 100 mL) is exceeded whether there were CSOs and SSOs (upper panel) for a specific time period or whether there were no overflows for the same time period (CSO and SSO inputs removed from model analysis - lower panel).

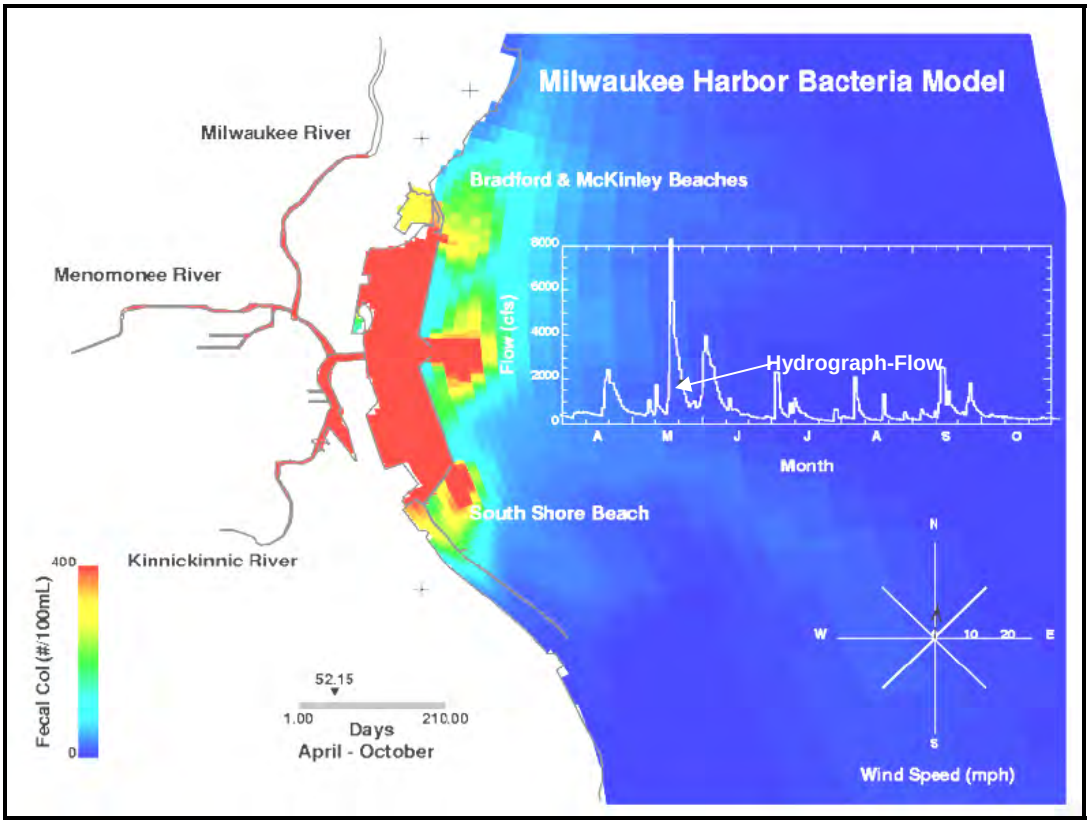


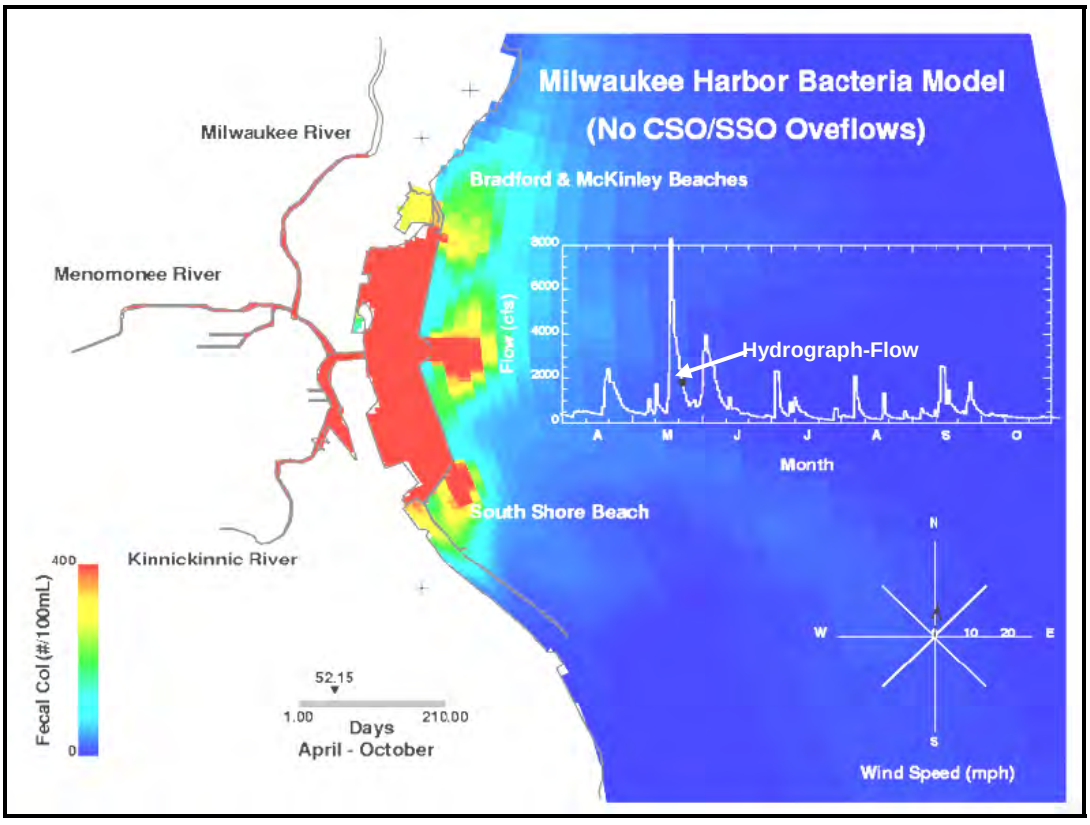


Hydrodynamic animation of Fecal coliform bacteria (FC) dispersion in to Milwaukee's Outer Harbor and Lake Michigan during extreme wet weather (May, 2000). The water quality standard for Fecal coliform bacteria (400 counts/ 100 mL) is exceeded whether there were CSOs and SSOs (upper panel) for a specific time period or whether there were no overflows for the same time period (CSO and SSO inputs removed from model analysis - lower panel).



Hydrodynamic animation of Fecal coliform bacteria (FC) dispersion in to Milwaukee's Outer Harbor and Lake Michigan during extreme wet weather (May, 2000). The water quality standard for Fecal coliform bacteria (400 counts/ 100 mL) is exceeded whether there were CSOs and SSOs (upper panel) for a specific time period or whether there were no overflows for the same time period (CSO and SSO inputs removed from model analysis - lower panel).





Hydrodynamic animation of Fecal coliform bacteria (FC) dispersion in to Milwaukee's Outer Harbor and Lake Michigan during extreme wet weather (May, 2000). The water quality standard for Fecal coliform bacteria (400 counts/ 100 mL) is exceeded whether there were CSOs and SSOs (upper panel) for a specific time period or whether there were no overflows for the same time period (CSO and SSO inputs removed from model analysis - lower panel).

APPENDIX VI

PUBLIC COMMUNICATIONS (2003-2005)

INVITED SPEAKER

Hollis, E.J. Sierra Club Great Lakes Chapter Meeting. *Sewer Overflows and Stormwater Pollution in Lake Michigan.* January 19, 2006. Kenosha, WI.

