**UNITED STATES ENVIRONMENTAL PROTECTION AGENCY**
GREAT LAKES NATIONAL PROGRAM OFFICE
77 WEST JACKSON BOULEVARD
CHICAGO, IL 60604-3590

Stephen Galarneau, Director
Office of Great Waters – Great Lakes & Mississippi River
Wisconsin Department of Natural Resources
PO Box 7921
Madison, WI 53707-7921

Dear Mr. Galarneau:

Thank you for your December 8, 2020 request to remove the *Degradation of Benthos* Beneficial Use Impairment (BUI) from the Sheboygan River Area of Concern (AOC) located near Sheboygan, WI. As you know, we share your desire to restore all the Great Lakes AOCs and to formally delist them.

Based upon a review of your submittal and supporting information, the U.S. Environmental Protection Agency (EPA) approves your request to remove this BUI from the Sheboygan River AOC. EPA will notify the International Joint Commission (IJC) of this significant positive environmental change at this AOC.

We congratulate you and your staff as well as the many federal, state and local partners who have been instrumental in achieving this environmental improvement. Removal of this BUI will benefit not only the people who live and work in the AOC, but all residents of Wisconsin and the Great Lakes basin as well.

We look forward to the continuation of this important and productive relationship with your agency as we work together to delist this AOC in the years to come. If you have any further questions, please contact me at (312) 353-8320 or your staff can contact Leah Medley at (312) 886-1307.

Sincerely,

CHRISTOPHER KORLESKI

Digitally signed by CHRISTOPHER KORLESKI
Date: 2020.12.15 16:11:06 -06'00'

Chris Korleski, Director
Great Lakes National Program Office

cc: Kendra Axness, WDNR
Brennan Dow, WDNR
Rebecca Fedak, WDNR
Madeline Magee, WDNR
Michelle Soderling, WDNR
Raj Bejankiwar, IJC

**State of Wisconsin**
**DEPARTMENT OF NATURAL RESOURCES**
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WISCONSIN DEPT. OF NATURAL RESOURCES

December 8, 2020

Mr. Chris Korleski, Director
Great Lakes National Program Office
U.S. Environmental Protection Agency
77 West Jackson Boulevard (G-17J)
Chicago IL 60604-3507

Subject: Removal of the Degradation of Benthos Beneficial Use Impairment in the Sheboygan River Area of Concern

Dear Mr. Korleski:

I am writing to request the U.S. Environmental Protection Agency (U.S. EPA) Great Lakes National Program Office's (GLNPO's) concurrence with the removal of the Degradation of Benthos Beneficial Use Impairment (BUI) in the Sheboygan River Area of Concern (AOC).

The Wisconsin Department of Natural Resources (DNR) has assessed the status of the Degradation of Benthos BUI in accordance with the BUI removal target that was established in 2008. We are pleased to report that all actions associated with this impairment have been completed and the target has been met. The U.S. EPA Technical Review Lead (TRL) has reviewed the BUI removal document and has provided their support for removal of this BUI. Following TRL review, the Sheboygan River AOC Advisory Committee met on September 29th, 2020 and expressed support for the removal. A 30-day public review and comment period for the BUI removal document was held from October 9 through November 6, 2020. DNR did not receive any comments regarding the proposed removal. As a result, we are recommending that the Degradation of Benthos BUI be removed from the list of impairments in the Sheboygan River AOC.

Please find the enclosed documentation to support this recommendation, including the Degradation of Benthos BUI Removal document prepared by DNR and letters of support from the City of Sheboygan, Sheboygan County, and Maywood Environmental Park.

We value our continuing partnership in the AOC Program and look forward to working closely with U.S. EPA GLNPO in the removal of BUIs and the delisting of Wisconsin's AOCs. If you need additional information, please contact Brennan Dow, DNR, at 920-366-1371, Rebecca Fedak, DNR, 920-207-8380, or you may contact me.

Sincerely,

Stephen G. Galarneau, Director
Office of Great Waters – *Great Lakes and Mississippi River*
608-266-1956 Stephen.Galarneau@Wisconsin.gov

Cc: Kendra Axness, WDNR
Brennan Dow, WDNR
Rebecca Fedak, WDNR
Madeline Magee, WDNR
Michelle Soderling, WDNR
Todd Nettesheim, USEPA
Marc Tuchman, USEPA
Amy Pelka, USEPA
Leah Medley, USEPA
Nick Green, USEPA

Enclosures:

Removal Recommendation for the Degradation of Benthos Beneficial Use Impairment in the Sheboygan River Area of Concern

# Removal Recommendation for the Degradation of Benthos Beneficial Use Impairment in the Sheboygan River Area of Concern

Submitted to:

U.S. EPA-GLNPO
77 W. Jackson Blvd.
Chicago, IL 60604

By:
Wisconsin Department of Natural Resources
December 2020

## Acknowledgments

Prepared by:

Michelle Soderling, Water Resources Management Specialist
Office of Great Waters
Wisconsin Department of Natural Resources

With Input and Contributions From:

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Diane Packett, WDNR Data Manager
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Mike Shupryt, WDNR
Stacy Hron, WDNR Lake Michigan LAMP Coordinator

The Wisconsin Department of Natural Resources would like to acknowledge the support provided by the Sheboygan River Area of Concern stakeholders in the development of the Degradation of Benthos Beneficial Use Impairment Removal Recommendation Document. Your local input and associated efforts were an invaluable part of the process to remove the Degradation of Benthos Beneficial Use Impairment and reflects the incredible ongoing efforts that will enable us to continue forging the path to delisting. The Wisconsin Department of Natural Resources and partners continue to work toward the shared goal of a healthy, self-sustaining Sheboygan River for people as well as for fish and wildlife.

Cover photo taken by Debbie Beyer.

## Disclaimer

The Great Lakes Water Quality Agreement (GLWQA) is a non-regulatory agreement between the United States and Canada, and criteria developed under its auspices are non-regulatory. The actions identified in this document were needed to meet beneficial use impairment removal targets leading to the delisting of the AOC.

# Executive Summary

The Sheboygan River was designated as an Area of Concern (AOC) in 1987 under the Great Lakes Water Quality Agreement (GLWQA). The lower 14 miles of the Sheboygan River downstream from the Sheboygan Falls Dam, including the entire harbor and nearshore waters of Lake Michigan, became the Sheboygan River AOC primarily due to contamination from polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs). These contaminants were discharged directly into the river from municipal and industrial sources and settled to the river bottom, leading to many contamination-related beneficial use impairments (BUIs) within the AOC. This document recommends removal of one of those BUIs: the Degradation of Benthos BUI.

Benthic invertebrates are organisms that live on or in the bottom sediment of a waterbody. The effects of environmental contaminants to species vary but include adverse impacts to every level of the food chain, beginning with those organisms which live on or in the sediment. Because benthic organisms are in direct contact with the sediment and water, they are sensitive to poor water and sediment quality, including chemical contaminants (such as PCBs and PAHs), low dissolved oxygen, high ammonia, and poor substrate conditions.

Final delisting targets for the AOC were developed in 2008 and in 2011 a final list of management actions was developed in order to address the remaining sources of impairment. The target to remove this BUI included three parts: 1) known contamination sources have been identified and control measures have been implemented, 2) all remediation actions for contaminated sediment have been completed and are monitored according to their approved plan, and 3) that the site is evaluated as statistically similar to a reference site with similar habitat and minimal sediment contamination. By July of 2013 all management actions for the Sheboygan AOC had been completed, including the following remediation actions for contaminated sediment, all of which have been or are being monitored according to their approved plans: the Camp Marina Superfund Alternative Remediation; the Sheboygan Harbor Navigational Improvement Dredging; the Sheboygan River and Harbor Superfund Remediation; and the Sheboygan River Great Lakes Legacy Act Project.

As such, the first and second portions of the target have been met. The third portion of the target states that the AOC is statistically similar to a non-AOC reference site, and this portion of the target was assessed by using the USGS studies conducted in 2012 and 2014 that compare the benthos of the Sheboygan River AOC to two non-AOC reference sites. USGS studies and additional lines of evidence from WDNR and other studies show that the AOC is similar to the reference site. Therefore, the Wisconsin Department of Natural Resources proposes to remove the Degradation of Benthos Beneficial Use Impairment in the Sheboygan River Area of Concern.

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# Purpose

The purpose of this document is to provide information in support of the recommendation to remove of the Degradation of Benthos Beneficial Use Impairment (BUI) from the Sheboygan River Area of Concern (AOC). This document presents the data that supported the listing of this BUI, the remedial actions taken to address the significant damages caused to benthic habitat and populations, and the data collected following the completion of remedial actions that support the recommendation to remove this BUI. More information on the AOC can be found on the Wisconsin DNR website at: http://dnr.wi.gov/topic/greatlakes/sheboygan.html.

# Background

### Rationale for AOC Designation

The Sheboygan River was designated as an AOC in 1987 under the Great Lakes Water Quality Agreement (GLWQA) due to severe degradation. The AOC designation was established mainly due to contaminated river sediment. The primary sources of contamination were municipal treatment plants, industries, and agricultural and urban runoff.

The Sheboygan River Remedial Action Plan (RAP) (WDNR, 1989) and RAP Update (WDNR, 1995) identified the following nine of fourteen possible BUIs in the AOC:

1. Fish tumors or other deformities
2. Bird or animal deformities or reproductive problems
3. Restrictions on fish and wildlife consumption
4. Restrictions on dredging activities (removed in 2015)
5. Degradation of benthos
6. Degradation of phytoplankton and zooplankton populations
7. Loss of fish and wildlife habitat
8. Degradation of fish and wildlife populations
9. Eutrophication or undesirable algae (removed in 2015)

### AOC Boundary

The AOC is located in east central Wisconsin, about 55 miles north of the City of Milwaukee. The Sheboygan River headwaters are located in Fond du Lac County and the river flows east, southeast approximately 80 river miles before reaching the western shore of Lake Michigan in the City of Sheboygan. The AOC encompasses the lower 14 miles of the Sheboygan River downstream from the Sheboygan Falls Dam, including the entire harbor and nearshore waters of Lake Michigan (Figure 1).

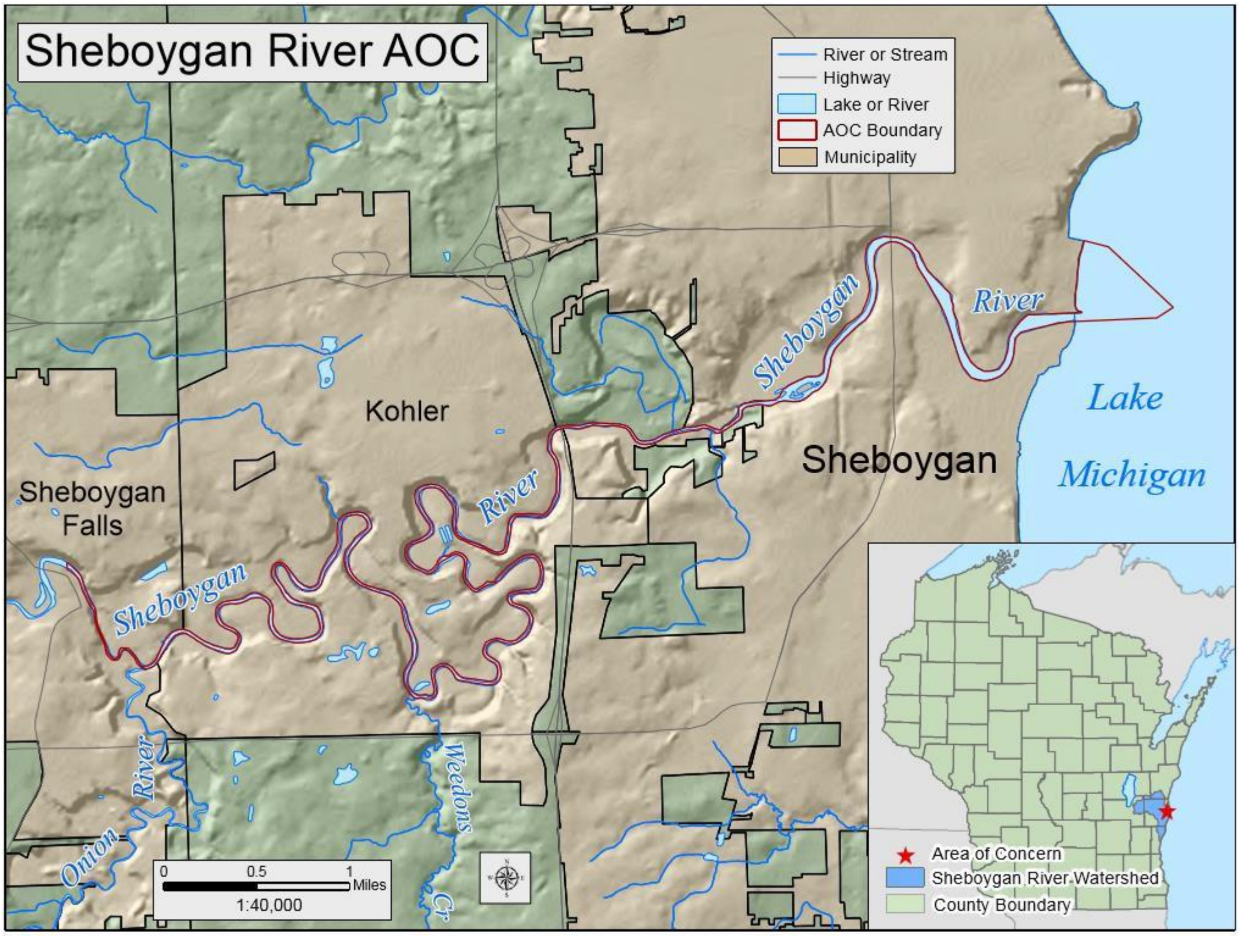
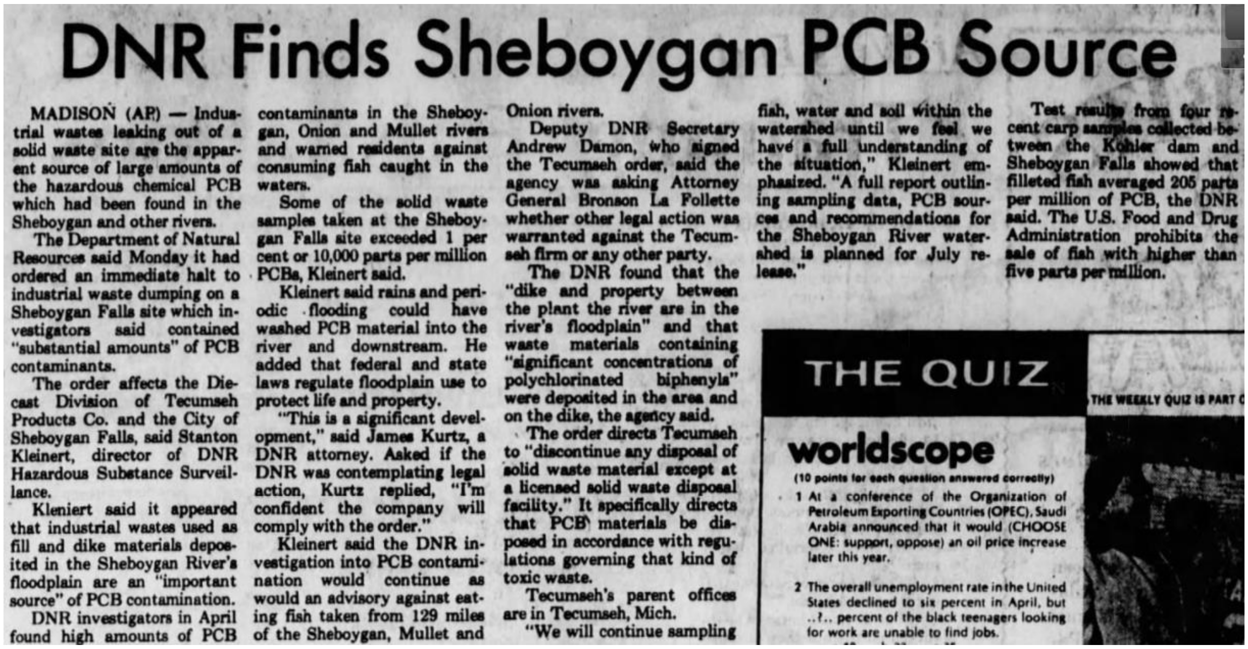



**Figure 1. Sheboygan River AOC Boundaries**

## Rationale for BUI Listing

While agriculture and timber production dominated the Sheboygan River area in the 1800’s, urbanization and industrialization boomed throughout the 1900’s, bringing with it various municipal and industrial effluents disposed directly into the Sheboygan River (WDNR, 1989). These effluents contained environmental contaminants including polychlorinated biphenyls (PCBs), polynuclear aromatic hydrocarbons (PAHs), and various heavy metals resulting in sediment contamination and a degraded benthic community. According to the 1989 RAP, PCBs constituted the most significant sediment contaminant in the AOC, with Tecumseh Products Company identified as the responsible party (RP) due to its disposal of PCB contaminated material in the Sheboygan River floodplain (Figure 2).



# DNR Finds Sheboygan PCB Source

MADISON (AP) — Industrial wastes leaking out of a solid waste site are the apparent source of large amounts of the hazardous chemical PCB which had been found in the Sheboygan and other rivers.

The Department of Natural Resources said Monday it had ordered an immediate halt to industrial waste dumping on a Sheboygan Falls site which investigators said contained "substantial amounts" of PCB contaminants.

The order affects the Diecast Division of Tecumseh Products Co. and the City of Sheboygan Falls, said Stanton Kleinert, director of DNR Hazardous Substance Surveillance.

Kleniert said it appeared that industrial wastes used as fill and dike materials deposited in the Sheboygan River's floodplain are an "important source" of PCB contamination.

DNR investigators in April found high amounts of PCB contaminants in the Sheboygan, Onion and Mullet rivers and warned residents against consuming fish caught in the waters.

Some of the solid waste samples taken at the Sheboygan Falls site exceeded 1 per cent or 10,000 parts per million PCBs, Kleinert said.

Kleinert said rains and periodic flooding could have washed PCB material into the river and downstream. He added that federal and state laws regulate floodplain use to protect life and property.

"This is a significant development," said James Kurtz, a DNR attorney. Asked if the DNR was contemplating legal action, Kurtz replied, "I'm confident the company will comply with the order."

Kleinert said the DNR investigation into PCB contamination would continue as would an advisory against eating fish taken from 129 miles of the Sheboygan, Mullet and Onion rivers.

Deputy DNR Secretary Andrew Damon, who signed the Tecumseh order, said the agency was asking Attorney General Bronson La Follette whether other legal action was warranted against the Tecumseh firm or any other party.

The DNR found that the "dike and property between the plant the river are in the river's floodplain" and that waste materials containing "significant concentrations of polychlorinated biphenyls" were deposited in the area and on the dike, the agency said.

The order directs Tecumseh to "discontinue any disposal of solid waste material except at a licensed solid waste disposal facility." It specifically directs that PCB materials be disposed in accordance with regulations governing that kind of toxic waste.

Tecumseh's parent offices are in Tecumseh, Mich.

"We will continue sampling fish, water and soil within the watershed until we feel we have a full understanding of the situation," Kleinert emphasized. "A full report outlining sampling data, PCB sources and recommendations for the Sheboygan River watershed is planned for July release."

Test results from four recent carp samples collected between the Kohler dam and Sheboygan Falls showed that filleted fish averaged 205 parts per million of PCB, the DNR said. The U.S. Food and Drug Administration prohibits the sale of fish with higher than five parts per million.

THE QUIZ

THE WEEKLY QUIZ IS PART

worldscope

(10 points for each question answered correctly)

1 At a conference of the Organization of Petroleum Exporting Countries (OPEC), Saudi Arabia announced that it would (CHOOSE ONE: support, oppose) an oil price increase later this year.

2 The overall unemployment rate in the United States declined to six percent in April, but ..?.. percent of the black teenagers looking for work are unable to find jobs.

**Figure 2. Newspaper article published in the Green Bay Press Gazette on May 16, 1978 detailing the WDNR's identification of Tecumseh Products Company as the RP for substantial amounts of PCB contamination in the Sheboygan, Onion, and Mullet Rivers.**

One primary source of PAHs was a manufactured gas plant (MGP) operated by Wisconsin Public Service Corporation (WPSC). The MGP was known as Camp Marina, and it provided fuel and electricity from coal. The coal tar byproducts produced from the coal gasification process used by the Camp Marina plant were composed primarily of PAHs, and these substances were discharged into the Sheboygan River.

The Kohler Landfill was historically a source of various pollutants, including volatile organic compounds (VOCs) and heavy metals. The Kohler Company Landfill was declared a Superfund site in 1984 after contaminated surface water runoff was detected. Kohler Company had operated this landfill since 1950 for foundry and manufacturing waste disposal. Certain cells were used for disposal of chrome plating sludges, enamel powder, hydraulic oils, solvents, and paint wastes. Not only was surface water runoff contaminating the river, but groundwater in the shallow aquifer beneath the site was contaminated with VOCs and heavy metals, which were also flowing into the Sheboygan River (Geraghty and Miller, 1992).

The Sheboygan River RAP (WDNR, 1989) and RAP Update (WDNR, 1995) identified nutrients and solids as significant pollutants for the AOC. The 1995 RAP identified that damages to the benthic community were likely due to industrial and agricultural habitat modifications in the Sheboygan River, which resulted in elevated levels of suspended solids and nutrients.

Benthic populations were suspected to be negatively impacted because of the many sources of environmental contaminants and the known polluted sediments present in the river. An Aquatic Ecological Risk Assessment (EVS & NOAA, 1998), found that macroinvertebrate populations in sediment depositional areas of the AOC were degraded due to legacy chemical contamination, confirming the impairment to benthic organisms within the AOC.

Then, an assessment of the Sheboygan River food chain and sediments found invertebrate bioaccumulation of PCBs evident at all sampling sites, and that total PAH concentrations in larval and emergent macroinvertebrate tissues increased with increasing urbanization throughout the watershed (Burzynski, 2000). This study provided further evidence that sediments contaminated with PCBs and PAHs contributed to impairment of benthos in the AOC.

# BUI Removal Criteria

### Delisting Targets

Delisting targets for the AOC were developed through a highly collaborative process, and included significant input from technical experts, local stakeholders and the public. The process of determining targets included a review of the region’s historical background, land use transformations, ecological conditions, and previous progress toward restoration. Previous studies related to the BUIs were reviewed and considered, including documents concerning contaminated sediments and their effects on fish, wildlife, and benthic organisms. Delisting targets prepared for other Great Lakes AOCs were also reviewed for their relevance and applicability to the Sheboygan River.