McLellan, S. L. University of Cincinnati, Department of Environmental Health. *Sewage overflows and urban stormwater pollution in Lake Michigan*. January 11, 2006. Milwaukee, WI.

McLellan, S. L. Wisconsin Great Lakes Coalition, Inc. *Sources of pollution in Lake Michigan's waters.* December 10, 2005. Mequon, WI.

McLellan, S. L. University of Wisconsin-Parkside, Department of Biological Sciences. *Microbial source tracking in Lake Michigan*. November 11, 2005. Kenosha, WI.

McLellan, S. L. State of the Lake Conference. *Occurrence of genetic markers of fecal indicator bacteria in Lake Michigan*. November 3, 2005. Green Bay, WI.

Hollis, E. J. State of the Lake Conference. *The Impacts of Stormwater Runoff on Lake Michigan Beaches*. November 3, 2005. Green Bay, WI.

McLellan, S.L. Sheboygan River Basin Partnership. *Beach closings on the Great Lakes.* November 16, 2005. Sheboygan, WI.

McLellan, S.L. American Society of Civil Engineers Regional Meeting. *Survival and persistence of fecal indicator organisms in the coastal environment and molecular approaches to bacterial source tracking.* September 23, 2005. Green Bay, WI.

McLellan, S.L. Beach Closing Special, interview with Liz Coerner, Wisconsin Public Television. September 2005. Milwaukee, WI.

McLellan, S.L. Wisconsin Department of Natural Resources, SE District. Brown Bag Seminar Series. *E. coli Distribution in Lake Michigan and Integration into Hydrodynamic Modeling*. June 14, 2005. Milwaukee, WI.

McLellan, S.L. Milwaukee County Board of Supervisors. *Best Management Practices for Beaches.* May 17, 2005. Milwaukee, WI.

McLellan, S.L. Milwaukee River Revitalization Watershed Tour- State of Wisconsin Natural Resources Council. May 11, 2005. Milwaukee, WI.

McLellan, S.L. Southeast WI Beach Task Force. Public meeting and research update. May 11, 2005. Milwaukee, WI.

McLellan, S.L. Water Tower Trust Association. *Beach closings and Cladophora*. Lake Park Pavilion. May 4, 2005. Milwaukee, WI.

McLellan, S.L. National Council for Air and Stream Improvement Northern Regional Meeting. *E. coli survival and persistence in the environment and approaches to bacterial source tracking.* May 2005. Green Bay, WI.

Jensen, E.T. Bacterial Source Tracking Workshop. *Microbial Source Tracking Methods: Lake Michigan Case Studies*. April 14-15, 2005. Egg Harbor, WI.

Jensen, E.T. Wisconsin Environmental Health Association. *Sources of Bacterial Contamination at Lake Michigan Beaches and the Public Health Implications.* April 6, 2005. Wisconsin Rapids, WI.

McLellan, S.L. Intergovernmental Cooperation Council of Milwaukee. *Water quality data as it relates to beach closings*. March 14, 2005. Bayside, WI.

McLellan, S.L. Illinois Regional American Society of Microbiology. *Fate and Transport of Escherichia coli in nearshore Lake Michigan.* February 24, 2005. Des Plains, IL.

McLellan, S.L. Watershed Planning Conference. *Wet weather Impacts: Implications for regional and local contamination of beaches.* February 23, 2005. Milwaukee, WI.

Jensen, E.T. Nuisance Algae in Lake Michigan Public Forum. *The Abundance of Escherichia coli in Cladophora Mats at Lake Michigan Beaches*. February 18, 2005. Cleveland, WI.

McLellan, S.L. Lake Park Pavilion Town Hall Meeting. *Cladophora*, the odor-causing algae affecting our neighborhood shores of Lake Michigan. June 29, 2004. Milwaukee, WI.

McLellan, S.L. Presentation to the Community Action Committee, MMSD, December 7, 2004. Milwaukee, WI.

McLellan, S.L. Presentation to the Milwaukee Metropolitan Sewerage District Commission. September 27, 2004. Milwaukee, WI.

McLellan, S.L. Mayors Audit Committee for the Milwaukee Metropolitan Sewage District, Scientific Panel. July 16, 2004. Milwaukee, WI.

McLellan, S.L. Presentation of the Milwaukee Metropolitan Sewerage District Commission. July 12, 2004. Milwaukee, WI.

McLellan, S.L. Meeting organized by State Rep. Jon Richards. *The 2004 Beach/Swimming Season.* May 3, 2004. Bay View, WI.

McLellan, S.L. UW Ag Alumni Association-Manitowoc County. *Water quality and beach closings: What we know and what we don't know.* April 14, 2004. Manitowoc, WI.

McLellan, S.L. Southeast Environmental Enforcement Network/Midwest Environmental Enforcement Association Spring Conference. *Microbial source tracking approaches and application to investigating environmental contamination.* April 27-30, 2004. Memphis, TN.

Jensen, E.T. Wisconsin Bird Conservation Initiative Conference. *Sources of Bacterial Contamination at Lake Michigan Beaches*. March 11, 2004. Milwaukee, WI.

McLellan, S.L. Southeastern Regional Watershed Planning Conference. *Bacterial loading and sources: From watershed inputs to Lake Michigan beaches.* February 10, 2004. Milwaukee, WI.

McLellan, S.L. Manitowoc Soil and Water Conservation Department. S*ources of Escherichia coli (E. coli) at Manitowoc County Beaches*. January 27, 2004. Manitowoc, WI.

McLellan, S.L. Testimony to the Legislative audit committee (WI State Legislature). November 18, 2003. Madison, WI.

McLellan, S.L. Northeast Regional Watershed Conference. *Local and regional water quality in Lake Michigan and the link to beach closings.* July 15, 2003. Green Bay, WI.

McLellan, S.L. Heftner Center Town Hall Meeting. *Cladophora*, the odor-causing algae affecting the shores of Lake Michigan. June 2003. Milwaukee, WI.

McLellan, S.L. Emerging Environmental Health Challenges (sponsored by the Milwaukee Health Department). *Beach closings and the implications for public health*. May 14, 2003. Milwaukee, WI.

McLellan, S.L. Public Beach Closings Conference (sponsored by the Milwaukee Area Society for Public Administration). *Sources of* Escherichia coli *on our beaches*. May 8, 2003. Milwaukee, WI.

Jensen, E.T. Public Beach Closing Conference. *Citizen Science Outreach: A tool for Environmental Education in the Community*. May 8, 2003. Milwaukee, WI.

INTERACTIONS AND TECHNICAL ADVISING

Jim Ritchie, Wisconsin Department of Natural Resources
Gary Mick, Milwaukee County Parks Department
Steve Keith, Milwaukee County Parks Department
Erick Schambarger, City of Milwaukee, Budget Office
Jeff Polenske, City of Milwaukee, Department of Engineering
Mark Pfister, Lake County Illinois Health Department
SEWRPC, Water Quality Modeling Subcommittee
Kathy Schmidt, Wisconsin Seagrant
Milwaukee River Basin Partnership
Manitowoc Soil and Water Conservation District
Door County Soil and Water Conservation District
Scott Gunderson, UW-Extension Manitowoc County
Milwaukee Health Department
Racine Health Department
Senator Robert Cowles
Representative Jon Richards
Pier Wisconsin
Sustainable Racine
Friends of Milwaukee's Rivers

INTERVIEWS

Marie Rhode, Milwaukee Journal Sentinel
Steve Schultze, Milwaukee Journal Sentinel
Don Boehm, Milwaukee Journal Sentinel
Lee Bergquist, Milwaukee Journal Sentinel
Bruce Murphy, Milwaukee Magazine
David Steinkraus, Racine Times
Doug Hissom, Shepherd Express