WDNR established the following removal criteria based on recommendations from the 2008 delisting targets report (ECT & SEH, 2008):

**Table 1. BUI removal target**

| Target |
| --- |
| Known contaminant sources contributing to sediment contamination and degraded benthos have been identified and control measures implemented. |
| All remediation actions for contaminated sediments are completed and monitored according to the approved plan with consideration to using consensus-based sediment quality guidelines and equilibrium partitioning sediment benchmarks. |
| The benthic community within the site being evaluated is statistically similar to a reference site with similar habitat and minimal sediment contamination. |

# Actions Taken to Restore the BUI

## Contaminated Sediment Events Timeline

The following is a summary of events, Superfund projects, and remediation efforts which have taken place in the Sheboygan River AOC:

- 1976 DNR discovers PCBs in river
- 1984 Kohler Company Landfill listed as Superfund site
- 1986 Sheboygan River and Harbor listed as Superfund site
- 1986 Camp Marina site listed as Superfund site
- 1987 Sheboygan River designated as an AOC
- 1995-1998 Superfund remediation of Kohler Company Landfill, including treatment of groundwater and leachate
- 2002 Superfund remediation of upland portion of Camp Marina site
- 2004 Superfund Phase I of remediation in the upper river of soils, groundwater, and adjoining riverbank soils of Sheboygan River and Harbor Superfund site
- 2006-2007 Superfund Phase II of remediation in the upper river of PCB contaminated sediment of the Sheboygan River and Harbor Superfund site
- 2009 Superfund characterization and sediment sampling in the lower river of the Sheboygan River and Harbor Superfund site
- 2009 Sheboygan River Dredging Workgroup forms
- 2011 Superfund remediation of sediment and shoreline at the Camp Marina site
- 2011-2012 Superfund remediation of lower river of Sheboygan River and Harbor
- 2013 Great Lakes Legacy Act Dredging Project completed
- 2013 Strategic Navigational Dredging Project completed

## Completion of Contaminated Sediment Management Actions

The management actions for the Degradation of Benthos BUI were to complete sediment remediation projects. By 2013, all management actions for the AOC were completed (Appendix A) and in 2015 the Restrictions on Dredging Activities BUI was removed (WDNR, 2015).

The following four sediment remediation projects (fig. 3) were the final management actions to restore the Degradation of Benthos BUI and meet the first and second portions of the target:

- Camp Marina Superfund Alternative Remediation
- Sheboygan Harbor Navigational Improvement Dredging
- Sheboygan River & Harbor Superfund Remediation

- Sheboygan River Great Lakes Legacy Act Project

In 2011, the Camp Marina Superfund Alternative Remediation was completed and in 2012, the Sheboygan Harbor Navigational Improvement Dredging was completed. By the end of 2012, over 400,000 cubic yards of contaminated sediment were removed from the river. Then in 2013 both the Sheboygan River and Harbor Superfund Remediation and the Sheboygan River Great Lakes Legacy Act (GLLA) Project were completed. Through the GLLA project, approximately 160,000 cubic yards of PCB- and PAH-contaminated sediment were removed from the lower river (Kiwanis Park to the 8th Street Bridge).

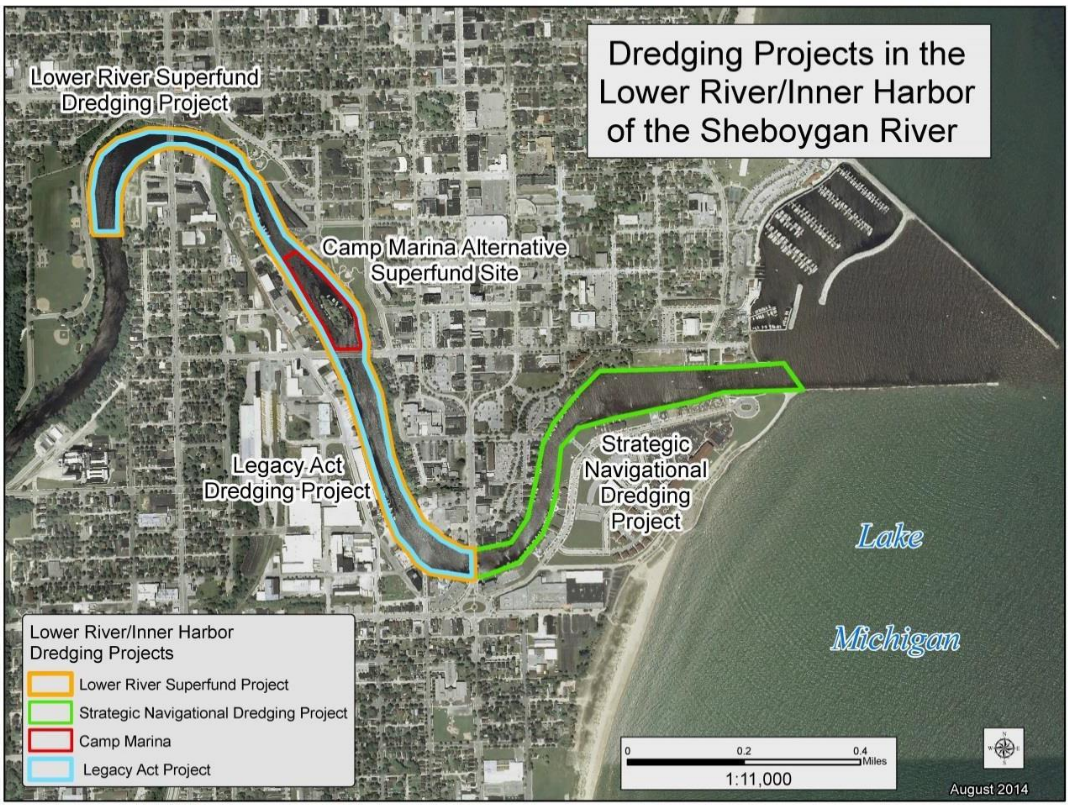



**Figure 3. Sediment Remediation Projects in the Sheboygan River AOC**

Upon completion of these four projects, known contaminant sources contributing to sediment contamination and degraded benthos had been identified and control measures implemented, thus meeting the first portion of the target.

The second portion of the target stipulates that the projects are not only completed, but also monitored according to their approved plan with consideration to using consensus-based sediment quality guidelines (WDNR, 2003) and equilibrium partitioning sediment benchmarks.

Post-dredging sampling of the non-Superfund projects confirmed that remediation actions for contaminated sediment had met the goals of the approved remediation plans. For the Superfund projects, however, continued monitoring is necessary. There are compliance requirements under Superfund that require the responsible parties to monitor fish and sediments to ensure that remedial objectives continue to be met. U.S. EPA (United States Environmental Protection Agency) conducts 5-year reviews for this purpose. Continued monitoring is being conducted as necessary under the Superfund program. All projects have met, or are meeting, the goals of their approved remediation plans, thus the second portion of the target is being met.

For more information on contaminated sediment projects, please see the Restrictions on Dredging BUI Removal Recommendation found on the following web page: https://dnr.wi.gov/topic/greatlakes/sheboygan.html.

### Additional Projects

Although not required management actions for this BUI, there were additional projects completed in the AOC which alleviated some of the agricultural sediment and nutrient impacts to the benthic organisms within the Sheboygan River.

The Sheboygan River Priority Watershed Project, which ran from 1993 to 2003, resulted in installation of agricultural best management practices throughout the watershed that reduced nonpoint source pollution entering the river.

Several AOC habitat projects incorporated elements that were beneficial to the benthos, such as in-stream habitat improvements, shoreline restoration, and wetland restoration. The following habitat projects may promote benthic community recolonization within the AOC:

- In-Stream Habitat Improvements
- Kiwanis Park Shoreline Restoration
- Schuchardt Property Conservation Planning and Invasive Species Management Planning
- Shoreline Stabilization in Problem Areas
- Targeted Invasive Species Control
- Taylor Drive & Indiana Ave Riparian Area and Wetland Restoration
- Wildwood Island Area Restoration

# Benthos-related assessments within the AOC

For the third component of the target to be met, the benthic community within the AOC had to be evaluated as statistically similar to a reference site with similar habitat and minimal sediment contamination. To assess whether the target was met, the U.S. Geological Survey (USGS) conducted benthos assessments in 2012 and 2014. Additional assessments to support the restoration of the Degradation of Benthos BUI included pre- and post- sediment remediation mussel surveys, a sediment toxicity testing study, and benthos monitoring completed by WDNR pre- and post- sediment remediation.

## USGS Wisconsin Lake Michigan AOC Benthos and Plankton Studies

As part of 2012 and 2014 research projects to assess the benthos and plankton BUIs at Wisconsin's Lake Michigan AOCs, the USGS sampled the Sheboygan, Milwaukee, Lower Menominee River, and Green Bay AOCs along with six non-AOC sites to evaluate communities at those sites (Scudder Eikenberry et al., 2019; APPENDIX B).

The Sheboygan River AOC was compared to two non-AOC reference sites, the Manitowoc River and the Kewaunee River. The less degraded non-AOC sites were chosen because they were never designated as AOCs, and they have similar environmental conditions to the Sheboygan AOC. The analysis completed by USGS was done under the assumption that biological assemblages at the reference sites are similar to what would be in place in the AOC if it were not degraded.

The Kewaunee and Manitowoc Rivers are nearby tributaries to Lake Michigan, and these sites were selected because of similar climate (Albert, 1995), latitude, geology, and land use. The Manitowoc River and Sheboygan River have similar drainage areas (1,341 and 1,043 square kilometers [$km^2$], respectively), but the Kewaunee River is smaller (329 $km^2$). Surficial deposits for all three rivers are primarily clay with some areas of sand and gravel (Robertson and Saad, 1995). All three rivers are low gradient, warmwater rivers. All three rivers flow through agricultural land and wetlands, then flow through urban land use at the mouth where they connect to Lake Michigan.

Sample collection in 2012 occurred during ongoing dredging, and the USGS sampling location was downstream of dredging activities that year. Sediment remediation was completed in 2013, then USGS sampling in 2014 took place in the same location as the 2012 sampling. The sample collection in 2014 was post-remediation.

Following sampling, USGS compared benthic communities in the AOC to the non-AOC reference sites and found that several metrics, including density and richness of taxa in combined benthos, which is combined dredge and Hester-Dendy samples, did not significantly differ between the AOCs and non-AOCs in 2014 (Scudder Eikenberry et al., 2019).

**Table 2. Metric means and standard deviations for benthos samples from the Sheboygan AOC site and non-AOC comparison sites in 2012 and 2014. Adapted from Table 5 in Scudder Eikenberry et al., 2019**

| | | | Dredge | | Hester-Dendy | | | Combined Benthos | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Statistic | Year | Richness | Diversity | Richness | Diversity | IBI | Richness | Diversity | Density |
| Sheboygan AOC site | Mean | 2012 | 13 | 0.5 | 25.7 | 2 | 8.3 | 35.3 | 1.1 | 48,318 |
| | SD | 2012 | 6.6 | 0.4 | 6.7 | 0.9 | 2.9 | 14.2 | 0.6 | 33,987 |
| | Mean | 2014 | 16.7 | 1.1 | 27.3 | 2.3 | 15 | 39 | 1.5 | 37,748 |
| | SD | 2014 | 5.1 | 0.5 | 4.7 | 0.4 | 5 | 4.4 | 0.6 | 10,629 |
| Kewaunee non-AOC comparison site | Mean | 2012 | 9 | 0.6 | 12.7 | 1.3 | 8.3 | 20.7 | 1.6 | 53,986 |
| | SD | 2012 | 1.7 | 0.2 | 7.5 | 0.7 | 7.6 | 8.5 | 0.6 | 15,497 |
| | Mean | 2014 | 7.7 | 0.4 | 23 | 2 | 3.3 | 30 | 1.4 | 38,329 |
| | SD | 2014 | 2.3 | 0.2 | 5.3 | 0.4 | 2.9 | 7.5 | 0.5 | 5,672 |
| Manitowoc non-AOC comparison site | Mean | 2012 | 7.3 | 0.6 | 33.7 | 2.1 | 8.3 | 38.3 | 1 | 61,637 |
| | SD | 2012 | 0.6 | 0.1 | 9.5 | 0.3 | 7.6 | 10.3 | 0.4 | 41,640 |
| | Mean | 2014 | 14.3 | 0.8 | 29.3 | 2.4 | 15 | 40.3 | 1.2 | 25,405 |
| | SD | 2014 | 5.9 | 0.2 | 9.5 | 0.4 | 10 | 13.1 | 0 | 1,281 |

Diversity was determined by using the Shannon Diversity Index (Shannon, 1948), which is a popular diversity index in ecological studies, and is calculated by taking richness (the number of different species) as well as abundance (the number of individuals of a species present) into account. Dredge samples from the study showed similar diversity for all three sites, and even increased in the AOC from 2012 to 2014 (Table 2). The combined benthos showed richness, diversity, and density were similar for the AOC site and the non-AOC reference sites (Table 2). Results from the USGS study indicate that the AOC benthic community is not significantly statistically different from the non-AOC comparison sites.

Along with richness and diversity, the Index of Biotic Integrity (IBI) was also calculated for the Hester-Dendy samples. The IBI was designed for use with the Hester-Dendy sampler data for large, nonwadable rivers of Wisconsin (Weigel and Dimick, 2011). An IBI can be more effective than a single metric for defining differences in assemblages because it combines both structural metrics such as richness and diversity with tolerance metrics such as percentage of tolerant taxa to generate a numeric value for assemblage condition. Hester-Dendy samples showed an increase in richness, diversity, and IBI scores for the AOC post-remediation, and the scores for the AOC fell within scores for the non-AOC comparison sites (Table 2).

In summary, post remediation sampling in 2014 showed no significant differences between the AOC and the non-AOC comparison sites.

## Sediment Toxicity

USGS collected sediment samples from three locations within the AOC in 2016. Results (Scudder Eikenberry et al., 2017; APPENDIX C; Scudder Eikenberry et al., 2020) show that site quality was rated as “reference” to “low-hazard”, similar to non-AOC comparison sites for Lake Michigan. This rating means that the sites were similar and not considered toxic to the benthos.

## WDNR Benthos Monitoring

In 2010 and 2011, prior to restoration work, WDNR completed baseline surveys of physical and biological conditions in the AOC, then, from 2014-2016 similar ecological assessment studies were conducted as verification monitoring to determine macroinvertebrate responses to sediment remediation (Masterson, 2018; APPENDIX D). Verification monitoring was repeated at 8 sites along the Sheboygan River in 2014, 2015, and 2016 to determine if sediment remediation and habitat restoration projects improved the water quality and biological communities within the lower 14-miles of the Sheboygan River. This verification monitoring within the AOC included surveys for benthic macroinvertebrates, macrophyte communities, and stream habitat. Metrics used to evaluate conditions included condition category thresholds for wadable stream Macroinvertebrate Index of Biotic Integrity (M-IBI) (Weigel, 2003), condition category thresholds for nonwadable river Macroinvertebrate Index of Biotic Integrity (M-IBI) (Weigel & Dimick, 2011), and Hilsenhoff Biotic Index (HBI) water quality rating values (Hilsenhoff, 1987). Mean macroinvertebrate IBI scores were analyzed for all sites, aggregated by river, and compared from pre- to post-sediment remediation.

Although contaminated sediment has been removed, the downstream sites at and near the mouth of the Sheboygan River present more challenges for recolonization of the benthic community. These highly manipulated river sections lack shoreline habitat and have substrate that is dominated by fine sediment. The two lower reaches showed almost no change and had M-IBI ratings ranging from “poor” to “fair”. Despite the "poor" and "fair" M-IBI ratings near the river mouth, upstream sites within the Sheboygan AOC have excellent habitat and M-IBI ratings ranging from "good" to "excellent". M-IBI scores in the Sheboygan River increased in most of the middle and upper reaches, except for one site, which is located between two dams in the Village of Kohler. Among mean M-IBI scores, there was an increase of 17% on the Sheboygan River (Masterson, 2018), which could be evidence that macroinvertebrates are responding to restoration activities. Upward trends are a good sign that the benthic community is rebounding after sediment remediation.

## Mussel Surveys

Mussels are very sensitive to contaminants (Havlik and Marking 1987, Farris and Van Hassel 2007). Past and current pollution can disrupt the endocrine system of mussels (Ciocan et al. 2010). Endocrine disruptors may influence the reproduction of fish and amphibian mussel hosts, as well as mussels themselves. Contaminants such as metals, PCBs, and PAHs can impact mussel populations. Many of these pollutants are minimally soluble in water which means that they will concentrate and adhere to sediments of aquatic systems. Exposure to contaminated sediments can be detrimental to juvenile mussels that feed and live in the river bottom.

Two mussel surveys were conducted within the lower AOC restoration areas at the request of WDNR. The surveys found that the mussel community is moderately diverse and has varying abundance, depending on the site.

In 2011, WDNR hired Dare Ecosystem Management, LLC to complete an assessment of freshwater mussels within the AOC (APPENDIX E). The primary goals of this project were to determine the presence and distribution of native unionid mussel species (especially state listed species) and to develop a baseline of the presence and distribution throughout the survey area. The study found and identified eleven native unionid mussel species. Juvenile mussels were found, although they made up a small portion of the observed living mussels. The most widely distributed species found were the Floater (*Pyganodon grandis),* Fatmucket (*Lampsilis siliquoidea*), Creeper *(Strophitus undulatus*), and White Heelsplitter (*Lasmigona complanata*).

In 2016, post-remediation, WDNR hired Dare Ecosystem Management, LLC to complete another assessment of freshwater mussels within the AOC (APPENDIX F). During this survey eleven unionid mussel species were found, including Elktoe (*Alasmidonta marginata*) which is a Species of Local Conservation Interest (SLCI) for the Sheboygan River. Although the report stated the mussel community is moderately diverse, the recommendation of this study was that downstream areas should be given recovery time of at least ten years. It may take time for these areas to rebound after restoration work due to the distance and dynamics of upstream populations. However, fish species that are present in the Sheboygan River may act as hosts to translocate these mussels around and allow for recolonization for many of the live species found. It was found that throughout the AOC there is suitable substrate for mussels to inhabit (Dare J. M., 2017). Considering the suitable substrate and the removal of toxic sediments through the AOC dredging projects, more mussel species should be able to colonize and inhabit stretches of the AOC that were historically highly degraded. The ability of mussel species to recolonize the AOC after recovery time supports the decision to remove this BUI.

# Public Involvement and Stakeholder Recommendations

Based on results of the studies described in this BUI removal, the AOC Coordinator started communication with members of the previous Technical Advisory Committee that last met in 2017. WDNR reconvened an Advisory Committee in September 2020 to discuss the proposed BUI removal. Participants of the Advisory Committee meeting expressed support for the removal of this BUI and provided letters of support (APPENDIX G). Future meetings will be held semi-annually to provide updates to the Advisory Committee on recovery monitoring efforts of remaining BUIs.

Following the Advisory Committee meeting, a 30-day public review and comment period for the BUI removal document was held from October 9 to November 6, 2020. Information was distributed via GovDelivery and through e-mail (APPENDIX H). No comments were received regarding the proposed removal.

## Conclusion

In summary, post remediation sampling of benthos by USGS in 2014 showed no significant differences between the AOC and the non-AOC comparison sites (Scudder Eikenberry et al., 2019; APPENDIX B). Sediment samples collected by USGS in the AOC rated quality as “reference” to “low-hazard”, similar to the non-AOC comparison sites for Lake Michigan (Scudder Eikenberry et al., 2020; APPENDIX C). Benthos monitoring conducted by WDNR showed that stream sites rated fair to excellent for invertebrate communities and stream habitat, and IBI scores increased post-remediation (Masterson, 2018; APPENDIX D). Mussel studies indicate that there is suitable substrate and mussel species will be able to recolonize the AOC after recovery time (Dare J. M., 2017; APPENDIX F). The results of these studies support the decision to remove this BUI.

In consideration of all management actions for sediment remediation having been completed in the AOC, the Superfund projects being monitored according to their required plans, and the benthic community being statistically similar to non-AOC reference sites, all three criteria for the removal target are now met, and therefore, WDNR and stakeholders recommend removal of this BUI.

## Removal Statement

The Wisconsin Department of Natural Resources Area of Concern staff recommend the removal of the Degradation of Benthos Beneficial Use Impairment from the Sheboygan River Area of Concern.

## List of Acronyms

| | |
|---|---|
| AOC | Area of Concern |
| BUI | Beneficial Use Impairment |
| GLLA | Great Lakes Legacy Act |
| GLNPO | Great Lakes National Program Office |
| GLWQA | Great Lakes Water Quality Agreement |
| IBI | Index of Biotic Integrity |
| MGP | Manufactured Gas Plant |
| M-IBI | Macroinvertebrate Index of Biotic Integrity |
| PAHs | Polycyclic aromatic hydrocarbons |
| PCBs | Polychlorinated biphenyls |
| RAP | Remedial Action Plan |
| RP | Responsible Party |
| SLCI | Species of Local Conservation Interest |

| | |
|---|---|
| U.S. EPA | United States Environmental Protection Agency |
| USGS | United States Geological Survey |
| VOCs | Volatile organic compounds |
| WDNR | Wisconsin Department of Natural Resources |
| WPSC | Wisconsin Public Service Corporation |

# Definitions

**Area of Concern**
A region where legacy pollution— from industrial, agricultural, and urban sources— severely interferes with the public's use of water resources for activities such as swimming and fishing. Defined by Annex 2 of the 1987 Protocol to the U.S.-Canada Great Lakes Water Quality Agreement as "geographic areas that fail to meet the general or specific objectives of the Agreement where such failure has caused or is likely to cause impairment of beneficial use of the area's ability to support aquatic life." These areas are the "most contaminated" areas of the Great Lakes, and the goal of the AOC program is to bring these areas to a point at which they are not environmentally degraded more than other comparable areas of the Great Lakes. When that point has been reached, the AOC can be removed from the list of AOCs in the Annex, or "delisted."

**Beneficial Use Impairment (BUI)**
A "beneficial use" is any way that a water body can improve the quality of life for humans or for fish and wildlife (for example, providing fish that are safe to eat). If the beneficial use is unavailable due to environmental problems (for example if it is unsafe to eat the fish because of contamination) then that use is impaired. The International Joint Commission provided a list of 14 possible beneficial use impairments in the 1987 Great Lakes Water Quality Agreement amendment.

**Benthos**
Community of organisms that live on, or in, the bottom sediments

**Combined Benthos**
In the USGS study, combined benthos is the combination of the dredge sample and the Hester-Dendy sample.

**Delisting Target**
Specific goals and objectives established for beneficial use impairments, with measurable indicators to track progress and determine when delisting can occur.

**Diversity**
Diversity was determined by using the Shannon Diversity Index (Shannon, 1948), which is a popular diversity index in ecological studies, and is calculated by taking richness

(the number of different species) as well as abundance (the number of individuals of a species present) into account.

**Dredging**
Dredging is the operation of excavating material from an aquatic environment. In this document, dredging refers to excavating sediment from the river bottom.

**Hester-Dendy**
A multi-plate invertebrate sampler uses to assess aquatic species assemblages.

**Macroinvertebrate**
Animals without a vertebral column and which are visible to the unaided eye.

**Macrophyte**
A rooted aquatic plant.

**Nonpoint Source Pollution**
Pollution whose sources cannot be traced to a single point such as a municipal or industrial wastewater treatment plant discharge pipe. Nonpoint sources include eroding farmland and construction sites, urban streets, and barnyards. Pollutants from these sources reach water bodies in runoff, which can best be controlled by proper land management.

**Nutrient**
Substances such as nitrogen or phosphorus which are necessary for and therefore promote the growth of plants and algae.

**Plankton**
Tiny plants (phytoplankton or algae) and animals (zooplankton) that live in the water column. Note that attached algae and invertebrates are not plankton.

**Pollution**
The presence of materials or energy whose nature, location, or quantity produces undesired environmental effects.

**Remedial**
Tending to remedy something, to restore to natural conditions, to correct or improve.

**Remedial Action Plan (RAP)**
According to the 1987 Protocol to the U.S.-Canada Great Lakes Water Quality Agreement, a RAP is a document that provides "a systematic and comprehensive ecosystem approach to restoring and protecting beneficial uses in Areas of Concern…" RAPs are required to be submitted to the International Joint Commission at three

stages: Stage 1: Problem definition Stage 2: When remedial and regulatory measures are selected Stage 3: When monitoring indicates that identified beneficial uses have been restored. Note that a renegotiated Great Lakes Water Quality Agreement was signed in 2012 by the U.S. and Canada which removed the “stage” terminology from the AOC Annex, and simply requires Remedial Action Plans to be “developed, periodically updated, and implemented for each AOC.”

**Richness**
Richness is computed as the number of unique taxa in the sample.

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# Appendices

**Appendix A – Completion of Management Actions Letter**

**Appendix B – Benthos and Plankton of Western Lake Michigan Areas of Concern in Comparison to Non-Areas of Concern for Selected Rivers and Harbors, 2012 and 2014**

**Appendix C – Sediment Toxicity Assessment in Two Wisconsin Areas of Concern and Selected Lake Michigan Tributaries**

**Appendix D – Verification Monitoring of Biological Communities and Physical Habitat in Select Streams within the Sheboygan River Area of Concern 2014-2016**

**Appendix E – Qualitative Unionid Mussel Surveys and Habitat Assessment of the Sheboygan River AOC**

**Appendix F – Lower Sheboygan River Restoration Area of Concern Mussel Inventories**

**Appendix G – Letters of Support for BUI Removal**

**Appendix H – GovDelivery Announcement for Public Comment Period**

# Appendix A – Completion of Management Actions Letter

**State of Wisconsin**
**DEPARTMENT OF NATURAL RESOURCES**
**101 S. Webster Street**
**Box 7921**
**Madison WI 53707-7921**

**Scott Walker, Governor**
**Cathy Stepp, Secretary**
Telephone 608-266-2621
Toll Free 1-888-936-7463
TTY Access via relay - 711

July 6, 2015

Mr. Chris Korleski, Director
United States Environmental Protection Agency
Great Lakes National Program Office
77 West Jackson Boulevard (G-17J)
Chicago, IL 60604-3511

Subject: Completion of Management Actions for the Sheboygan River Area of Concern

Dear Mr. Korleski:

This letter serves to document the completion of management actions for the Sheboygan River Area of Concern. The AOC has nine beneficial use impairments:

- Restrictions on dredging activities;
- Restrictions on fish and wildlife consumption;
- Degradation of benthos;
- Degradation of fish and wildlife populations;
- Loss of fish and wildlife habitat;
- Bird or animal deformities or reproduction problems;
- Fish tumors or other deformities;
- Degradation of phytoplankton and zooplankton populations; and,
- Eutrophication or undesirable algae.

Following designation as an AOC in 1987, many partners worked together to make progress toward restoring the Sheboygan River. In particular, notable progress was made in addressing point and nonpoint sources of nutrients to address eutrophication issues. Important groundwork was laid for the eventual cleanup of contaminated sediment sites, with state and federal agency staff engaging responsible parties in discussions about cleanup.

In 2011, Wisconsin DNR's Office of the Great Lakes (OGL) worked with local stakeholders and U.S. EPA's Great Lakes National Program Office to identify a final set of actions that would address the remaining sources of impairment. The management actions that were identified and subsequently completed include the following:

- Camp Marina Superfund Alternative Remediation*
- Sheboygan Harbor Navigational Improvement Dredging
- Sheboygan River & Harbor Superfund Remediation*
- Sheboygan River Great Lakes Legacy Act Project
- In-Stream Habitat Improvements
- Kiwanis Park Shoreline Restoration
- Schuchardt Property Conservation Planning & Invasive Species Management Planning
- Shoreline Stabilization in Problem Areas
- Targeted Invasive Species Control

- Taylor Drive & Indiana Ave Riparian Area and Wetland Restoration
- Wildwood Island Area Restoration

  *This work was completed in the field by the responsible parties. The EPA Superfund program has not yet issued final completion documents for these projects and some long term responsibility will remain for the foreseeable future.

Completing these management actions would not have been possible without strong partnerships between DNR, U.S. EPA's Great Lakes Legacy Act program, City of Sheboygan, Sheboygan County, and many others. We are grateful for the efforts of all of the partners and for the funds provided by the Great Lakes Restoration Initiative.

While we have completed the management actions that we believe were necessary to delist the AOC, we are undertaking verification monitoring to ensure that AOC targets have been met. We have documented the achievement of targets for the Restrictions on Dredging Activities and Eutrophication or Undesirable Algae BUIs and will propose them for removal in 2015. PCBs are a pollutant of concern in this AOC and they are persistent in the environment. Natural attenuation of PCBs was part of the approach the EPA Superfund program employed in their record of decision for portions of the river. BUIs related to PCB contamination will need time for the system to recover. The state will be reviewing the results of verification monitoring to determine the appropriate timeframes for considering additional BUIs for removal.

We thank you for your support in completing the identified management actions and look forward to your continued support and collaboration in monitoring and documenting progress in the AOC. If you have any questions about the management actions, verification monitoring, or BUI removals, please contact me at (608) 266-1956 or by e-mail at Stephen.Galarneau@Wisconsin.gov; or you may contact Vic Pappas, Lake Michigan Team Leader, at (920) 893-8512 or by e-mail at Victor.Pappas@Wisconsin.gov.

Sincerely,

Stephen Galarneau, Director
Office of the Great Lakes

Cc: Vic Pappas, WDNR
Camille Bruhn, WDNR
Kendra Axness, WDNR
Ted Smith, USEPA
Marc Tuchman, USEPA

# Appendix B – Benthos and Plankton of Western Lake Michigan Areas of Concern in Comparison to Non-Areas of Concern for Selected Rivers and Harbors, 2012 and 2014

USGS
science for a changing world

Prepared in cooperation with the Wisconsin Department of Natural Resources and the U.S. Environmental Protection Agency, Great Lakes National Program Office

# Benthos and Plankton of Western Lake Michigan Areas of Concern in Comparison to Non-Areas of Concern for Selected Rivers and Harbors, 2012 and 2014

Scientific Investigations Report 2019–5051

U.S. Department of the Interior
U.S. Geological Survey

**Cover.** Photograph showing Sheboygan River South Pier (photograph by Amanda Bell, U.S. Geological Survey).

# Benthos and Plankton of Western Lake Michigan Areas of Concern in Comparison to Non-Areas of Concern for Selected Rivers and Harbors, 2012 and 2014

By Barbara C. Scudder Eikenberry, Hayley T. Olds, Daniel J. Burns, Amanda H. Bell, and James L. Carter

Prepared in cooperation with the Wisconsin Department of Natural Resources and the U.S. Environmental Protection Agency, Great Lakes National Program Office

Scientific Investigations Report 2019–5051

**U.S. Department of the Interior**
**U.S. Geological Survey**

**U.S. Department of the Interior**
DAVID BERNHARDT, Secretary

**U.S. Geological Survey**
James F. Reilly II, Director

U.S. Geological Survey, Reston, Virginia: 2019



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Kassidy T. Mapel, U.S. Geological Survey (USGS), assisted with all sampling in 2014; Nicolas Buer (USGS) assisted with Hester-Dendy sampler deployment in 2012. Michelle A. Nott and James L. Kennedy (USGS) assisted with geographic information systems and the creation of the map in figure 1; Leah Kammel (USGS) assisted with the finalization of figure 1. Scott A. Grotheer (USGS), Daniel J. Sullivan (USGS), and two anonymous reviewers provided technical comments on an earlier version of the report.

# Contents

## Figures

## Tables

# Conversion Factors

International System of Units to U.S. customary units

| Multiply | By | To obtain |
|---|---|---|
| | Length | |
| micrometer (μm) | 0.00003937 | inch (in.) |
| meter (m) | 3.281 | foot (ft.) |
| meter (m) | 1.094 | yard (yd.) |
| kilometer (km) | 0.6214 | mile (mi.) |
| | Area | |
| square kilometer ($km^2$) | 0.3861 | square mile ($mi^2$) |
| square meter ($m^2$) | 1.19599 | square yard ($yd^2$) |
| | Volume | |
| liter (L) | 0.2624 | gallon (gal.) |
| cubic meter ($m^3$) | 264.2 | gallon (gal.) |
| | Mass | |
| kilogram (kg) | 2.205 | pound (lb.) |

# Datum

Vertical coordinate information is referenced to the North American Vertical Datum of 1988 (NAVD 88).

Horizontal coordinate information is referenced to the North American Datum of 1983 (NAD 83).

# Supplemental Information

Specific conductance is given in microsiemens per centimeter at 25 degrees Celsius (μS/cm at 25 °C).

Concentrations of chemical constituents in water are given in either milligrams per liter (mg/L) or micrograms per liter (μg/L). The mesh opening size for the plankton net is given in micrometers (μm).

## Abbreviations

| | |
|---|---|
| ANOSIM | analysis of similarity |
| AOC | Area of Concern |
| BUI | Beneficial Use Impairment |
| EPT | Ephemeroptera-Plecoptera-Trichoptera |
| HD | Hester-Dendy (artificial substrate sampler) |
| IBI | Index of Biotic Integrity |
| MDS | multidimensional scaling |
| MMSD | Milwaukee Metropolitan Sewerage District |
| PCBs | polychlorinated biphenyl compounds |
| SIMPER | similarity percentage |
| TSS | total suspended solids |
| USGS | U.S. Geological Survey |
| VOI | volatile on ignition |
| VSS | volatile suspended solids |
| WDNR | Wisconsin Department of Natural Resources |

# Benthos and Plankton of Western Lake Michigan Areas of Concern in Comparison to Non-Areas of Concern for Selected Rivers and Harbors, 2012 and 2014

By Barbara C. Scudder Eikenberry, Hayley T. Olds, Daniel J. Burns, Amanda H. Bell, and James L. Carter

## Abstract

Since their designation in the 1980s, Areas of Concern (AOCs) around the Great Lakes have been the focus of multi-State and international cleanup efforts that were needed after decades of human activity resulted in severely contaminated sediment, water-quality degradation, loss of habitat for aquatic organisms, and impaired public use. Although individual Great Lake States had been working to cleanup and mitigate environmental concerns, there was insufficient funding and little coordination between Federal and State efforts to address the large and complex set of problems. The Great Lakes Ecosystem Protection Act was passed in 2010, providing for comprehensive multi-State planning and dedicating Federal funds to accelerate cleanup and improve conditions at the AOCs with a particular focus on 14 beneficial use impairments, such as degradation of benthos and degradation of phytoplankton and zooplankton populations. Of Wisconsin's five AOCs, four lie adjacent to Lake Michigan: Lower Menominee River, Lower Green Bay and Fox River, Sheboygan River, and Milwaukee Estuary (which includes the Milwaukee River, Menomonee River, Kinnickinnic River, and Milwaukee Harbor). The Wisconsin Department of Natural Resources has focused much of the cleanup on removal of contaminated sediment from these AOCs because many beneficial use impairments were a result of contaminated sediment. However, recent and quantitative assessments of the status of benthos and plankton at the AOCs were lacking. Therefore, to inform management decisions regarding the status of benthos and plankton at AOCs, the U.S. Geological Survey, in cooperation with the Wisconsin Department of Natural Resources (WDNR) and the U.S. Environmental Protection Agency, Great Lakes National Program Office, assessed the condition of benthos (benthic invertebrates) and plankton (zooplankton and phytoplankton) at sites in the 4 AOCs and at 6 less-degraded comparison sites (hereafter referred to as "non-AOCs").

The U.S. Geological Survey collected benthos, plankton, sediment, and water three times per year in 2012 and 2014 between May and August at the AOC and non-AOC comparison sites. Except for Lower Green Bay and Milwaukee Harbor, each AOC site or subsite was paired with sites in two non-AOCs with similar environmental conditions. Community-based metrics were compared using univariate and multivariate statistics between each AOC and the mean of all non-AOCs and between each AOC and the mean of two non-AOC comparison sites. Although it was assumed that, because of their designation as AOCs, the relationships would indicate degraded conditions compared to the non-AOC sites, several metrics for the AOCs did not significantly differ between the AOCs and non-AOCs in 2014. Of all four AOCs examined for benthos, only the Lower Menominee River AOC differed from its two non-AOC comparison sites; the density and richness of taxa in insect orders Ephemeroptera-Plecoptera-Trichoptera (mayflies, stoneflies, and caddisflies) in combined benthos (dredge and artificial substrate samples) were lower at the AOC. For plankton, the assemblages for zooplankton at the Fox River near Allouez (a subsite in the Lower Green Bay AOC) and the Milwaukee River differed from their two non-AOC comparison sites; density of zooplankton was lower at both AOCs. Metrics for combined benthos and combined phytoplankton (soft algae and diatoms) at the Sheboygan River AOC did not differ from the two non-AOC comparison sites; however, the diversity of zooplankton in 2014 was lower at the Sheboygan River AOC than at the two non-AOC comparison sites. The combination of univariate and multivariate statistics provided a way to evaluate the status of the aquatic assemblage at each AOC and whether or not the assemblage differed from less-degraded non-AOC comparison sites. Results for this study provide multiple lines of evidence for evaluating the status of aquatic communities at AOC sites in Wisconsin along the western Lake Michigan shoreline in 2012 and 2014.

# Introduction

Aquatic biological communities have been used for more than a century as sentinels and endpoints for quantifying the degree of water and sediment quality degradation as well as improvement after remediation. However, recent ecological assessments are few in river mouths and harbors of the Great Lakes, especially along the shoreline of Lake Michigan (Canfield and others, 1996; Scudder Eikenberry and others, 2016a). Benthic invertebrates (organisms living near, on, or in the bottom of a waterbody, hereafter referred to as “benthos”) are considered good indicators of water quality and especially good indicators of sediment quality because they have direct contact with the sediment, are mostly sedentary compared to fish, and are constantly exposed to any chemical contaminants, low dissolved oxygen, high ammonia, and poor substrate conditions. In general, much less is known about the benthos of nonwadeable freshwater rivers, river mouths, and harbors than about wadeable riverine environments (Flotemersch and others, 2006; Larson and others, 2013; Weigel and Dimick, 2011; Wells and Demos, 1979). Zooplankton and phytoplankton (hereafter referred to as “plankton,” mostly microscopic organisms living in the water column) are important food sources for many organisms and are useful indicators of water quality. Together, benthos and plankton can provide a more complete assessment of conditions and effectiveness of remediation at Great Lakes river mouths and harbors than either benthos or plankton can alone.

With the long period of human effects on ecosystems in Great Lakes river mouths and harbors, characterization of the taxa or abundances of aquatic organisms that should compose an unimpaired benthic or planktonic assemblage is a challenge. Also, the hydrodynamic effect of the large lakes can be significant because of their proximity as well as the effect of seiche and tidal action that can periodically transport lake water and organisms upriver for varying distances. Nevertheless, the primary effect is from the river and the benthos and plankton in the river mouth, and harbor samples should reflect this dynamic.

Relatively diverse fauna with at least modest abundances of various taxa in a healthy, downstream assemblage would be expected in a temperate river mouth or harbor (Larson and others, 2013). A study of benthos at 50 nearshore reference sites in lakes Superior, Huron, Erie, and Ontario by Bailey and others (1995) found that the 4 most abundant taxa were midges, oligochaetes, bivalves, and sponges; however, that study found considerable variation in benthos across sites and indicated that there was not a single, well-defined healthy ecosystem. The benthos of soft bottom sediment is usually dominated by worms (oligochaetes) and midges (chironomids), with some bivalves and occasional crustaceans, and less so water mites, flatworms, and various insect larvae, and the number of taxa usually decreases with depth (Wiederholm, 1980). For plankton, the zooplankton is usually dominated by rotifers and microcrustaceans, such as cladocerans and copepods, and protozoans. As secondary producers in aquatic food webs, benthos and zooplankton are important food sources for fish, aquatic birds, and other animals. As primary producers, phytoplankton play a major role at the base of aquatic food webs in large rivers and lakes, and assemblages are usually dominated by diatoms. The percentage of diatoms tends to decrease with pollution, and changes in the assemblage from dominance by diatoms to dominance by green algae or cyanobacteria (also known as “blue-green algae”) can have a cascading effect on secondary consumers (Flotemersch and others, 2006; Wisconsin Department of Natural Resources, 1993).

In the 1987 Amendment to the Great Lakes Water Quality Agreement, the United States and Canada designated 43 Areas of Concern (AOCs). Of Wisconsin’s five AOCs, four lie adjacent to Lake Michigan (International Joint Commission United States and Canada, 1987) and include the Lower Menominee River, the Lower Green Bay and Fox River, the Sheboygan River, and the Milwaukee Estuary (which includes the Milwaukee River, Menomonee River, Kinnickinnic River, and Milwaukee Harbor). AOCs are severely degraded areas that fail to meet quality objectives of the Agreement because of the presence of at least 1 of 14 beneficial use impairments (BUIs), including BUIs for the degradation of benthos and the degradation of phytoplankton and zooplankton populations. Historical and ongoing anthropogenic activities contribute to degraded sediment, benthos, and plankton at many AOCs. Removal or remediation of contaminated sediment has played a key role in Great Lakes Restoration Initiative efforts at AOCs. Recent data are lacking to assess whether or not the benthos and plankton have recovered.

In 2012 and 2014, the U.S. Geological Survey (USGS), in cooperation with the Wisconsin Department of Natural Resources (WDNR) and the U.S. Environmental Protection Agency, Great Lakes National Program Office, completed a study of the benthos and plankton at 10 sites in rivers and harbors along the western Lake Michigan shoreline. A total of 4 sampling sites (plus subsites) were in AOCs and 6 sites were in less-degraded sites (hereafter referred to as “non-AOCs”). The purpose of this study is to collect and evaluate data for determining whether or not the assemblages of benthos or plankton at four Wisconsin AOCs differ from the assemblages at presumptively less-degraded sites with comparable physical and chemical characteristics. This report presents an assessment of the status of assemblage structure of the benthos and plankton at the 4 AOC sites and 6 non-AOC comparison sites in 2014. The 2014 results are then compared to the results of the 2012 study (Scudder Eikenberry and others, 2016a), as well as to results for the AOCs from selected historical studies that used similar sampling methods, to provide context and evaluate potential progress in site remediation benefits in the four AOCs. State governments, citizen groups, and the U.S. Environmental Protection Agency can use the results of this study in making their BUI status determinations and as baseline information for future studies.

# Methods

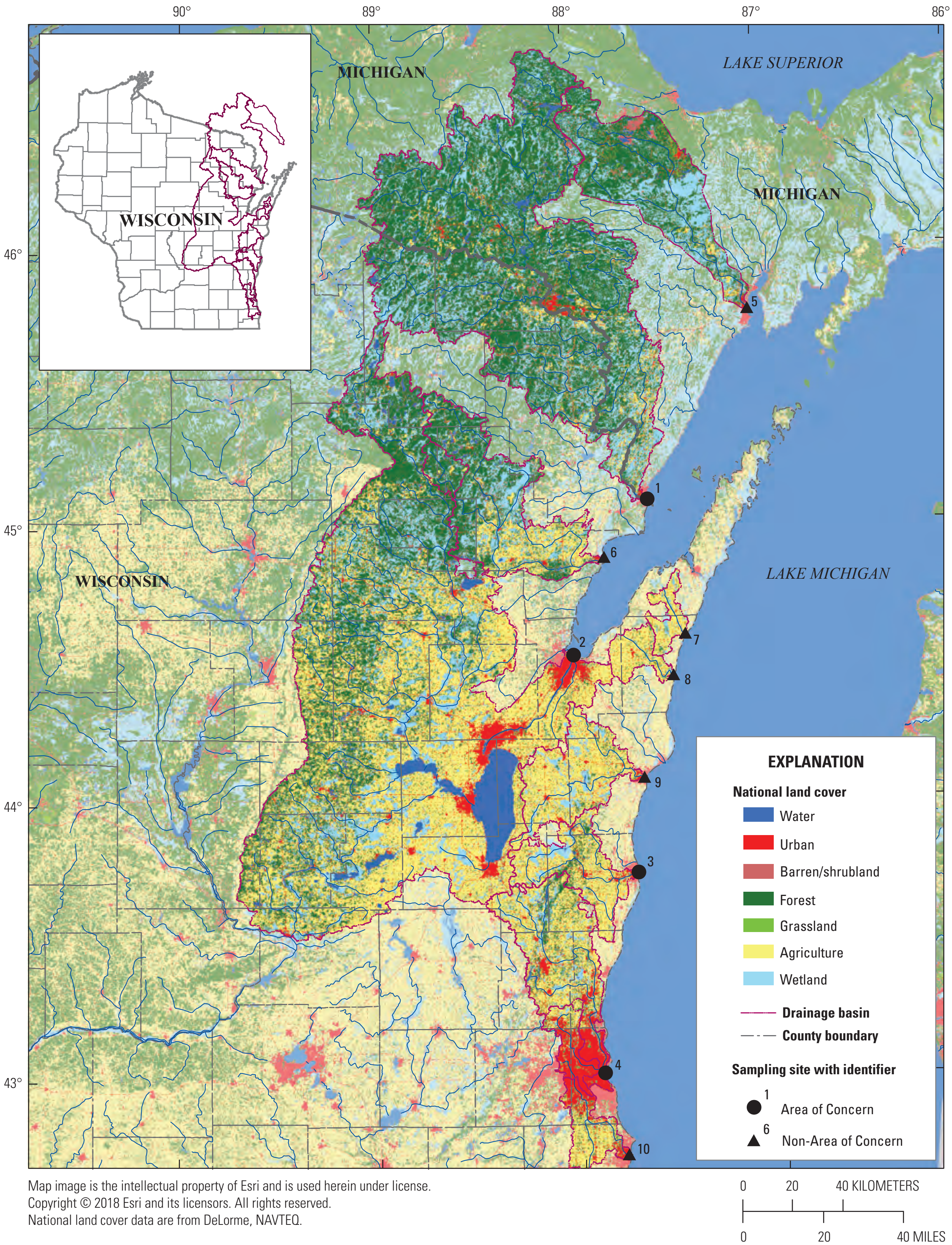
A total of 4 AOC sites and 6 non-AOC comparison sites, on the western shore of Lake Michigan, were selected for this study (fig. 1, table 1). Although all the river mouths or harbors along the western Lake Michigan shoreline are degraded to some degree, the non-AOCs selected for comparison with the AOCs have natural physical and chemical characteristics that are as close as possible to those of the AOCs, are presumptively less degraded because they are not designated AOCs, and are assumed to have biological assemblages similar to those that would be present in the AOCs if it were not for the specific contamination that was identified during the designation and listing of each AOC. That is, in the absence of effect, the less-degraded non-AOCs were assumed to have similar biological potential to the AOCs. The AOC sites sampled were the Lower Menominee River AOC at 1 site (hereafter referred to as "MENI") and the Lower Green Bay and Fox River AOC (1 subsite [hereafter referred to as "FOXR"] was sampled at the Fox River near Allouez). A total of 6 subsites were sampled in lower Green Bay; only 1 subsite (the Lower Green Bay subsite, hereafter referred to as "GREE") was sampled for benthos and plankton and the other 5 subsites were sampled for benthos only. The Sheboygan River AOC was sampled at 1 site (hereafter referred to as "SHEB"). The Milwaukee Estuary AOC is the largest Wisconsin AOC with respect to geographic area, population size, and the complexity of its drainage system. In the Milwaukee Estuary AOC, samples were collected at subsites in the Milwaukee River (1 subsite hereafter referred to as "MILR") and the Menomonee River (1 subsite hereafter referred to as "MENO"), as well as the Milwaukee Harbor (1 subsite hereafter referred to as "MILH"), which lies downstream from the confluence of these two rivers and the Kinnickinnic River (not sampled). The terms "location" or "subsite" in this study are used when more than one area was sampled within an AOC site. Detailed site information is provided elsewhere (Scudder Eikenberry and others, 2014, 2016b).

## Sample Collection and Processing

Detailed method descriptions are available elsewhere (Scudder Eikenberry and others, 2014, 2016b). Briefly, benthos and plankton were collected during three sampling events about 6 weeks apart in late May/early June, mid-July, and late August 2014. For simplicity, the three sampling events are hereafter referred to as the "spring," "summer," and "fall" seasonal samples. Unless otherwise specified, use of the term plankton in this report implies zooplankton and phytoplankton. High heat and drought during the summer and fall sampling periods in 2012 resulted in lower stream discharges at some sampling locations when compared to historical mean discharge. The sites most notably affected were MENI, the Milwaukee Estuary subsite MENO, and ROOT where annual mean discharges in 2012 were about two-thirds or less of the historical mean annual discharges at nearby streamgages. For this reason, and because remediation was completed at the Sheboygan River in 2013, benthos and plankton were sampled again in 2014 at all sites using the same methods. All sites were nonwadeable, so samples were collected from a boat. To quantify heterogeneity or "patchiness" of the organisms at sites, primary and replicate samples were collected at SHEB and its non-AOC comparison site on the Manitowoc River (hereafter referred to as "MANI"). Water quality at each site was determined during assemblage sampling by measuring pH, specific conductance, and water temperature with a Yellow Springs Instrument sonde.

Samples of the benthos were collected at most sites using two methods: (1) a standard Ponar dredge for grab samples of surficial bottom sediment and (2) Hester-Dendy (HD) artificial substrate samplers. HD samplers were deployed at the Fox River near Allouez subsite but were not deployed at the Green Bay subsites because of inadequate deployment conditions. A total of three to four grab samples of surficial sediment were collected and combined into one composite sample per site (U.S. Environmental Protection Agency, 2010a). A small amount of sediment (less than 50 grams) from each composite sample was split between two plastic bags for analysis of sand-silt-clay fractions and the volatile-on-ignition (VOI) component of the sediment. Large debris and empty shells in the remaining composite sample were examined for any attached invertebrates before being discarded, and the rest of the composite sample was washed through a 500-micrometer (μm) sieve. The retained debris and organisms were collected, and the organisms were identified and counted. A total of four individual HDs were deployed for 6 weeks at each site during each season (two each anchored to a cinder block). HD samplers were placed in areas with good flow to ensure velocities averaged at least 0.09 meters per second (m/s) as recommended (Ohio Environment Protection Agency, 1987). Once retrieved, three of the four HD samples were randomly chosen to represent the site and all organisms were scraped off and composited into one sample per season per site. Each dredge and HD sample was stained with rose bengal and preserved with 10-percent buffered formalin. Benthic invertebrates in samples were identified and counted by the Lake Superior Research Institute at the University of Wisconsin-Superior (U.S. Environmental Protection Agency, 2010b). Sediment samples were analyzed for sand-silt-clay fractions by the University of Wisconsin Soil and Plant Analysis Laboratory through the Wisconsin State Laboratory of Hygiene, except for five samples analyzed by the USGS Kentucky Water Science Center Sediment Laboratory because of low mass. Sediment samples were analyzed at the USGS in Middleton, Wis., using a VOI combustion method (U.S. Geological Survey, 1989; Wentworth, 1922) to provide an estimate of the organic content of sediment samples.

Artificial substrates such as the HD samplers measure short-term (1 month) colonization potential, and therefore, the attached invertebrates may not reflect the benthos of the location. Regardless, they may provide estimates of the organisms

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National land cover data are from DeLorme, NAVTEQ.

**Figure 1.** Sampling sites and subsites investigated for the evaluation of benthic and planktonic assemblages at Wisconsin's 4 Lake Michigan Areas of Concern and 6 non-Area of Concern comparison sites in Wisconsin and Michigan. Site and subsite numbers with names are provided in table 1.

**Table 1.** U.S. Geological Survey sampling locations at Wisconsin's Lake Michigan Areas of Concern and non-Area of Concern comparison sites in Wisconsin and Michigan, including site or subsite number, latitude, longitude, and drainage area.

[All locations except historical Green Bay sites were also sampled in 2012. Plankton samples in the Lower Green Bay and Fox River Area of Concern were collected only at subsites GREE (2a) and FOXR (2b). A subsite, or additional sampling location within the geographic area of a site, is indicated by the addition of an alphabet letter to a site number. $km^2$, square kilometer; NA, not applicable]

| Site or subsite name | Abbreviated name | Site or subsite number | Latitude[1] (decimal degrees) | Longitude[2] (decimal degrees) | Drainage[3] area ($km^2$) | Comparison site or subsite number |
|---|---|---|---|---|---|---|
| | | | Areas of Concern | | | |
| Lower Menominee River | MENI | 1 | 45.09810 | −87.60772 | 10,490 | 5, 6 |
| Lower Green Bay and Fox River | NA | 2 | NA | NA | NA | NA |
| Lower Green Bay | GREE | 2a | 44.57751 | −87.98600 | 16,584 | NA |
| Green Bay Historical Subsite 3–1 | GB03 | GB03 | 44.56611 | −87.99158 | 16,584 | NA |
| Green Bay Historical Subsite 5 | GB05 | GB05 | 44.54444 | −87.99444 | 16,584 | NA |
| Green Bay Historical Subsite 8 | GB08 | GB08 | 44.54861 | −87.94861 | 16,584 | NA |
| Green Bay Historical Subsite 16 | GB16 | GB16 | 44.55972 | −87.95972 | 16,584 | NA |
| Green Bay Historical Subsite 17 | GB17 | GB17 | 44.57222 | −87.93889 | 16,584 | NA |
| Fox River near Allouez | FOXR | 2b | 44.49499 | −88.02424 | 16,178 | 7, 8 |
| Sheboygan River | SHEB | 3 | 43.74887 | −87.70352 | 1,043 | 8, 9 |
| Milwaukee Estuary | NA | 4 | NA | NA | NA | NA |
| Milwaukee River | MILR | 4a | 43.04789 | −87.91269 | 1,779 | 9, 10 |
| Menomonee River | MENO | 4b | 43.03220 | −87.92156 | 381 | 9, 10 |
| Milwaukee Harbor | MILH | 4c | 43.02501 | −87.89722 | 2,193 | NA |
| | | | Non-Area of Concern comparison sites | | | |
| Escanaba River, Michigan | ESCA | 5 | 45.77845 | −87.06325 | 2,393 | 1 |
| Oconto River | OCON | 6 | 44.89198 | −87.83678 | 2,502 | 1 |
| Ahnapee River | AHNA | 7 | 44.60979 | −87.43484 | 274 | 2b |
| Kewaunee River | KEWA | 8 | 44.46073 | −87.50205 | 354 | 2b, 3 |
| Manitowoc River | MANI | 9 | 44.09190 | −87.66183 | 1,341 | 3, 4a, 4b |
| Root River | ROOT | 10 | 42.72866 | −87.78827 | 514 | 4a, 4b |

[1]Vertical coordinate information is referenced to the North American Vertical Datum of 1988.

[2]Horizontal coordinate information is referenced to the North American Datum of 1983.

[3]Drainage area determined using Hydrologic Unit Codes as described in Seaber and others, 1987.

associated with firmer (and potentially less contaminated) substrate than exists at a site. One advantage of using artificial substrates in assessments is to minimize the effect of habitat differences and allow the comparison of colonization potential on a single consistent substrate across all sites.

Samples of plankton for each site consisted of a plankton net sample to collect larger zooplankton and a set of whole-water samples to collect phytoplankton. Zooplankton were collected using a 63-µm mesh plankton net towed vertically from a depth of 5 meters (m) to the surface (U.S. Environmental Protection Agency, 2010c). If the available depth was less than 5 m, multiple tows were taken from just above the bottom to the surface until a 5-m total depth was sampled. A Kemmerer vertical water sampler was used to collect a set of five whole-water samples at 1-m depth intervals from 1 m below the surface to just above the bottom or, if the available depth was less than 5 m, samples were repeated at available 1-m intervals until five whole-water samples were collected. Subsamples were collected from the whole-water sample for the identification and counting of "soft" algae phytoplankton (cyanobacteria or "blue-greens," cryptomonads, desmids, dinoflagellates, euglenoids, and greens) and diatom phytoplankton, and analysis of chlorophyll-*a*, total suspended solids (TSS), and volatile suspended solids (VSS; U.S. Environmental Protection Agency, 2010d). Samples of zooplankton and phytoplankton were preserved with glutaraldehyde to a 1-percent final solution. Soft algae were identified and counted at the Wisconsin State Laboratory of Hygiene (Karner, 2005). Zooplankton and diatoms were identified and counted at the WDNR (U.S. Environmental Protection Agency, 2010e, f).

Analyses of chlorophyll-*a*, TSS, and VSS were done at the Wisconsin State Laboratory of Hygiene (American Public Health Association and others, 2006; Kennedy-Parker, 2011).

## Data Analyses

Potential differences in assemblages between AOCs and non-AOCs were first determined within a year and then between years. Except for the Lower Green Bay and Milwaukee Harbor subsites, each AOC site and associated subsite was matched to two non-AOC sites (hereafter referred to as "non-AOC comparison sites") based on the similarity of available environmental data as described earlier in the "Methods" section. Some non-AOCs were used for more than one AOC in comparisons. Metrics were computed from the assemblage data for comparisons between sites and years. The metrics used for comparisons were total taxon richness (the total number of taxa), the Shannon diversity index (Shannon, 1948), and total abundance (density) for dredge and HD sampler data combined (hereafter referred to as "combined benthos"), zooplankton, and soft algae and diatoms combined (hereafter referred to as "combined phytoplankton"). Additional metrics were computed for the benthos. These metrics included richness, density, and percentage of individuals in insect orders Ephemeroptera-Plecoptera-Trichoptera (EPT; mayflies, stoneflies, and caddisflies) for combined benthos and a macroinvertebrate index of biotic integrity (IBI) based on HD sampler data only. The IBI was designed for use with HD sampler data for large, nonwadeable rivers of Wisconsin (Weigel and Dimick, 2011). An IBI is a multimetric that combines structural metrics (for example, richness, diversity, and relative abundance), functional metrics (for example, feeding groups), and tolerance metrics (for example, percentage of tolerant taxa) to generate a numeric value that indicates the assemblage condition. The combination of structural and functional metrics can make IBIs more effective than a single metric for defining differences or change in assemblages. Indices to evaluate the benthos of deep freshwater environments are still in development. At present, no IBIs exist for zooplankton or phytoplankton in river mouths or harbors; therefore, seven metrics/multimetrics were used when comparing benthos and three metrics were used when comparing plankton. Means of metric values for non-AOCs were calculated within a sampling event (season).

Paired *t*-tests were used to compare metrics between sites. Comparisons were made between AOCs and the mean of all non-AOCs and between AOCs and their two matched non-AOC comparison sites. Some non-AOCs were compared with more than one AOC. In all, the sample size ($n$) was 3; unless otherwise stated, use of the term "significant" refers to statistical values of probability ($p$) less than (<) 0.05 in data comparisons. To satisfy conditions of normality, all total densities for benthos and plankton were log-10 transformed ($\log_{10}$) before statistical comparisons between samples; other data transformations were done as needed on a case by case basis. Replicate sample data (SHEB and MANI only) were not used in comparisons between AOCs and non-AOCs. Comparisons were begun at a broad level by comparing each AOC site to all non-AOCs as a group across all seasons using the means of non-AOCs within a season ($n$=3). Comparisons were then narrowed to comparing each AOC site or subsite with its two non-AOC comparison sites across all seasons, again using the means of the two non-AOC comparison sites within season. Comparing each AOC to a matched pair of non-AOCs provided a more robust measure of potential difference. If a metric value was lower at the AOC than at the non-AOCs, then the AOC was rated as degraded for that metric. Lack of a significant difference does not imply that the AOC assemblage is not degraded but that it was not rated as degraded in comparison to the selected non-AOCs. Sample size for comparisons ($n$=3), with just 1 value per site for each of the 3 seasons in a year, was low in this study. The lower the sample size or number of samples, the lower the statistical power and the lower the ability to detect a true difference between samples or sites when a difference exits (Gotelli and Ellison, 2004). In some statistical comparisons, between-site seasonal differences may have led to high variances and contributed to an inability to detect differences between AOCs and non-AOCs. Also, values for some metrics differed between non-AOC comparison sites. High variability is also likely among the group of six non-AOCs; however, this metric was not tested.

A total of four PRIMER software (Clarke and Gorley, 2006) routines were used for multivariate analyses with relative abundances of taxa. Relative abundance was used because of the possibility of uneven effort among samples. The routines used were (1) DIVERSE—to calculate diversity in $\log_e$; (2) similarity percentage (SIMPER)—to assess differences in the relative abundances of taxa between each AOC and its non-AOC comparison sites, among primary and replicate samples collected each season at SHEB and MANI, and among subsites within the Lower Green Bay and Fox River (benthos only) and Milwaukee Estuary AOCs; (3) multidimensional scaling (MDS), a nonmetric method based on relative abundances of taxa—to derive assemblage site scores and create ordination plots of sites and (or) samples; and (4) analysis of similarity (ANOSIM)—to compare assemblages among sites and samples using similarity matrices in a procedure analogous to an analysis of variance.

For multivariate analyses with PRIMER software, the relative abundance of each taxon was determined for each sample and then fourth-root transformed to allow common and rare taxa to affect outcomes (Clarke and Gorley, 2006). A Bray-Curtis similarity matrix was calculated between each set of samples, and these similarity matrices formed the basis of SIMPER and ANOSIM comparisons. A one-way ANOSIM was used to determine the extent to which benthos and plankton varied across sites by sampling event and across sampling seasons. Differences between AOCs and non-AOCs as indicated by multivariate test results do not signify degradation at an AOC but only differences in the relative abundances of taxa making up the benthic assemblages at each AOC in comparison with the non-AOC comparison sites. Multivariate results allow for an evaluation of how similar or different the assemblages at each AOC and its two non-AOC comparison sites are and aid in understanding differences in metrics. However, because we assumed that non-AOCs represent the best available nondegraded condition, large differences between AOC and non-AOC assemblages may indicate that the AOC was not meeting expectations.

Ambiguous taxa, taxa whose abundances are reported for multiple and related taxonomic levels, were resolved on a per sample basis before calculating metrics and before completing multivariate analyses by distributing counts for the parent to the children present within each site, based on the proportion of counts already assigned to each child, and removing the counts for the parent (Cuffney and others, 2007). If no children were present in the sample, then counts were left with the parent as originally identified. This procedure for dealing with ambiguous taxa was applied to the benthos and zooplankton; there were no ambiguous soft algae in samples of phytoplankton, so this procedure was used on only diatoms in the phytoplankton.

Richness was computed by totaling the number of unambiguous taxa; diversity was calculated using the Shannon diversity index (in $\log_e$) on raw abundances of taxa without data standardization or transformation using all unambiguous taxa. Richness and diversity were calculated separately for the two benthic sampling types—dredge and HDs—as well as for the combined (dredge and HDs) benthic samples. The macroinvertebrate IBI was calculated only for the HD samples as described by Weigel and Dimick (2011). The IBI values or “scores” range from 0 (worst) to 100 (best) and are rated as follows: very poor (less than or equal to [≤] 19), poor (20–39), fair (40–59), good (60–79), and excellent (greater than or equal to 80). Richness and diversity were also calculated separately for soft algae and diatom phytoplankton, as well as for combined phytoplankton (soft algae and diatoms combined). Relative abundance or dominance of taxonomic groups in the phytoplankton was computed from densities in the original soft algal dataset, which also included the density of diatoms as a group.

## Chemical and Physical Comparisons between Areas of Concern and Non-Area of Concern Sites

All physical and chemical data are available in Scudder Eikenberry and others (2014, 2016b). There were no differences between years within each site/subsite with respect to water temperature, pH, and specific conductance except at the MILH subsite in the Milwaukee Estuary AOC (table 2). Specific conductance at MILH was higher in 2014 than in 2012, reflecting differences in the type and (or) amount of dissolved major ions in the water. In 2014, one or more water-quality values differed between an AOC and non-AOC comparison sites. Values for mean specific conductance at MENI and FOXR in the Green Bay and Fox River AOC were lower than at their two respective non-AOC comparison sites, and specific conductance was higher at SHEB than at its two non-AOC comparison sites. Johnson and others (2015) found that values higher than 363 microsiemens per centimeter (µS/cm) inhibited the growth of mayfly larvae. Although mean specific conductances at MENI and one of its non-AOC comparison sites, the Oconto River non-AOC comparison site (hereafter referred to as “OCON”), were below this value in 2012 and 2014, the mean specific conductance at the other non-AOC comparison site, the Escanaba River, Michigan (hereafter referred to as “ESCA”), was below this value in 2014 only. Mean specific conductances at FOXR and its two non-AOC comparison sites, as well as at SHEB and its two non-AOC comparison sites, were all above 363 µS/cm. Water temperatures in 2014 were higher at MENI, FOXR, SHEB, and MENO in the Milwaukee Estuary AOC when compared to their non-AOC comparison sites. Higher water temperatures have implications for comparisons of plankton at these AOCs and non-AOC comparison sites because temperature is one control of growth for plankton.

Chlorophyll-*a* and suspended solids (TSS and VSS) are indicators of algal biomass (table 3). Nondetections for VSS data in summer and fall at MENI and MENO precluded testing VSS values for these two sites. Paired *t*-tests indicated that values for these measurements were not different between any AOC and non-AOC comparison sites in 2012 or 2014, and there were no differences within each site/subsite between 2012 and 2014 with respect to these three parameters. This result for chlorophyll-*a* and suspended solids indicates that the biomass of phytoplankton did not differ between AOCs and non-AOCs during these periods.

Although each AOC site or subsite except Green Bay sites and the MILH subsite was paired with two non-AOCs based on similar watershed characteristics, sediment size fraction and organic carbon content (as estimated by VOI) differed between AOCs and their non-AOC comparison sites (table 4). Results for size fraction and organic carbon content are included with results for benthic communities at each AOC.

## Condition of the Benthos and Plankton of Areas of Concern in Comparison to Non-Areas of Concern for Selected Rivers and Harbors

Differences in benthos and plankton at AOCs were evaluated by comparing computed biological metrics as well as relative abundances of individual taxa comprising the aquatic assemblages at each site. Results for each AOC are discussed separately in the following sections to allow the reader to focus on the benthos or plankton of a single AOC

**Table 2.** Mean and standard deviation for water-quality measurements made in situ with a Yellow Springs Instrument sonde at about a 1-meter depth in 2012 and 2014 at Areas of Concern and non-Area of Concern comparison sites in Wisconsin and Michigan.

[The number of samples is 3 for each mean and standard deviation. °C, degree Celsius; µS/cm at 25 °C, microsiemens per centimeter at 25 °C; ±, plus or minus; MENI, Lower Menominee River; FOXR, Fox River near Allouez (Lower Green Bay and Fox River subsite); SHEB, Sheboygan River; MILR, Milwaukee River; MENO, Menomonee River; MILH, Milwaukee Harbor (MILR, MENO, and MILH are Milwaukee Estuary subsites); ESCA, Escanaba River, Mich.; OCON, Oconto River; AHNA, Ahnapee River; KEWA, Kewaunee River; MANI, Manitowoc River; ROOT, Root River]

| Site | 2012 | | | 2014 | | |
|---|---|---|---|---|---|---|
| | Water temperature (°C) | pH | Specific conductance (µS/cm at 25 °C) | Water temperature (°C) | pH | Specific conductance (µS/cm at 25 °C) |
| Areas of Concern | | | | | | |
| MENI | 24.1±1.9 | 7.60±0.16 | 283±39 | 22.0±1.5 | 7.77±0.08 | 256±34 |
| FOXR | 24.4±4.1 | 8.18±0.71 | 434±20 | 23.5±0.6 | 8.53±0.45 | 385±9 |
| SHEB | 19.8±2.7 | 8.28±0.23 | 485±144 | 21.2±0.7 | 7.96±0.15 | 594±53 |
| MILR | 22.6±4.4 | 8.15±0.53 | 805±171 | 22.3±0.3 | 7.88±0.11 | 656±45 |
| MENO | 23.4±2.9 | 7.47±0.40 | 621±74 | 24.1±1.8 | 7.70±0.08 | 875±230 |
| MILH | 21.1±3.4 | 7.91±0.43 | 524±74 | 21.0±2.4 | 7.76±0.08 | 734±70 |
| Non-Area of Concern comparison sites | | | | | | |
| ESCA | 23.1±1.5 | 7.44±0.10 | 647±148 | 20.4±1.1 | 7.49±0.13 | 352±72 |
| OCON | 23.7±2.5 | 7.75±0.37 | 305±28 | 20.6±1.3 | 7.76±0.13 | 328±10 |
| AHNA | 17.5±6.1 | 8.15±0.11 | 422±109 | 17.9±1.3 | 7.72±0.23 | 584±6 |
| KEWA | 20.7±3.8 | 8.34±0.08 | 412±42 | 18.7±1.7 | 7.97±0.35 | 498±10 |
| MANI | 21.1±2.3 | 7.95±0.63 | 544±80 | 21.3±1.0 | 7.88±0.28 | 535±98 |
| ROOT | 22.8±1.9 | 7.94±0.13 | 800±263 | 20.6±2.9 | 8.01±0.39 | 930±83 |

of interest, and results for all comparisons are summarized. Because the Green Bay subsites and MILH were not compared to non-AOCs, they are presented in a separate section later in this report. Results and data for the 2012 sampling have been previously published (Scudder Eikenberry and others, 2014, 2016a), and data for the 2014 sampling are provided in Scudder Eikenberry and others (2016b).

*Dreissena polymorpha* (zebra mussels), an invasive species in Lake Michigan and many tributaries, were present in many samples from the benthos and plankton. Although *Dreissena* in the benthic samples were not identified to species, they were likely zebra mussels because all immature *Dreissena* ("veligers") in samples of zooplankton were identified as zebra mussels. Because of extremely high numbers of zebra mussel veligers in three samples of zooplankton, counts of this taxon were estimated at MILR and MILH (more than 2,000 at each) and ROOT (more than 4,000) in fall 2014.

There was minimal variability among field replicates within each season for most taxonomic groups. Primary and replicate samples were collected at two sites, SHEB and its non-AOC comparison site, MANI. Within each site, replicate samples had Bray-Curtis similarities higher than 60 percent except for fall diatom samples, which had only a 34- to 35-percent similarity. Because of the low similarity for fall diatom samples, similarities for fall combined phytoplankton were also low. In 2014, for example, fall diatom densities in the Sheboygan River primary and replicate samples were dominated (more than 75 percent) by one colony-forming centric taxon, but overall, there were fewer taxa and higher densities in the replicate sample. Also, fall diatom densities in the Manitowoc River primary and replicate samples in 2014 were dominated by other colony-forming centric taxa. Using relative abundances for samples of combined phytoplankton in comparisons with AOCs lessened the effect of differences in the fall diatom taxa. Results of paired *t*-tests indicated that there were no differences between metrics computed for primary and replicate samples of benthos, zooplankton, and combined phytoplankton for either SHEB or MANI in 2014.

## Benthic Assemblage Comparisons between Areas of Concern and Non-Areas of Concern

The benthic assemblage that was compared between an AOC and non-AOCs was based on the combination of dredge and HD samples (hereafter referred to as "combined benthos") to better represent the potential assemblage at each site.

**Table 3.** Mean and standard deviation for chlorophyll-*a*, total suspended solids, and volatile suspended solids for composited water samples collected in 2012 and 2014 at Areas of Concern and non-Area of Concern comparison sites in Wisconsin and Michigan.

[The limit of detection for suspended solids is 2 mg/L. The number of samples is 3 for each mean and standard deviation. µg/L, microgram per liter; mg/L, milligram per liter; MENI, Lower Menominee River; ±, plus or minus; FOXR, Fox River near Allouez (Lower Green Bay and Fox River subsite); SHEB, Sheboygan River; MILR, Milwaukee River; MENO, Menomonee River; MILH, Milwaukee Harbor (MILR, MENO, and MILH are Milwaukee Estuary subsites); ESCA, Escanaba River, Mich.; OCON, Oconto River; AHNA, Ahnapee River; KEWA, Kewaunee River; MANI, Manitowoc River; ROOT, Root River]

| Site | 2012 | | | 2014 | | |
|---|---|---|---|---|---|---|
| | Chlorophyll-*a* (µg/L) | Total suspended solids (mg/L) | Volatile suspended solids (mg/L) | Chlorophyll-*a* (µg/L) | Total suspended solids (mg/L) | Volatile suspended solids (mg/L) |
| | | | Areas of Concern | | | |
| MENI | 3.44±1.65 | 4.0±1.0 | 2.67±1.15 | 4.51±1.82 | 3.60±1.98 | 7.67 |
| FOXR | 72.4±27.6 | 45.3±29.4 | 19.7±13.6 | 91.9±57.3 | 46.1±20.9 | 22.9±10.0 |
| SHEB | 44.4±33.3 | 16.0±8.9 | 6.67±3.06 | 15.2±11.9 | 16.8±9.7 | 9.17±8.25 |
| MILR | 22.6±13.4 | 17.0±14.0 | 9.00±9.54 | 7.26±4.43 | 20.9±5.9 | 8.72±5.88 |
| MENO | 18.5±18.2 | 7.67±4.04 | 4.50±2.12 | 11.0±3.8 | 16.2±12.9 | 17.0 |
| MILH | 23.3±22.5 | 5.0±3.0 | 3.50±2.12 | 6.99±4.16 | 7.55±3.08 | 6.33±2.83 |
| | | | Non-Area of Concern comparison sites | | | |
| ESCA | 1.37±0.33 | 4.3±2.1 | 4.0±0.0 | 1.70±0.71 | 4 | 6.7 |
| OCON | 3.72±1.76 | 3.33±1.15 | 2.0±0.0 | 4.06±0.53 | 4.24±1.17 | 8.3 |
| AHNA | 22.0±16.7 | 11.7±5.0 | 7.7±5.1 | 19.3±5.3 | 7.78±6.26 | 11.7±11.8 |
| KEWA | 23.3±10.8 | 12.3±7.5 | 6.3±2.3 | 21.7±28.0 | 41.0±9.9 | 15.1±0.6 |
| MANI | 18.5±10.5 | 14.0±9.9 | 7.0±4.6 | 17.5±22.0 | 29.3±14.4 | 9.1±6.8 |
| ROOT | 19.9±4.0 | 20.7±19.4 | 7.3±4.2 | 13.9±12.2 | 33.2±33.5 | 9.8±8.8 |

Except for the IBI metric (computed from HD sampler data), all metrics used in comparisons were for combined benthos even though metrics were also computed for dredge and HD sampler data (table 5). Benthic communities collected by dredge in 2014 were dominated by oligochaetes (68 percent) and (or) midges (20 percent; chironomids). Of the 68 percent of oligochaetes, most were immature Tubificinae. Benthic assemblages collected by HD samplers in 2014 were dominated by midges (38 percent) and oligochaetes (21 percent). Statistical comparisons between AOCs and non-AOCs for combined benthos indicated differences in one or more metric values for every AOC. Differences in the relative abundance and distribution of combined benthic taxa at AOCs and non-AOCs in 2014 are shown in the MDS ordination plots (as described in the “Data Analyses” section). More similar samples appear closer together, indicating greater similarity, and less similar samples plot farther apart.

## Lower Menominee River Area of Concern

The Lower Menominee River was designated an AOC because of sediment contamination with arsenic, polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (also known as PAHs or coal tars), paint sludge, and heavy metals including cadmium, chromium, copper, lead, mercury, nickel, and zinc (U.S. Environmental Protection Agency, 2013a; Wisconsin Department of Natural Resources and Michigan Department of Environmental Quality, 2011). Sediment remediation was completed in November 2014 at the Lower Menominee River AOC and was therefore ongoing upstream when the 2014 samples were collected. The Escanaba River and Oconto River sites (ESCA and OCON) were the two non-AOC sites selected for comparisons to MENI because they have similar climate (cooler temperatures and higher snowfall than the more southern AOCs; Albert, 1995), latitude, and geology. All three are cold-water rivers (based on maximum daily mean temperatures less than about 20–22 °C with resultant fish assemblages; Lyons and others, 1996; Epstein, 2017) that have relatively high gradients, mostly sand and gravel (glaciated) surficial deposits, and parts that flow over bedrock. The Oconto River drains more clay surficial deposits than the other two rivers, mostly in the lower reaches (Robertson and Saad, 1995). Land cover/land is primarily forested and used for pulp production, with little

**Table 4.** Mean and standard deviation for sediment size fractions and volatile-on-ignition solids in bottom sediment collected in 2012 and 2014 at Areas of Concern and non-Area of Concern comparison sites in Wisconsin and Michigan.

[The number of samples is 3 for each mean and standard deviation. MENI, Lower Menominee River; ±, plus or minus; FOXR, Fox River near Allouez (Lower Green Bay and Fox River subsite); SHEB, Sheboygan River; MILR, Milwaukee River; MENO, Menomonee River; MILH, Milwaukee Harbor (MILR, MENO, and MILH are Milwaukee Estuary subsites); ESCA, Escanaba River, Mich.; OCON, Oconto River; AHNA, Ahnapee River; KEWA, Kewaunee River; MANI, Manitowoc River; ROOT, Root River]

| Site | 2012 | | | | 2014 | | | |
|---|---|---|---|---|---|---|---|---|
| | Sand (percent) | Silt (percent) | Clay (percent) | Volatile-on-ignition solids (percent) | Sand (percent) | Silt (percent) | Clay (percent) | Volatile-on-ignition solids (percent) |
| Areas of Concern | | | | | | | | |
| MENI | 89.7±5.1 | 6.3±4.2 | 4.0±1.0 | 3.42±1.47 | 90.3±4.6 | 3.0±5.2 | 6.7±0.6 | 1.18±0.32 |
| FOXR | 61.0±19.2 | 32.7±17.6 | 6.3±2.1 | 18.3±13.9 | 78.0±12.5 | 13.3±10.1 | 8.7±2.5 | 8.70±5.31 |
| SHEB[1] | 88.7±8.1 | 6.33±5.0 | 5.0±3.5 | 2.21±1.34 | 67.0±11.1 | 23.7±9.1 | 9.3±2.9 | 3.33±1.13 |
| MILR | 72.0±9.2 | 21.0±6.0 | 7.0±3.5 | 5.15±2.12 | 90.7±2.1 | 3.3±3.1 | 6.0±1.7 | 3.06±2.04 |
| MENO | 53.3±13.3 | 38.3±9.9 | 8.3±4.2 | 14.3±8.4 | 20.3±6.4 | 64.3±5.9 | 15.3±2.1 | 13.2±2.6 |
| MILH | 50.3±20.6 | 33.3±5.5 | 16.3±17.0 | 7.42±1.19 | 34.0±6.1 | 42.6±8.1 | 23.4±13.9 | 16.4±6.2 |
| Non-Area of Concern comparison sites | | | | | | | | |
| ESCA | 89.3±8.3 | 7.7±9.0 | 6.3±5.1 | 5.04±5.43 | 92.5±5.0 | 3.5±2.1 | 4.0±2.8 | 6.33±7.65 |
| OCON | 97.3±1.5 | 2.0±1.7 | 0.67±0.58 | 1.46±1.74 | 95.7±1.5 | 0.67±0.58 | 3.7±1.2 | 0.95±0.19 |
| AHNA | 60.0±29.5 | 31.3±27.5 | 8.7±3.2 | 12.3±6.3 | 50 | 36 | 14 | 27.8±11.8 |
| KEWA | 45.7±28.9 | 44.7±24.0 | 9.7±4.9 | 28.6±9.4 | 34 | 50 | 16 | 29.9±8.2 |
| MANI | 28.3±1.5 | 58.0±4.4 | 13.7±3.5 | 12.0±2.2 | 18.0±2.0 | 58.0±2.0 | 24.0±3.5 | 9.58±0.33 |
| ROOT | 89.7±3.5 | 6.0±1.7 | 4.3±2.3 | 2.77±0.41 | 86.3±5.8 | 5.7±4.9 | 8.0±1.0 | 2.14±0.21 |

[1]Values for SHEB in 2012 are for the replicate sample because of missing data in the primary sample.

other agriculture. Because of these similarities, the three rivers were expected to have similar benthic assemblages, despite the smaller drainage areas of the Escanaba and Oconto Rivers compared to the Lower Menominee River. The City of Oconto dredged the lower part of the Oconto River for navigation in 2012 through 2014, and it is possible that one or more of the 2014 dredge samples may have been affected (Jeremy Wusterbarth, City of Oconto, written commun., August 8, 2017) even though the samples were collected at a site upstream from and outside of the area where maps indicated planned dredging was done. No dredging was recorded in the lower Escanaba River during 2012–14 (Ryan McCone, Michigan Department of Environmental Quality, written commun., August 28, 2017).

Sediment size fraction and organic carbon content (estimated by VOI of solids) in sediment did not differ between MENI and its two non-AOC comparison sites (table 4). Similar to ESCA and OCON, the substrate at MENI was primarily hard sand (90 percent), making sediment difficult to obtain with the dredge; VOI analyses indicated low amounts of organic matter in the samples. Substrate that is mostly sand is a poor substrate for a variety of organisms (Wood and Armitage, 1997), especially if it contains only low amounts of organic matter to provide nutrients for benthic organisms.

At MENI in 2014, results were mixed for metric comparisons with non-AOCs using combined benthos (fig. 2, table 5). Diversity, total density, and EPT density differed between MENI and the mean of all non-AOCs in 2014; diversity at MENI was higher, indicating a less degraded condition, and both densities were lower, indicating a more degraded condition (table 6). Only EPT density and EPT richness differed between MENI and the mean of the two non-AOC comparison sites, ESCA and OCON; both metrics at MENI were lower. Lower EPT density and richness indicate poorer quality assemblages and, therefore, these metrics were rated as degraded at MENI relative to mean of the two non-AOC comparison sites in 2014. The mean IBI in 2014 was 25.0 plus or minus (±) 8.7, and this score is in the "poor" rating category that ranges from 20 to 39 (fig. 2*B*, table 5). The mean IBI for the two non-AOC comparison sites in 2014 was 38.3±3.8, which is also "poor." Metrics did not differ between 2012 and 2014 at MENI. This result was not unexpected because sediment remediation was still ongoing during both years and the sampling site was downstream from contaminated areas.

**Table 5.** Metric means and standard deviations for benthos sampled in 2012 and 2014 at 4 Lake Michigan Areas of Concern in Wisconsin and 6 non-Area of Concern comparison sites in Wisconsin and Michigan.

[Richness was computed as the number of unique taxa in the sample. Diversity is Shannon diversity, calculated as $\log_e$. EPT, Ephemeroptera-Plecoptera-Trichoptera; IBI, index of biotic integrity; MENI, Lower Menominee River; SD, standard deviation; ESCA, Escanaba River, Mich.; OCON, Oconto River; FOXR, Fox River near Allouez (FOXR is a Lower Green Bay and Fox River subsite); AHNA, Ahnapee River; KEWA, Kewaunee River; SHEB, Sheboygan River; MANI, Manitowoc River; MILR, Milwaukee River; MENO, Menomonee River; ROOT, Root River; MILH, Milwaukee Harbor (MILR, MENO, and MILH are Milwaukee Estuary subsites)]

| Statistic | Year | Dredge | | Hester-Dendy | | | Combined benthos[1] | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | All taxa | | All taxa | | | All taxa | | | EPT taxa | | |
| | | Richness | Diversity | Richness | Diversity | IBI[2] | Richness | Diversity | Density[3] | Richness | Density[3] | EPT percentage[4] |
| MENI Area of Concern site | | | | | | | | | | | | |
| Mean | 2012 | 13.3 | 2.1 | 27.7 | 1.9 | 18.3 | 38.7 | 2.4 | 4,414 | 6.7 | 342.6 | 7.8 |
| SD | 2012 | 4.0 | 0.3 | 9.7 | 0.9 | 2.9 | 13.1 | 0.9 | 2,239 | 2.3 | 192.2 | 3.0 |
| Mean | 2014 | 16.3 | 2.2 | 32 | 2.9 | 25 | 45.7 | 3.1 | 3,459 | 7.7 | 149.1 | 4.3 |
| SD | 2014 | 1.2 | 0.5 | 7.2 | 0.1 | 8.7 | 6.7 | 0.3 | 1,291 | 2.1 | 54.5 | 0.3 |
| ESCA non-Area of Concern comparison site | | | | | | | | | | | | |
| Mean | 2012 | 16.7 | 2.1 | 23.3 | 2.3 | 26.7 | 34.7 | 2.6 | 5,776 | 6.7 | 847.8 | 16.9 |
| SD | 2012 | 4.9 | 0.4 | 3.2 | 0.2 | 2.9 | 7.6 | 0.5 | 2,260 | 0.6 | 117.7 | 8.9 |
| Mean | 2014 | 28.3 | 2.8 | 26.3 | 2.1 | 30 | 49 | 3.1 | 9,478 | 8.3 | 991.0 | 15.3 |
| SD | 2014 | 16.7 | 0.4 | 11.2 | 0.6 | 5 | 9.6 | 0 | 6,085 | 1.5 | 294.5 | 11.2 |
| OCON non-Area of Concern comparison site | | | | | | | | | | | | |
| Mean | 2012 | 20.0 | 2.0 | 36.3 | 2.6 | 35 | 49 | 2.4 | 12,968 | 13.0 | 1,217.7 | 17.0 |
| SD | 2012 | 3.6 | 0.6 | 11 | 0.5 | 13.2 | 12.1 | 0.5 | 10,723 | 1.0 | 638.2 | 13.9 |
| Mean | 2014 | 28.7 | 2.5 | 41.7 | 3.0 | 46.7 | 63 | 3.0 | 10,937 | 14.3 | 1,000.7 | 10.7 |
| SD | 2014 | 6.7 | 0.2 | 14 | 0.1 | 10.4 | 9.6 | 0.1 | 3,939 | 3.1 | 447.2 | 6.9 |
| ESCA–OCON non-Area of Concern comparison sites | | | | | | | | | | | | |
| Mean | 2012 | 18.3 | 2.0 | 29.8 | 2.5 | 30.8 | 41.8 | 2.5 | 9,372 | 9.8 | 1,032.7 | 17.0 |
| SD | 2012 | 4.3 | 0.1 | 5.9 | 0.2 | 8.0 | 8.5 | 0.2 | 6,294 | 0.8 | 376.5 | 11.2 |
| Mean | 2014 | 28.5 | 2.6 | 34.0 | 2.6 | 38.3 | 56.0 | 3.0 | 10,207 | 11.3 | 995.9 | 13.0 |
| SD | 2014 | 9.6 | 0.2 | 10.4 | 0.3 | 3.8 | 9.5 | 0.1 | 5,011 | 1.6 | 319.3 | 8.9 |
| FOXR Area of Concern subsite | | | | | | | | | | | | |
| Mean | 2012 | 10.7 | 1.1 | 23.7 | 1.3 | 16.7 | 29.3 | 1.5 | 40,157 | 1.3 | 99.1 | 0.4 |
| SD | 2012 | 2.3 | 0.4 | 18.5 | 1.4 | 5.8 | 17.1 | 0.6 | 39,557 | 0.6 | 43.7 | 0.2 |
| Mean | 2014 | 15.0 | 1.7 | 27.3 | 2.6 | 13.3 | 38.7 | 2.4 | 18,841 | 4.3 | 476.2 | 3.7 |
| SD | 2014 | 4.4 | 0.1 | 4.2 | 0.1 | 10.4 | 9.0 | 0.2 | 13,046 | 0.6 | 224.2 | 2.7 |

**Table 5.** Metric means and standard deviations for benthos sampled in 2012 and 2014 at 4 Lake Michigan Areas of Concern in Wisconsin and 6 non-Area of Concern comparison sites in Wisconsin and Michigan.—Continued

[Richness was computed as the number of unique taxa in the sample. Diversity is Shannon diversity, calculated as $\log_e$. EPT, Ephemeroptera-Plecoptera-Trichoptera; IBI, index of biotic integrity; MENI, Lower Menominee River; SD, standard deviation; ESCA, Escanaba River, Mich.; OCON, Oconto River; FOXR, Fox River near Allouez (FOXR is a Lower Green Bay and Fox River subsite); AHNA, Ahnapee River; KEWA, Kewaunee River; SHEB, Sheboygan River; MANI, Manitowoc River; MILR, Milwaukee River; MENO, Menomonee River; ROOT, Root River; MILH, Milwaukee Harbor (MILR, MENO, and MILH are Milwaukee Estuary subsites)]

| Statistic | Year | Dredge | | Hester-Dendy | | | Combined benthos[1] | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | All taxa | | All taxa | | | All taxa | | | EPT taxa | | |
| | | Richness | Diversity | Richness | Diversity | IBI[2] | Richness | Diversity | Density[3] | Richness | Density[3] | EPT percentage[4] |
| AHNA non-Area of Concern comparison site | | | | | | | | | | | | |
| Mean | 2012 | 3.7 | 0.8 | 24.7 | 2.2 | 11.7 | 27.7 | 1.9 | 6,917 | 3.3 | 175.1 | 2.5 |
| SD | 2012 | 1.5 | 0.5 | 10.2 | 0.6 | 2.9 | 8.4 | 0.1 | 927 | 2.1 | 48.0 | 0.7 |
| Mean | 2014 | 9.0 | 1.7 | 27.7 | 2.3 | 6.7 | 35.0 | 2.3 | 11,249 | 3.0 | 68.9 | 0.6 |
| SD | 2014 | 1.7 | 0.1 | 7.1 | 0.3 | 2.9 | 7.9 | 0.3 | 3,779 | 0 | 33.6 | 0.4 |
| KEWA non-Area of Concern comparison site | | | | | | | | | | | | |
| Mean | 2012 | 9.0 | 0.6 | 12.7 | 1.3 | 8.3 | 20.7 | 1.6 | 53,986 | 1.0 | 126.3 | 0.3 |
| SD | 2012 | 1.7 | 0.2 | 7.5 | 0.7 | 7.6 | 8.5 | 0.6 | 15,497 | 0 | 130.4 | 0.4 |
| Mean | 2014 | 7.7 | 0.4 | 23 | 2.0 | 3.3 | 30.0 | 1.4 | 38,329 | 2.3 | 137.8 | 0.4 |
| SD | 2014 | 2.3 | 0.2 | 5.3 | 0.4 | 2.9 | 7.5 | 0.5 | 5,672 | 1.2 | 119.3 | 0.3 |
| AHNA–KEWA non-Area of Concern comparison site | | | | | | | | | | | | |
| Mean | 2012 | 6.3 | 0.7 | 18.7 | 1.7 | 10.0 | 24.2 | 1.7 | 30,452 | 2.2 | 150.7 | 1.4 |
| SD | 2012 | 1.0 | 0.4 | 8.4 | 0.6 | 2.5 | 8.1 | 0.3 | 7,297 | 1.0 | 74.1 | 0.3 |
| Mean | 2014 | 8.3 | 1.1 | 25.3 | 2.2 | 5.0 | 32.5 | 1.9 | 24,789 | 2.7 | 103.4 | 0.5 |
| SD | 2014 | 2.0 | 0.2 | 1.9 | 0.1 | 2.5 | 4.6 | 0.4 | 4,247 | 0.6 | 70.4 | 0.2 |
| SHEB Area of Concern site | | | | | | | | | | | | |
| Mean | 2012 | 13.0 | 0.5 | 25.7 | 2.0 | 8.3 | 35.3 | 1.1 | 48,318 | 1.3 | 15.8 | 0.1 |
| SD | 2012 | 6.6 | 0.4 | 6.7 | 0.9 | 2.9 | 14.2 | 0.6 | 33,987 | 1.2 | 17.4 | 0.1 |
| Mean | 2014 | 16.7 | 1.1 | 27.3 | 2.3 | 15 | 39.0 | 1.5 | 37,748 | 2.3 | 57.2 | 0.2 |
| SD | 2014 | 5.1 | 0.5 | 4.7 | 0.4 | 5 | 4.4 | 0.6 | 10,629 | 1.2 | 43.0 | 0.1 |
| KEWA non-Area of Concern comparison site | | | | | | | | | | | | |
| Mean | 2012 | 9.0 | 0.6 | 12.7 | 1.3 | 8.3 | 20.7 | 1.6 | 53,986 | 1.0 | 126.3 | 0.3 |
| SD | 2012 | 1.7 | 0.2 | 7.5 | 0.7 | 7.6 | 8.5 | 0.6 | 15,497 | 0 | 130.4 | 0.4 |
| Mean | 2014 | 7.7 | 0.4 | 23 | 2.0 | 3.3 | 30.0 | 1.4 | 38,329 | 2.3 | 137.8 | 0.4 |
| SD | 2014 | 2.3 | 0.2 | 5.3 | 0.4 | 2.9 | 7.5 | 0.5 | 5,672 | 1.2 | 119.3 | 0.3 |

**Table 5.** Metric means and standard deviations for benthos sampled in 2012 and 2014 at 4 Lake Michigan Areas of Concern in Wisconsin and 6 non-Area of Concern comparison sites in Wisconsin and Michigan.—Continued

[Richness was computed as the number of unique taxa in the sample. Diversity is Shannon diversity, calculated as $\log_e$. EPT, Ephemeroptera-Plecoptera-Trichoptera; IBI, index of biotic integrity; MENI, Lower Menominee River; SD, standard deviation; ESCA, Escanaba River, Mich.; OCON, Oconto River; FOXR, Fox River near Allouez (FOXR is a Lower Green Bay and Fox River subsite); AHNA, Ahnapee River; KEWA, Kewaunee River; SHEB, Sheboygan River; MANI, Manitowoc River; MILR, Milwaukee River; MENO, Menomonee River; ROOT, Root River; MILH, Milwaukee Harbor (MILR, MENO, and MILH are Milwaukee Estuary subsites)]

| Statistic | Year | Dredge | | Hester-Dendy | | | Combined benthos[1] | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | All taxa | | All taxa | | | All taxa | | | EPT taxa | | |
| | | Richness | Diversity | Richness | Diversity | IBI[2] | Richness | Diversity | Density[3] | Richness | Density[3] | EPT percentage[4] |
| MANI non-Area of Concern comparison site | | | | | | | | | | | | |
| Mean | 2012 | 7.3 | 0.6 | 33.7 | 2.1 | 8.3 | 38.3 | 1.0 | 61,637 | 1.7 | 14.4 | 0 |
| SD | 2012 | 0.6 | 0.1 | 9.5 | 0.3 | 7.6 | 10.3 | 0.4 | 41,640 | 2.9 | 24.9 | 0 |
| Mean | 2014 | 14.3 | 0.8 | 29.3 | 2.4 | 15 | 40.3 | 1.2 | 25,405 | 3.0 | 53.1 | 0.2 |
| SD | 2014 | 5.9 | 0.2 | 9.5 | 0.4 | 10 | 13.1 | 0 | 1,281 | 2.0 | 17.4 | 0.1 |
| KEWA–MANI non-Area of Concern comparison sites | | | | | | | | | | | | |
| Mean | 2012 | 8.2 | 0.6 | 23.2 | 1.7 | 8.3 | 29.5 | 1.3 | 57,811 | 1.3 | 70.3 | 0.2 |
| SD | 2012 | 1.0 | 0.1 | 8.4 | 0.4 | 3.8 | 9.3 | 0.1 | 13,417 | 1.4 | 77.6 | 0.2 |
| Mean | 2014 | 11.0 | 0.6 | 26.2 | 2.2 | 9.2 | 35.2 | 1.3 | 31,867 | 2.7 | 95.5 | 0.3 |
| SD | 2014 | 3.8 | 0.0 | 7.3 | 0.2 | 6.3 | 10.3 | 0.2 | 2,954 | 1.5 | 61.5 | 0.1 |
| MILR Area of Concern subsite | | | | | | | | | | | | |
| Mean | 2012 | 12.3 | 0.6 | 18.7 | 1.8 | 6.7 | 27.7 | 1.1 | 41,406 | 1.0 | 251.2 | 0.9 |
| SD | 2012 | 3.8 | 0.3 | 6 | 0.5 | 5.8 | 7.2 | 0.4 | 19,031 | 1.0 | 227.6 | 1.2 |
| Mean | 2014 | 14.3 | 1.1 | 32.7 | 2.4 | 30.0 | 42.0 | 1.4 | 30,574 | 6.7 | 676.1 | 4.1 |
| SD | 2014 | 2.1 | 0.5 | 14.6 | 0.6 | 15.0 | 13 | 0.3 | 23,330 | 3.2 | 295.6 | 4.7 |
| MENO Area of Concern subsite | | | | | | | | | | | | |
| Mean | 2012 | 13.7 | 1.0 | 21 | 1.8 | 5.0 | 31.3 | 1.2 | 74,158 | 1.3 | 144.1 | 0.2 |
| SD | 2012 | 2.1 | 0.2 | 9.5 | 0.6 | 5.0 | 5.0 | 0.2 | 32,908 | 0.6 | 156.5 | 0.2 |
| Mean | 2014 | 11.0 | 1.1 | 26.3 | 2.5 | 10.0 | 32.3 | 1.2 | 110,579 | 1.7 | 228.3 | 0.3 |
| SD | 2014 | 0 | 0 | 10.1 | 0.5 | 5.0 | 12.0 | 0.1 | 65,789 | 1.2 | 339.4 | 0.4 |
| MANI non-Area of Concern comparison site | | | | | | | | | | | | |
| Mean | 2012 | 7.3 | 0.6 | 33.7 | 2.1 | 8.3 | 38.3 | 1.0 | 61,637 | 1.7 | 14.4 | 0 |
| SD | 2012 | 0.6 | 0.1 | 9.5 | 0.3 | 7.6 | 10.3 | 0.4 | 41,640 | 2.9 | 24.9 | 0 |
| Mean | 2014 | 14.3 | 0.8 | 29.3 | 2.4 | 15.0 | 40.3 | 1.2 | 25,405 | 3.0 | 53.1 | 0.2 |
| SD | 2014 | 5.9 | 0.2 | 9.5 | 0.4 | 10.0 | 13.1 | 0 | 1,281 | 2.0 | 17.4 | 0.1 |

**Table 5.** Metric means and standard deviations for benthos sampled in 2012 and 2014 at 4 Lake Michigan Areas of Concern in Wisconsin and 6 non-Area of Concern comparison sites in Wisconsin and Michigan.—Continued

[Richness was computed as the number of unique taxa in the sample. Diversity is Shannon diversity, calculated as $log_e$. EPT, Ephemeroptera-Plecoptera-Trichoptera; IBI, index of biotic integrity; MENI, Lower Menominee River; SD, standard deviation; ESCA, Escanaba River, Mich.; OCON, Oconto River; FOXR, Fox River near Allouez (FOXR is a Lower Green Bay and Fox River subsite); AHNA, Ahnapee River; KEWA, Kewaunee River; SHEB, Sheboygan River; MANI, Manitowoc River; MILR, Milwaukee River; MENO, Menomonee River; ROOT, Root River; MILH, Milwaukee Harbor (MILR, MENO, and MILH are Milwaukee Estuary subsites)]

| Statistic | Year | Dredge | | Hester-Dendy | | | Combined benthos[1] | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | All taxa | | All taxa | | | All taxa | | | EPT taxa | | |
| | | Richness | Diversity | Richness | Diversity | IBI[2] | Richness | Diversity | Density[3] | Richness | Density[3] | EPT percentage[4] |
| ROOT non-Area of Concern comparison site | | | | | | | | | | | | |
| Mean | 2012 | 10.7 | 0.9 | 24.0 | 1.9 | 13.3 | 31.0 | 1.6 | 25,264 | 3.0 | 165.1 | 0.8 |
| SD | 2012 | 3.8 | 0.1 | 13.5 | 1.0 | 7.6 | 12.5 | 0.5 | 13,403 | 1.7 | 126.0 | 0.6 |
| Mean | 2014 | 16.0 | 1.0 | 30.3 | 2.4 | 10.0 | 39.7 | 1.6 | 35,482 | 1.7 | 87.1 | 0.2 |
| SD | 2014 | 1.0 | 0.2 | 10.2 | 0.3 | 10.0 | 9.1 | 0.2 | 9,797 | 0.6 | 30.7 | 0 |
| MANI–ROOT non-Area of Concern comparison sites | | | | | | | | | | | | |
| Mean | 2012 | 9.0 | 0.7 | 28.8 | 2.0 | 10.8 | 34.7 | 1.3 | 43,451 | 2.3 | 89.7 | 0.4 |
| SD | 2012 | 2.2 | 0.1 | 11.4 | 0.6 | 7.6 | 11.4 | 0.1 | 15,152 | 1.3 | 58.8 | 0.3 |
| Mean | 2014 | 15.2 | 0.9 | 29.8 | 2.4 | 12.5 | 40.0 | 1.4 | 30,443 | 2.3 | 70.1 | 0.2 |
| SD | 2014 | 3.1 | 0.2 | 6.3 | 0.2 | 10.0 | 8.2 | 0.1 | 4,260 | 1.3 | 16.9 | 0.03 |
| MILH Area of Concern subsite | | | | | | | | | | | | |
| Mean | 2012 | 13.0 | 0.9 | 23.7 | 1.7 | 18.3 | 31.3 | 1.0 | 61,650 | 0.3 | 5.7 | 0 |
| SD | 2012 | 4.4 | 0.3 | 14.8 | 1.3 | 7.6 | 12.3 | 0.2 | 44,509 | 0.6 | 9.9 | 0.1 |
| Mean | 2014 | 6.0 | 0.6 | 29.0 | 1.9 | 26.7 | 33.3 | 1.1 | 46,815 | 2.0 | 33.0 | 0.1 |
| SD | 2014 | 1.7 | 0.1 | 11.5 | 1.5 | 5.8 | 11.9 | 0.3 | 32,487 | 1.0 | 15.1 | 0.2 |
| All non-Area of Concern comparison sites | | | | | | | | | | | | |
| Mean | 2012 | 11.2 | 1.1 | 25.8 | 2.1 | 17.2 | 33.6 | 1.8 | 27,758 | 4.8 | 424.4 | 6.3 |
| SD | 2012 | 2.0 | 0.1 | 6.3 | 0.3 | 1.3 | 6.6 | 0.1 | 4,586 | 0.1 | 95.6 | 3.8 |
| Mean | 2014 | 17.3 | 1.5 | 29.7 | 2.4 | 18.6 | 42.8 | 2.1 | 21,813 | 5.4 | 389.8 | 4.6 |
| SD | 2014 | 4.5 | 0.1 | 5.9 | 0.1 | 4.6 | 7.4 | 0.1 | 2,902 | 1.1 | 91.8 | 3.0 |

[1]Metrics for combined benthos are for combined dredge and Hester-Dendy samples.

[2]IBI is designed for use with Hester-Dendy artificial substrates in nonwadeable rivers, calculated as in Weigel and Dimick (2011). The IBI score ranges from 0 (worst) to 100 (best): very poor (less than or equal to 19), poor (20–39), fair (40–59), good (60–79), and excellent (greater than or equal to 80).

[3]Density values for combined benthos are in count per square meter.

[4]Denotes the percentage of EPT individuals in the total sample.

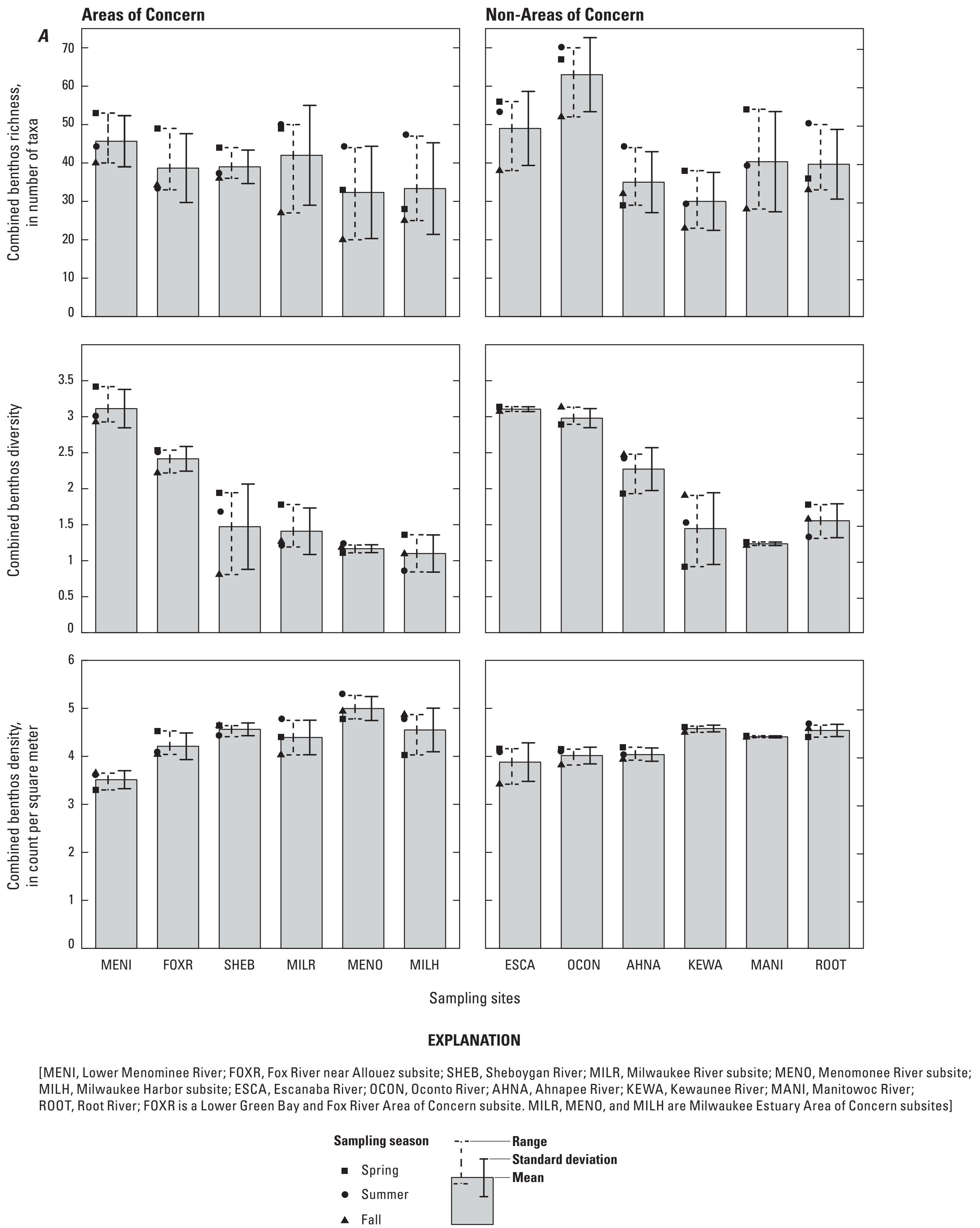
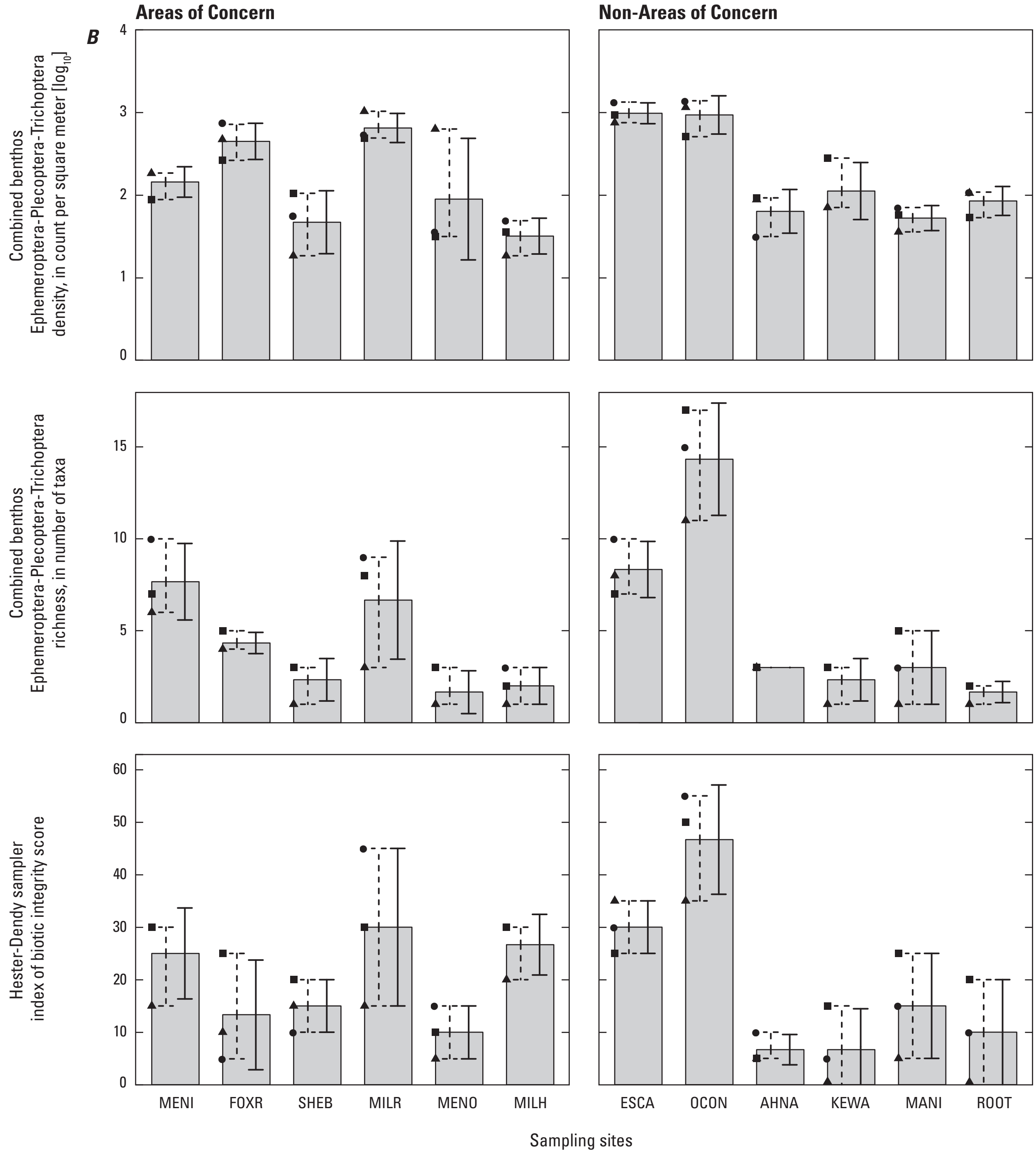



**Figure 2.** Metric values for benthos from 4 Lake Michigan Areas of Concern and 6 non-Area of Concern comparison sites. *A*, Richness, diversity, and total density of combined benthos (dredge and Hester-Dendy samples combined); and *B*, Ephemeroptera-Plecoptera-Trichoptera (EPT) density and EPT richness for combined benthos and the index of biotic integrity for Hester-Dendy samples.




**EXPLANATION**

[MENI, Lower Menominee River; FOXR, Fox River near Allouez subsite; SHEB, Sheboygan River; MILR, Milwaukee River subsite; MENO, Menomonee River subsite; MILH, Milwaukee Harbor subsite; ESCA, Escanaba River; OCON, Oconto River; AHNA, Ahnapee River; KEWA, Kewaunee River; MANI, Manitowoc River; ROOT, Root River; FOXR is a Lower Green Bay and Fox River Area of Concern subsite. MILR, MENO, and MILH are Milwaukee Estuary Area of Concern subsites]

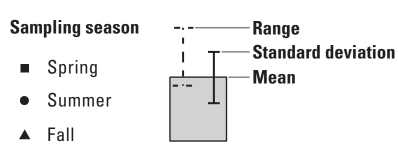



**Figure 2.** Metric values for benthos from 4 Lake Michigan Areas of Concern and 6 non-Area of Concern comparison sites. *A*, Richness, diversity, and total density of combined benthos (dredge and Hester-Dendy samples combined); and *B*, Ephemeroptera-Plecoptera-Trichoptera (EPT) density and EPT richness for combined benthos and the index of biotic integrity for Hester-Dendy samples.—Continued

A comparison of the benthic assemblage at MENI to non-AOCs by multivariate ordination indicated that MENI was similar to its two non-AOC comparison sites. MENI, ESCA, and OCON grouped together and away from the more southern sites in the MDS ordination plots, when seasons were combined (fig. 3*A*) and when seasons were separate (fig. 3*B*). The ANOSIM results did not indicate a difference between the assemblages at these sites, but results indicated that MENI was 61 percent dissimilar from its two non-AOC comparison sites. SIMPER analysis further indicated that the three taxa contributing most to this dissimilarity were (in order of contribution) the oligochaete *Nais simplex*, immature Tubificinae oligochaetes, and the pea clam *Pisidium*. In spring 2014, densities of *Nais simplex* at OCON were several times higher than at MENI or ESCA. *Nais simplex* is considered moderately tolerant to pollution (Bode and others, 2002). There were lower relative abundances of highly tolerant immature Tubificinae at MENI than at ESCA and OCON. *Pisidium* was common at MENI in all seasons, absent at ESCA, and present only in the fall at OCON. Pea clams such as *Pisidium* are moderately tolerant and common in Lake Michigan and its tributaries, and some species can be locally abundant and found in a variety of substrates (Barbour and others, 1999; Heard, 1962; Mackie and others, 1980). They are an important food source for fish.

Dominance of benthic taxa at MENI in 2014 was similar to dominance at its two non-AOC comparison sites. In all seasons, midges had the highest relative abundance of all taxa at MENI (more than 40 percent), ESCA (more than 30 percent), and OCON (more than 41 percent). Oligochaetes were moderately abundant at all three sites, and abundances at MENI were higher in the spring and summer (22 percent) than in the fall (9 percent), which likely reflects the life histories of these organisms. Abundances of pea clams were higher (28 percent) in the fall than in the spring or summer. Mayflies and caddisflies were rare or absent in 2014 samples from most sites. Together, they comprised 4–5 percent of the overall abundance in all three seasons at MENI and 3–6 percent in the spring and 17–28 percent in the fall at ESCA and OCON. Amphipods were found in low abundance (5–15 percent) in 2014 samples from MENI and ESCA, and they were rare or absent at OCON and other sites. Zebra mussels were present at all three sites but were absent from some samples or in low abundance in others (less than 3 percent).

In addition, there were differences in metrics between the two non-AOC comparison sites. The total richness of combined benthos at MENI (45.7±6.7) and ESCA (49.0±9.6) was similar in 2014; however, this metric was higher at OCON (63.0±9.6) than at ESCA. These differences in metrics highlight the fact that some non-AOC comparison sites were different from each other, and some non-AOCs were slightly degraded and thus similar to their AOCs; therefore, these slightly degraded non-AOCs may not have been appropriate as comparison sites for assessing the degradation status of their respective AOCs.

## Lower Green Bay and Fox River Area of Concern

Farther south, the Fox River historically received contaminant discharges, primarily PCBs, that were noted as the main cause of AOC designation because of the resultant severe sediment contamination; however, nutrient enrichment in nonpoint runoff from agricultural and urban lands was a contributing factor as well (U.S. Environmental Protection Agency, 2013b; Wisconsin Department of Natural Resources, 2013). Drainage of contaminants and nutrients from the Fox River into Green Bay led to lower Green Bay near the mouth of the Fox River being designated as part of the AOC. Sediment remediation was ongoing in the Lower Green Bay and Fox River AOC at the time of sampling. There is no river or estuary system on the western shoreline of Lake Michigan that can truly compare to Green Bay, and therefore, only the Fox River near Allouez subsite (FOXR) was compared to the non-AOC comparison sites. Despite smaller drainage areas, sites on the Ahnapee River (sampling site hereafter referred to as “AHNA”) and Kewaunee River (sampling site hereafter referred to as “KEWA”) were chosen for comparison to the Fox River based on similar climate (Albert, 1995), latitude, and geology. The Fox River, Ahnapee River, and Kewaunee River are all warm-water (based on maximum daily mean temperatures greater than about 24 °C with resultant fish assemblages; Lyons and others, 1996; Epstein, 2017), low-gradient streams that flow through predominantly agricultural land and wetlands. Surficial deposits are glaciated and clay is dominant (Robertson and Saad, 1995).

The substrate at FOXR in 2014 was mostly sand (average of 78±12.5 percent) with some silt and clay and generally low to moderate organic carbon content sites (table 4). Missing data (insufficient material) for sediment size fractions precluded comparisons between FOXR, AHNA, and KEWA in the spring and summer; however, results for the fall indicated that sediment at AHNA and KEWA was lower in sand and higher in silt and organic carbon content than FOXR. The percentage of clay in FOXR sediment was higher in 2014 compared to 2012 but was still low overall. Lower Green Bay is discussed later in this report in the “Overview of Benthos and Plankton in Lower Green Bay and Milwaukee Harbor” section.

For combined benthos, no metrics differed between FOXR and the mean of all non-AOCs in 2014. Only EPT richness differed in comparisons between FOXR and the mean of the two non-AOC comparison sites in 2014; EPT richness was higher at FOXR than at AHNA and KEWA (fig. 2, table 6). EPT (mayflies, stoneflies, and caddisflies) richness was actually low at all three sites in 2014 (fig. 2*B*, table 5). A total of one to three mayfly taxa were found at all three sites. No stonefly taxa were found at FOXR or KEWA, and only one stonefly taxon was found in the spring at AHNA. For caddisfly taxa, zero to two taxa were found at AHNA and only one taxon in one season was found at KEWA. In each season at FOXR, two to three caddisfly taxa were present: *Cheumatopsyche* in the spring and summer and *Cyrnellus fraternus*

**Table 6.** Probability values for significance in paired *t*-tests comparing metrics for benthos at Areas of Concern (AOCs) with the mean of all non-AOCs or the mean of the two non-AOC comparison sites.

[All metrics are for combined benthos (combined dredge and Hester-Dendy samples) except the index of biotic integrity (Hester-Dendy samples only). Values in bold italics indicate the AOC metrics were significantly lower than non-AOCs compared; the number of samples is 3 in all comparisons. MENI, Lower Menominee River; EPT, Ephemeroptera-Plecoptera-Trichoptera; IBI, index of biotic integrity; FOXR, Fox River near Allouez (Lower Green Bay and Fox River subsite); SHEB, Sheboygan River; MILR, Milwaukee River; MENO, Menomonee River (MILR and MENO are Milwaukee Estuary subsites)]

| Metric | 2012 | | 2014 | |
|---|---|---|---|---|
| | AOC: non-AOC group | AOC: non-AOC pair | AOC: non-AOC group | AOC: non-AOC pair |
| | | MENI site | | |
| Richness | 0.543 | 0.814 | 0.466 | 0.109 |
| Diversity | 0.371 | 0.844 | 0.043 | 0.722 |
| Total density[1] | ***0.025*** | 0.313 | ***0.023*** | 0.206 |
| EPT density[1] | 0.307 | ***0.017*** | ***0.029*** | ***0.005*** |
| EPT percent | 0.100 | 0.194 | 0.904 | 0.241 |
| EPT richness | 0.278 | 0.202 | 0.141 | ***0.037*** |
| IBI | 0.621 | 0.082 | 0.118 | 0.067 |
| | | FOXR subsite | | |
| Richness | 0.585 | 0.582 | 0.509 | 0.378 |
| Diversity | 0.423 | 0.461 | 0.201 | 0.218 |
| Total density[1] | 0.927 | 0.986 | 0.498 | 0.311 |
| EPT density[1] | 0.064 | 0.263 | 0.499 | 0.141 |
| EPT percent | 0.126 | ***0.041*** | 0.651 | 0.197 |
| EPT richness | ***0.008*** | 0.464 | 0.171 | 0.038 |
| IBI | 0.895 | 0.208 | 0.379 | 0.319 |
| | | SHEB site | | |
| Richness | 0.749 | 0.173 | 0.394 | 0.402 |
| Diversity | 0.117 | 0.499 | 0.268 | 0.806 |
| Total density[1] | 0.731 | 0.606 | 0.162 | 0.570 |
| EPT density[1] | 0.063 | 0.187 | 0.061 | 0.122 |
| EPT percent | 0.108 | 0.349 | 0.132 | 0.155 |
| EPT richness | ***0.038*** | 1.000 | ***0.0003*** | 1.000 |
| IBI | ***0.012*** | 1.000 | 0.370 | 0.423 |
| | | MILR subsite | | |
| Richness | 0.059 | 0.256 | 0.822 | 0.547 |
| Diversity | 0.083 | 0.315 | 0.105 | 0.919 |
| Total density[1] | 0.353 | 0.722 | 0.786 | 0.696 |
| EPT density[1] | 0.423 | 0.825 | 0.209 | 0.013 |
| EPT percent | 0.088 | 0.414 | 0.787 | 0.288 |
| EPT richness | ***0.019*** | ***0.015*** | 0.429 | 0.080 |
| IBI | 0.115 | 0.130 | 0.253 | 0.149 |

**Table 6.** Probability values for significance in paired *t*-tests comparing metrics for benthos at Areas of Concern (AOCs) with the mean of all non-AOCs or the mean of the two non-AOC comparison sites.—Continued

[All metrics are for combined benthos (combined dredge and Hester-Dendy samples) except the index of biotic integrity (Hester-Dendy samples only). Values in bold italics indicate the AOC metrics were significantly lower than non-AOCs compared; the number of samples is 3 in all comparisons. MENI, Lower Menominee River; EPT, Ephemeroptera-Plecoptera-Trichoptera; IBI, index of biotic integrity; FOXR, Fox River near Allouez (Lower Green Bay and Fox River subsite); SHEB, Sheboygan River; MILR, Milwaukee River; MENO, Menomonee River (MILR and MENO are Milwaukee Estuary subsites)]

| Metric | 2012 | | 2014 | |
|---|---|---|---|---|
| | AOC: non-AOC group | AOC: non-AOC pair | AOC: non-AOC group | AOC: non-AOC pair |
| | | MENO subsite | | |
| Richness | 0.268 | 0.458 | 0.096 | 0.168 |
| Diversity | ***0.037*** | 0.238 | ***0.004*** | 0.158 |
| Total density[1] | 0.048 | 0.114 | 0.039 | 0.043 |
| EPT density[1] | 0.102 | 0.832 | 0.283 | 0.833 |
| EPT percent | 0.110 | 0.535 | 0.105 | 0.892 |
| EPT richness | ***0.013*** | 0.438 | ***0.025*** | 0.270 |
| IBI | ***0.038*** | 0.317 | 0.053 | 0.667 |

[1]$Log_{10}$-transformed data.

in all seasons. Although different species of *Cheumatopsyche* can vary in their tolerance to pollution, *Cyrnellus fraternus* is highly tolerant (Hilsenhoff, 1987). Although higher EPT richness is a positive indicator, the mean IBI at FOXR was 13.3±10.4, and this score is in the “very poor” rating category that includes all scores less than or equal to 19 (fig. 2, table 5). The mean IBI for the two non-AOC comparison sites, AHNA and KEWA, was only 5.0±3.2 in 2014. Only EPT richness differed between 2012 and 2014 at FOXR, with 2014 higher than 2012.

Multivariate ordination indicated that the combined benthic assemblage at FOXR was distinct, plotting away from all other sites in MDS ordination plots when seasons were combined (fig. 3*A*); however, with seasons separate, the summer and fall samples at FOXR were less similar to the two non-AOC comparison sites (AHNA and KEWA) than the spring FOXR sample (fig. 3*B*). An ANOSIM indicated that the 2014 benthic assemblages at FOXR were different from benthic assemblages at its two non-AOC comparison sites. Additional SIMPER testing indicated that FOXR was 62 percent dissimilar from its non-AOC comparison sites, mostly because of higher relative abundances of oligochaetes *Limnodrilus cervix*, *Aulodrilus pigueti*, and *Branchiura sowerbyi* at FOXR. *Limnodrilus cervix* is tolerant of highly polluted conditions including extremely eutrophic conditions; *A. pigueti* and *B. sowerbyi* are also pollution tolerant but less so than *L. cervix* (Bode and others, 2002; Rodriguez and Reynoldson, 2011). *Branchiura sowerbyi* is common around the Great Lakes but was not reported until the 1930s and is possibly nonnative (Spencer and Hudson, 2003; Great Lakes Aquatic Nonindigenous Species Information System, 2018).

Oligochaetes had the highest relative abundance in all seasons in 2014 at FOXR (more than 56 percent), and this was similar to AHNA and KEWA, except in the fall at AHNA when midges were higher in abundance (69 percent). Midges were moderately abundant (more than 16 percent) at FOXR, as well as at AHNA and KEWA (except in the spring at KEWA). Zebra mussels comprised less than 1 percent of the relative abundance at FOXR in 2014, were found at AHNA in the fall only and in low abundance (2 percent), and were not found at KEWA.

## Sheboygan River Area of Concern

The Sheboygan River AOC was designated because of concerns about sediment contamination from PCBs, polycyclic aromatic hydrocarbons, and heavy metals (Burzynski, 2000; Wisconsin Department of Natural Resources, 1995, 2012). Sediment remediation was completed in June 2013; therefore, sample collection in 2014 was postremediation. The sampling sites on the Kewaunee and Manitowoc Rivers were the two non-AOCs selected for comparison to the Sheboygan River AOC, the smallest AOC in Wisconsin. The Kewaunee and Manitowoc Rivers are nearby tributaries to the Sheboygan River, and sites on these rivers (KEWA and MANI) were selected because of similar climate (Albert, 1995), latitude, geology, and land use. The Manitowoc River and Sheboygan River have similar drainage areas (1,341 and 1,043 square kilometers [$km^2$], respectively), but the Kewaunee River is smaller (329 $km^2$). There is a U.S. Environmental Protection Agency Superfund site on the Manitowoc River, about 1 mile from the mouth (U.S. Environmental Protection Agency, 2019), but the river does not have an AOC designation.

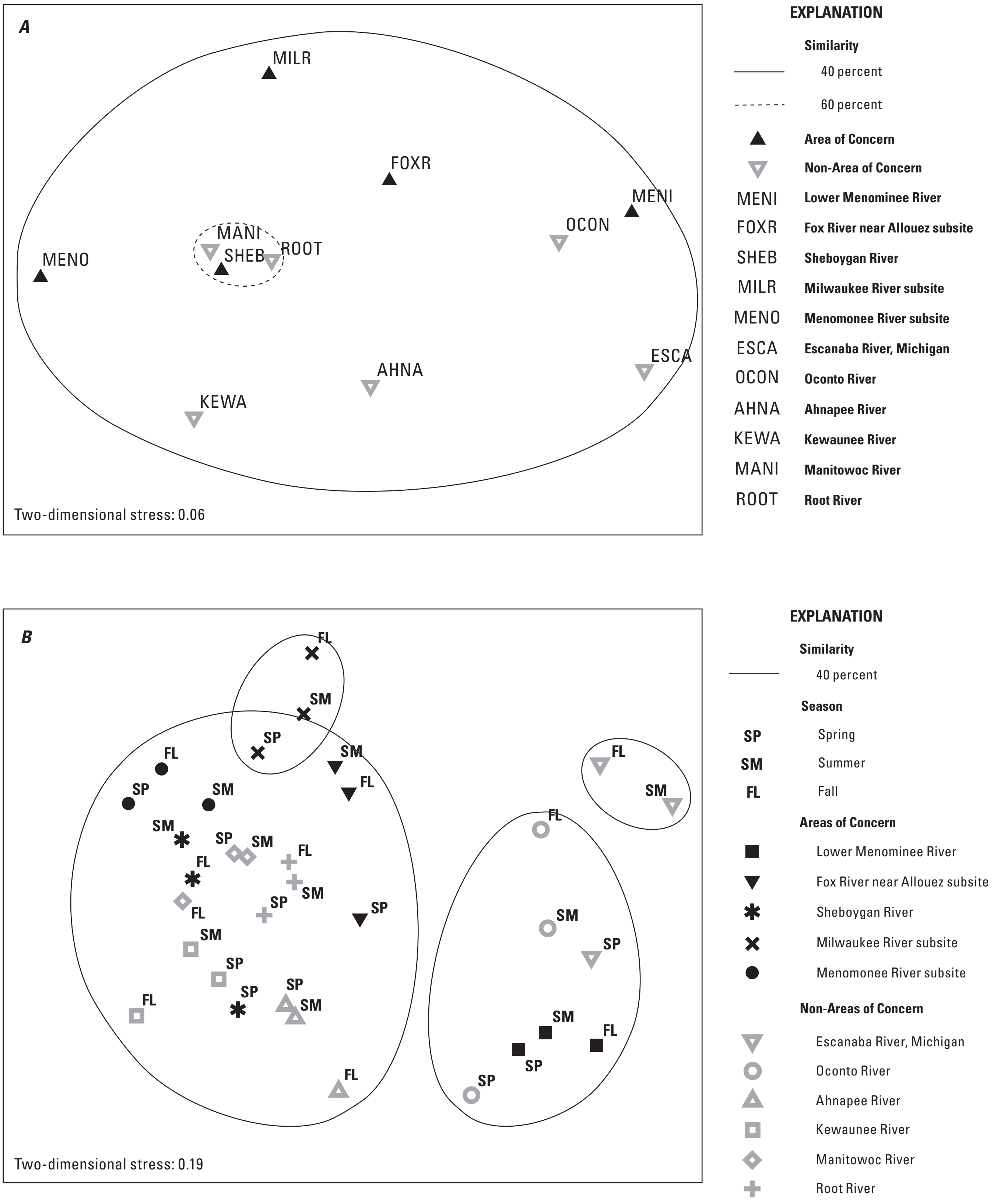



**Figure 3.** Multidimensional scaling ordination plots for combined benthos (dredge and Hester-Dendy samples combined) at 4 Lake Michigan Areas of Concern and 6 non-Area of Concern comparison sites, based on relative abundance with no rare or ambiguous taxa. *A*, Seasons combined; and *B*, seasons separate. Distances between sites are representative of their similarity or dissimilarity to each other. [The Fox River near Allouez is a subsite of the Green Bay and Fox River Area of Concern. The Milwaukee River and Menomonee River are subsites of the Milwaukee Estuary Area of Concern]

Surficial deposits for all three rivers are primarily clay with some areas of sand and gravel (Robertson and Saad, 1995). All three rivers are low gradient and flow through predominantly agricultural land and wetlands with urban land use at the mouth, and all are warm-water rivers.

Sediment percentages of silt and organic carbon were lower at SHEB than at MANI and KEWA in 2014, the percentages of clay did not differ, and the percentages of sand were higher at SHEB (table 4). Sediment at SHEB was mostly sand (average of 78±14 percent) followed by silt, with low organic content (less than 5 percent), whereas sediment at MANI and KEWA was about one-third sand and one-half silt with higher organic content.

Only EPT richness differed between SHEB and the mean of all non-AOCs, and SHEB was lower in 2012 and 2014. The IBI was lower at SHEB than at all non-AOCs in 2012 but not in 2014 after sediment remediation was complete. In 2014, the mean IBI at SHEB was 15.0±5.0, in the "very poor" rating category (≤19), and the mean IBI for the two non-AOC comparison sites was 9.2±9.2 (fig. 2*A*, table 5). No metrics differed between SHEB and the two non-AOC comparison sites, KEWA and MANI in 2014 (fig. 2*B*, table 6). Metrics did not differ between 2012 and 2014 at SHEB. In summary, no differences were found between SHEB and the non-AOC comparison sites in 2014, postremediation.

Multivariate ordination using ANOSIM indicated that the 2014 assemblage at SHEB for combined benthos was different from the two non-AOC comparison sites, KEWA and MANI. However, the MDS ordination plot indicated that this difference was due more to a difference between SHEB and KEWA for summer and fall (fig. 3*B*). Except for the spring sample at SHEB, relative abundances of benthic taxa were similar for SHEB and MANI, as evidenced by samples for these sites that plotted close to each other and away from KEWA when seasons were combined (fig. 3*A*). SIMPER results indicated that SHEB was 54 percent dissimilar from its two non-AOC comparison sites, mostly because of the midge *Glyptotendipes*, the oligochaete *Paranais*, and zebra mussels. *Glyptotendipes* was found in low abundance or was absent at the SHEB but was abundant at KEWA and uncommon to abundant at MANI. *Glyptotendipes* is highly tolerant of pollution (Barbour and others, 1999) and so is *Paranais* (Bode and others, 2002; Rodriguez and Reynoldson, 2011). *Paranais* and zebra mussels were relatively abundant at SHEB but were uncommon or absent at MANI and KEWA.

Oligochaetes had the highest relative abundance of all taxa at SHEB (more than 70 percent), as well as at KEWA (more than 52 percent) and MANI (more than 88 percent). The abundance of oligochaetes was lowest in the spring and highest in the fall at SHEB, but this was opposite of their abundance at KEWA; oligochaete abundance at MANI was only slightly lower in the summer than in the spring and fall. Although midges comprised 26 percent of the abundance at SHEB in spring 2014, midge abundance was only a fraction of that in other seasons (7 and 3 percent in summer and fall, respectively). In contrast, midge abundance was lowest in the spring and highest in the fall at KEWA, ranging from 3.5 percent in the spring to 44 percent in the fall. The abundance of midges at MANI was less than 7 percent in all seasons in 2014. Other insects, such as mayflies and caddisflies, made up less than 0.5 percent of the relative abundance at the three sites in any season.

## Milwaukee Estuary Area of Concern

Contaminants of concern in the Milwaukee Estuary AOC are mainly PCBs, polycyclic aromatic hydrocarbons, pesticides, and heavy metals such as cadmium, copper, and zinc (U.S. Environmental Protection Agency, 2013c; Wisconsin Department of Natural Resources, 1994, 2014). Sediment remediation was ongoing during both years of sampling for benthos and plankton. The MILH subsite was not compared to non-AOCs because of its size and complexity and, therefore, results for MILH are discussed in a separate section. The MILR and MENO subsites were compared to two non-AOC comparison sites, MANI and the Root River sampling site (hereafter referred to as "ROOT"), because of similar climate (Albert, 1995), geology, and land use. Surficial deposits in all these rivers are glaciated, with primarily clay and sand but also some areas of sand and gravel (Robertson and Saad, 1995). All these rivers have agricultural land in the headwaters transitioning to urban land near the mouth. The Milwaukee River and Manitowoc River are similar in drainage area and the Menomonee River and Root River are similar in drainage area. All are warm-water rivers water (based on maximum daily mean temperatures greater than about 24 °C with resultant fish assemblages; Lyons and others, 1996; Epstein, 2017).

Sediment contained more sand and less silt and clay at MILR than at MANI and ROOT, but organic carbon content was similar between the three sites (table 4). Organic carbon content at MILR was higher in 2012 than in 2014 but was still low both years. In contrast, sediment contained less sand and more silt at MENO than at MANI and ROOT, and higher values for organic carbon content were found at MENO; the percentage of sand at MENO was higher, and the percentage of silt was lower, in 2012 compared to 2014. Across 2012 and 2014, the substrate at MILR was mostly sand (81±12 percent) with low organic carbon content (4.1±2.2 percent), and the substrate at MENO was lower in sand (37±20 percent) and higher in silt (51±16 percent) and organic carbon content (14±5.6 percent; table 4). The sediment at MANI was more similar to MILR, whereas the sediment at ROOT was more similar to MENO.

For benthos at MILR in 2014, no metrics differed between MILR and the mean of all non-AOCs. Only EPT density differed between MILR and the mean of the two non-AOC comparison sites, MANI and ROOT, and the value at MILR was higher (fig. 2*B*, table 6). Densities of mayflies were low and there were no stoneflies at the three sites. Densities of most caddisflies were low to moderate at the sites. However, densities of the caddisfly *Cyrnellus fraternus* at MILR ranged from 108 to 965 individuals per square meter, which led to higher EPT densities at MILR compared to MANI and ROOT. As was mentioned earlier for the occurrence of this taxon at FOXR, *C. fraternus* is considered to be highly tolerant to pollution (Hilsenhoff, 1987). Although EPT richness in 2012 was lower than the mean of all non-AOCs as well as the two non-AOC comparison sites, no difference was found in 2014. Diversity was low at a mean of 1.4±0.3 (table 5). Surprisingly, there was no difference ($p$=0.060) between years at MILR for the IBI, which averaged 6.7±5.8 in 2012 (“very poor” rating category) and 30.0±15.0 (“poor” rating category) in 2014 (fig. 2*A*, table 5). The mean IBI for the two non-AOC comparison sites in 2014 was 12.5±10.0. There was no difference between 2012 and 2014 for any metrics at MILR.

Diversity, total density, and EPT richness differed between MENO and the mean of all non-AOCs in 2014, as well as in 2012. MENO was lower for diversity and EPT richness and was higher for total density. The relation for diversity was highly significant in 2014 ($p$<0.01; fig. 2*A*, table 6). Only total density differed between MENO and the mean of the two non-AOC comparison sites in 2014; total density at MENO was higher. The higher density at MENO was because of higher densities for oligochaetes, especially highly tolerant *Limnodrilus cervix*, *Limnodrilus hoffmeisteri*, and immature Tubificinae. The mean IBI was rated “very poor” in 2012 and 2014 at 5.0±5.0 and 10.0±5.0, respectively. Although the IBI at MENO was lower than the mean of all non-AOCs in 2012, the relation was not quite significant in 2014 ($p$=0.053), and the mean of the two non-AOC comparison sites was also rated “very poor” in 2012 and 2014 at 10.8±7.6 and 12.5±10.0, respectively. There was no difference between 2012 and 2014 for any metrics at MENO.

For multivariate ordination, all seasons for MILR plotted as a distinct grouping away from MANI and ROOT and closer or similar in makeup to MENO in 2014 (fig. 3*A*), especially the summer and fall samples (fig. 3*B*). The ANOSIM indicated that MILR was 58 percent dissimilar from MANI and ROOT, mostly because of differences in the abundances of the pea clam *Pisidium*, the oligochaete *Aulodrilus pluriseta*, and the caddisfly *Cyrnellus fraternus*. Abundances of *Pisidium* and *A. pluriseta* were relatively high at MILR in the spring and summer when compared to the low abundance or absence of these two taxa at MANI and ROOT; *C. fraternus* was found in higher abundance at MILR than the two non-AOC comparison sites. *Aulodrilus pluriseta* is moderately tolerant of pollution (Bode and others, 2002; Rodriguez and Reynoldson, 2011) and so is *C. fraternus* (Barbour and others, 1999). In 2014, the assemblage of combined benthos at MENO was different from its two non-AOC comparison sites MANI and ROOT. SIMPER results indicated that MENO was 51 percent dissimilar from these sites, primarily because of differences in the abundances of oligochaetes, *Aulodrilus pluriseta* and *Ilyodrilus templetoni*, and midges in the *Polypedilum halterale* group. There was a higher abundance of *A. pluriseta* in the summer and fall and a lack of *I. templetoni* and the *P. halterale* group at MENO.

As was seen at most other sites, oligochaetes were the dominant taxa at MILR and MENO in 2014. At MILR, the highest relative abundance for oligochaetes was in the spring (more than 88 percent) and the lowest was in the fall (more than 75 percent). Oligochaete abundance was similar across seasons (96–97 percent) at MENO. This abundance was similar to MANI (more than 88 percent) and ROOT (more than 75 percent). Midges were found in low abundance (less than 10 percent) at MILR, in lower abundance at MENO and MANI, and in moderate abundance at ROOT in all seasons (15 percent or more). Surprisingly, caddisflies made up 9 percent of the relative abundance in the fall at MILR but were never more than 1 percent at MENO or the non-AOC comparison sites. Zebra mussels were absent from MILR and were present in low abundance at MENO, MANI, and ROOT.

Of all four AOCs examined for benthos, only the Lower Menominee River AOC differed from its two non-AOC comparison sites; density and richness of EPT taxa (individuals in insect orders Ephemeroptera-Plecoptera-Trichoptera (EPT; mayflies, stoneflies, and caddisflies) in combined benthos (dredge and artificial substrate samples) were lower at the AOC.

## Planktonic Assemblage Comparisons between Areas of Concern and Non-Areas of Concern

Comparisons between each AOC and its non-AOC comparison sites were made for zooplankton and for combined phytoplankton (soft algae and diatoms combined). The metrics compared were richness, diversity, and total density (table 7). Assemblages of zooplankton at most sampled sites were dominated by rotifers in 2014, followed by copepods or zebra mussel veligers (means of 65, 17, and 13 percent abundance overall, respectively). The ANOSIM did not reveal differences between assemblages of zooplankton at any AOC when compared to the non-AOC comparison sites, possibly because there were often low similarities between the non-AOC comparison sites for zooplankton as indicated by SIMPER tests and MDS ordination plots., Differences in the relative abundances of taxa making up the assemblages at each AOC in comparison with the non-AOC comparison sites may signify degradation. Assemblages of phytoplankton at most sites were dominated by diatoms, followed by green algae and cryptophytes (means of 33-, 28-, and 22-percent abundance overall, respectively). Paired $t$-tests indicated no differences in chlorophyll-$a$ concentration or TSS and VSS between any AOCs and their non-AOC comparison sites in 2014, indicating that the biomass of phytoplankton was not different between the sites. This finding was supported in tests directly comparing densities of phytoplankton at sites. Missing data for VSS in two seasons at MENI and MENO precluded statistical analyses. Detailed assessments of planktonic assemblages at each AOC are provided in this section.

**Table 7.** Metric means and standard deviations for plankton sampled in 2012 and 2014 at 4 Lake Michigan Areas of Concern in Wisconsin and 6 non-Area of Concern comparison sites in Wisconsin and Michigan.

[Richness was computed as the number of unique taxa in the sample. Diversity is Shannon diversity, calculated as $\log_e$. MENI, Lower Menominee River; SD, standard deviation; ESCA, Escanaba River, Mich.; OCON, Oconto River; GREE, Lower Green Bay; FOXR, Fox River near Allouez (GREE and FOXR are Lower Green Bay and Fox River subsites); AHNA, Ahnapee River; KEWA, Kewaunee River; SHEB, Sheboygan River; MANI, Manitowoc River; MILR, Milwaukee River; MENO, Menomonee River; ROOT, Root River; MILH, Milwaukee Harbor (MILR, MENO, and MILH are Milwaukee Estuary subsites)]

| Statistic | Year | Zooplankton[1] | | | Soft algae | | Diatoms | | Combined phytoplankton[2] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Richness | Diversity | Density[3] | Richness | Diversity | Richness | Diversity | Richness | Diversity | Density |
| MENI Area of Concern site | | | | | | | | | | | |
| Mean | 2012 | 29.0 | 2.3 | 9,364 | 12.0 | 1.4 | 16.7 | 2.2 | 28.7 | 2.5 | 2,829 |
| SD | 2012 | 5.3 | 0.6 | 5,903 | 3.6 | 0.4 | 10.3 | 0.8 | 12.5 | 0.5 | 321 |
| Mean | 2014 | 33.0 | 2.3 | 9,958 | 7.0 | 1.1 | 75.0 | 3.6 | 82.0 | 3.0 | 2,725 |
| SD | 2014 | 7.9 | 0.3 | 688 | 1.7 | 0.1 | 9.5 | 0.5 | 8.9 | 0.2 | 1,499 |
| ESCA non-Area of Concern comparison site | | | | | | | | | | | |
| Mean | 2012 | 24.0 | 2.2 | 2,430 | 11.0 | 1.5 | 18.7 | 2.5 | 29.7 | 2.7 | 1,530 |
| SD | 2012 | 3.0 | 0.5 | 833 | 2.0 | 0.4 | 11.5 | 0.4 | 9.9 | 0.2 | 223 |
| Mean | 2014 | 32.3 | 2.4 | 6,668 | 8.0 | 1.3 | 67.3 | 3.1 | 75.3 | 2.9 | 2,087 |
| SD | 2014 | 6.7 | 0.4 | 2,116 | 2.0 | 0.7 | 0.6 | 0.4 | 1.5 | 0.5 | 1,975 |
| OCON non-Area of Concern comparison site | | | | | | | | | | | |
| Mean | 2012 | 22.0 | 2.2 | 2,123 | 10.0 | 1.4 | 23.3 | 2.6 | 33.3 | 2.8 | 3,841 |
| SD | 2012 | 3.6 | 0.2 | 1,379 | 5.3 | 0.6 | 11.7 | 0.3 | 15.1 | 0.4 | 2,051 |
| Mean | 2014 | 32.7 | 2.3 | 8,787 | 9.3 | 1.1 | 79.7 | 3.9 | 89.0 | 3.2 | 1,957 |
| SD | 2014 | 4.0 | 0.3 | 3,618 | 3.5 | 0.4 | 5.0 | 0.1 | 4.6 | 0.2 | 436 |
| ESCA–OCON non-Area of Concern comparison sites | | | | | | | | | | | |
| Mean | 2012 | 23.0 | 2.2 | 2,277 | 10.5 | 1.5 | 21.0 | 2.6 | 31.5 | 2.7 | 2,686 |
| SD | 2012 | 2.0 | 0.4 | 1,081 | 3.6 | 0.5 | 1.0 | 0.0 | 3.0 | 0.2 | 914 |
| Mean | 2014 | 32.5 | 2.4 | 7,727 | 8.7 | 1.2 | 73.5 | 3.5 | 82.2 | 3.0 | 2,022 |
| SD | 2014 | 5.2 | 0.1 | 950 | 2.5 | 0.6 | 2.8 | 0.2 | 2.0 | 0.4 | 816 |
| GREE Area of Concern subsite | | | | | | | | | | | |
| Mean | 2012 | 21.3 | 2.1 | 50,848 | 17.3 | 1.7 | 32.7 | 2.3 | 50.0 | 2.7 | 69,025 |
| SD | 2012 | 7.6 | 0.5 | 11,726 | 5.0 | 0.2 | 26.2 | 1.3 | 29.8 | 0.7 | 62,668 |
| Mean | 2014 | 30.3 | 2.5 | 725,831 | 14.0 | 1.6 | 62.0 | 3.3 | 76.0 | 3.1 | 24,816 |
| SD | 2014 | 1.5 | 0.3 | 492,988 | 1.7 | 0.5 | 16.1 | 0.4 | 16.0 | 0.2 | 18,669 |

**Table 7.** Metric means and standard deviations for plankton sampled in 2012 and 2014 at 4 Lake Michigan Areas of Concern in Wisconsin and 6 non-Area of Concern comparison sites in Wisconsin and Michigan.—Continued

[Richness was computed as the number of unique taxa in the sample. Diversity is Shannon diversity, calculated as $\log_e$. MENI, Lower Menominee River; SD, standard deviation; ESCA, Escanaba River, Mich.; OCON, Oconto River; GREE, Lower Green Bay; FOXR, Fox River near Allouez (GREE and FOXR are Lower Green Bay and Fox River subsites); AHNA, Ahnapee River; KEWA, Kewaunee River; SHEB, Sheboygan River; MANI, Manitowoc River; MILR, Milwaukee River; MENO, Menomonee River; ROOT, Root River; MILH, Milwaukee Harbor (MILR, MENO, and MILH are Milwaukee Estuary subsites)]

| Statistic | Year | Zooplankton[1] | | | Soft algae | | Diatoms | | Combined phytoplankton[2] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Richness | Diversity | Density[3] | Richness | Diversity | Richness | Diversity | Richness | Diversity | Density |
| FOXR Area of Concern subsite | | | | | | | | | | | |
| Mean | 2012 | 7.7 | 1.2 | 48,967 | 13.0 | 1.2 | 56.7 | 3.0 | 69.7 | 2.8 | 36,806 |
| SD | 2012 | 2.5 | 0.3 | 36,111 | 1.0 | 0.4 | 7.6 | 0.2 | 8.3 | 0.3 | 38,867 |
| Mean | 2014 | 20.3 | 2.0 | 83,012 | 10.3 | 0.7 | 61.0 | 3.0 | 71.3 | 2.6 | 23,717 |
| SD | 2014 | 5.1 | 0.1 | 62,916 | 3.5 | 0.6 | 6.0 | 0.1 | 5.5 | 0.3 | 9,741 |
| AHNA non-Area of Concern comparison site | | | | | | | | | | | |
| Mean | 2012 | 15.3 | 1.6 | 334,847 | 15.7 | 0.9 | 22.3 | 2.5 | 38.0 | 2.4 | 35,387 |
| SD | 2012 | 7.2 | 0.6 | 380,341 | 2.1 | 0.1 | 2.9 | 0.4 | 1 | 0.1 | 16,691 |
| Mean | 2014 | 29.7 | 2.0 | 342,654 | 12.0 | 0.8 | 84.3 | 3.9 | 96.3 | 3.0 | 26,045 |
| SD | 2014 | 8.6 | 0.7 | 491,362 | 3.6 | 0.1 | 4.2 | 0.1 | 4 | 0 | 13,055 |
| KEWA non-Area of Concern comparison site | | | | | | | | | | | |
| Mean | 2012 | 21.0 | 1.8 | 63,020 | 15.7 | 1.9 | 34.0 | 2.0 | 49.7 | 2.6 | 19,128 |
| SD | 2012 | 6.6 | 0 | 22,661 | 2.3 | 0.2 | 28.2 | 1.2 | 30.4 | 0.5 | 10,528 |
| Mean | 2014 | 29.7 | 2.2 | 954,523 | 11.3 | 1.6 | 69.7 | 3.3 | 81.0 | 3.1 | 5,661 |
| SD | 2014 | 9.0 | 0.3 | 873,820 | 3.5 | 0.4 | 15.5 | 0.2 | 12.8 | 0.2 | 4,857 |
| AHNA–KEWA non-Area of Concern comparison sites | | | | | | | | | | | |
| Mean | 2012 | 18.2 | 1.7 | 198,934 | 15.7 | 1.4 | 28.2 | 2.2 | 43.8 | 2.5 | 27,257 |
| SD | 2012 | 6.7 | 0.3 | 188,766 | 2.0 | 0.1 | 13.2 | 0.6 | 15.1 | 0.2 | 12,780 |
| Mean | 2014 | 29.7 | 2.1 | 648,588 | 11.7 | 1.2 | 77.0 | 3.6 | 88.7 | 3.1 | 15,853 |
| SD | 2014 | 3.2 | 0.4 | 507,750 | 3.5 | 0.2 | 9.5 | 0.1 | 7.0 | 0.1 | 4,104 |
| SHEB Area of Concern site | | | | | | | | | | | |
| Mean | 2012 | 20.3 | 2.0 | 47,985 | 11.7 | 1.5 | 45.3 | 2.5 | 57.0 | 2.7 | 24,485 |
| SD | 2012 | 3.2 | 0.2 | 29,636 | 3.2 | 0.3 | 21.7 | 0.8 | 18.5 | 0.3 | 17,681 |
| Mean | 2014 | 27.0 | 1.1 | 379,864 | 9.0 | 1.7 | 66.3 | 2.8 | 75.3 | 3.0 | 4,099 |
| SD | 2014 | 8.7 | 0.6 | 421,132 | 2.0 | 0.1 | 24.0 | 1.3 | 23.0 | 0.6 | 2,775 |

**Table 7.** Metric means and standard deviations for plankton sampled in 2012 and 2014 at 4 Lake Michigan Areas of Concern in Wisconsin and 6 non-Area of Concern comparison sites in Wisconsin and Michigan.—Continued

[Richness was computed as the number of unique taxa in the sample. Diversity is Shannon diversity, calculated as $\log_e$. MENI, Lower Menominee River; SD, standard deviation; ESCA, Escanaba River, Mich.; OCON, Oconto River; GREE, Lower Green Bay; FOXR, Fox River near Allouez (GREE and FOXR are Lower Green Bay and Fox River subsites); AHNA, Ahnapee River; KEWA, Kewaunee River; SHEB, Sheboygan River; MANI, Manitowoc River; MILR, Milwaukee River; MENO, Menomonee River; ROOT, Root River; MILH, Milwaukee Harbor (MILR, MENO, and MILH are Milwaukee Estuary subsites)]

| Statistic | Year | Zooplankton[1] | | | Soft algae | | Diatoms | | Combined phytoplankton[2] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Richness | Diversity | Density[3] | Richness | Diversity | Richness | Diversity | Richness | Diversity | Density |
| KEWA non-Area of Concern comparison site | | | | | | | | | | | |
| Mean | 2012 | 21.0 | 1.8 | 63,020 | 15.7 | 1.9 | 34.0 | 2.0 | 49.7 | 2.6 | 19,128 |
| SD | 2012 | 6.6 | 0.0 | 22,661 | 2.3 | 0.2 | 28.2 | 1.2 | 30.4 | 0.5 | 10,528 |
| Mean | 2014 | 29.7 | 2.2 | 954,523 | 11.3 | 1.6 | 69.7 | 3.3 | 81.0 | 3.1 | 5,661 |
| SD | 2014 | 9.0 | 0.3 | 873,820 | 3.5 | 0.4 | 15.5 | 0.2 | 12.8 | 0.2 | 4,857 |
| MANI non-Area of Concern comparison site | | | | | | | | | | | |
| Mean | 2012 | 20.7 | 1.6 | 104,977 | 12.7 | 1.1 | 20.7 | 2.4 | 33.3 | 2.4 | 17,938 |
| SD | 2012 | 5.9 | 0.7 | 139,569 | 5.8 | 0.2 | 10.7 | 0.4 | 7.4 | 0.3 | 16,548 |
| Mean | 2014 | 21.3 | 1.7 | 121,837 | 10.3 | 1.4 | 73.0 | 3.5 | 83.3 | 3.2 | 10,200 |
| SD | 2014 | 11.5 | 0.9 | 175,317 | 4.5 | 0.2 | 16.5 | 0.5 | 12.1 | 0.3 | 13,127 |
| KEWA–MANI non-Area of Concern comparison sites | | | | | | | | | | | |
| Mean | 2012 | 20.8 | 1.7 | 83,999 | 14.2 | 1.5 | 27.3 | 2.2 | 41.5 | 2.5 | 18,533 |
| SD | 2012 | 6.0 | 0.4 | 59,954 | 4.0 | 0.2 | 11.5 | 0.7 | 15.1 | 0.3 | 12,799 |
| Mean | 2014 | 25.5 | 2.0 | 538,180 | 10.8 | 1.5 | 71.3 | 3.4 | 82.2 | 3.1 | 7,931 |
| SD | 2014 | 10.0 | 0.6 | 514,780 | 3.6 | 0.1 | 15.7 | 0.4 | 12.3 | 0.1 | 8,980 |
| MILR Area of Concern subsite | | | | | | | | | | | |
| Mean | 2012 | 20.3 | 2.1 | 13,953 | 14.0 | 1.5 | 43.0 | 3.2 | 57.0 | 3.1 | 36,165 |
| SD | 2012 | 7.0 | 0.2 | 8,331 | 5.6 | 0.7 | 11.4 | 0.1 | 16.4 | 0.4 | 35,555 |
| Mean | 2014 | 28.7 | 1.8 | 29,488 | 8.0 | 1.4 | 72.0 | 3.6 | 80.0 | 3.2 | 3,865 |
| SD | 2014 | 9.3 | 0.6 | 35,897 | 2.0 | 0.2 | 11.1 | 0.4 | 12.0 | 0.2 | 1,715 |
| MENO Area of Concern subsite | | | | | | | | | | | |
| Mean | 2012 | 19.3 | 2.3 | 39,922 | 13.0 | 1.8 | 30.0 | 2.9 | 43.0 | 3.1 | 9,132 |
| SD | 2012 | 5.0 | 0.1 | 20,418 | 5.6 | 0.3 | 18.7 | 0.6 | 13.1 | 0.2 | 4,974 |
| Mean | 2014 | 28.7 | 1.9 | 45,744 | 8.0 | 1.6 | 64.7 | 3.2 | 72.7 | 3.1 | 3,696 |
| SD | 2014 | 7.2 | 0.6 | 22,668 | 1.0 | 0.1 | 10.6 | 0.2 | 11.2 | 0.1 | 832 |

**Table 7.** Metric means and standard deviations for plankton sampled in 2012 and 2014 at 4 Lake Michigan Areas of Concern in Wisconsin and 6 non-Area of Concern comparison sites in Wisconsin and Michigan.—Continued

[Richness was computed as the number of unique taxa in the sample. Diversity is Shannon diversity, calculated as $\log_e$. MENI, Lower Menominee River; SD, standard deviation; ESCA, Escanaba River, Mich.; OCON, Oconto River; GREE, Lower Green Bay; FOXR, Fox River near Allouez (GREE and FOXR are Lower Green Bay and Fox River subsites); AHNA, Ahnapee River; KEWA, Kewaunee River; SHEB, Sheboygan River; MANI, Manitowoc River; MILR, Milwaukee River; MENO, Menomonee River; ROOT, Root River; MILH, Milwaukee Harbor (MILR, MENO, and MILH are Milwaukee Estuary subsites)]

| Statistic | Year | Zooplankton[1] | | | Soft algae | | Diatoms | | Combined phytoplankton[2] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Richness | Diversity | Density[3] | Richness | Diversity | Richness | Diversity | Richness | Diversity | Density |
| MANI non-Area of Concern comparison site | | | | | | | | | | | |
| Mean | 2012 | 20.7 | 1.6 | 104,977 | 12.7 | 1.1 | 20.7 | 2.4 | 33.3 | 2.4 | 17,938 |
| SD | 2012 | 5.9 | 0.7 | 139,569 | 5.8 | 0.2 | 10.7 | 0.4 | 7.4 | 0.3 | 16,548 |
| Mean | 2014 | 21.3 | 1.7 | 121,837 | 10.3 | 1.4 | 73 | 3.5 | 83.3 | 3.2 | 10,200 |
| SD | 2014 | 11.5 | 0.9 | 175,317 | 4.5 | 0.2 | 16.5 | 0.5 | 12.1 | 0.3 | 13,127 |
| ROOT non-Area of Concern comparison site | | | | | | | | | | | |
| Mean | 2012 | 19.7 | 1.1 | 69,911 | 14.3 | 1.7 | 36.7 | 2.8 | 51.0 | 2.9 | 11,911 |
| SD | 2012 | 4.5 | 0.5 | 46,177 | 0.6 | 0.3 | 30.1 | 0.8 | 30.6 | 0.5 | 5,871 |
| Mean | 2014 | 27.0 | 1.5 | 59,270 | 9.7 | 1.3 | 49.7 | 2.3 | 59.3 | 2.5 | 5,813 |
| SD | 2014 | 0.0 | 1.0 | 65,990 | 2.1 | 0.3 | 29.4 | 1.4 | 27.6 | 0.8 | 5,720 |
| MANI–ROOT non-Area of Concern comparison sites | | | | | | | | | | | |
| Mean | 2012 | 20.2 | 1.3 | 87,444 | 13.5 | 1.4 | 28.7 | 2.6 | 42.2 | 2.7 | 14,925 |
| SD | 2012 | 5.1 | 0.5 | 90,648 | 2.6 | 0.2 | 18.9 | 0.4 | 16.4 | 0.3 | 10,785 |
| Mean | 2014 | 24.2 | 1.6 | 90,554 | 10.0 | 1.4 | 61.3 | 2.9 | 71.3 | 2.8 | 8,007 |
| SD | 2014 | 5.8 | 0.6 | 118,901 | 3.1 | 0.1 | 22.7 | 1.0 | 19.6 | 0.5 | 9,423 |
| MILH Area of Concern subsite | | | | | | | | | | | |
| Mean | 2012 | 20.7 | 1.5 | 74,702 | 10.3 | 1.6 | 12.7 | 2.0 | 23.0 | 2.5 | 6,843 |
| SD | 2012 | 4.6 | 0.3 | 24,965 | 4.0 | 0.5 | 8.7 | 0.9 | 10.4 | 0.6 | 3,856 |
| Mean | 2014 | 25.7 | 1.2 | 115,742 | 7.3 | 1.4 | 77.3 | 3.7 | 84.7 | 3.2 | 3,970 |
| SD | 2014 | 9.5 | 0.8 | 61,086 | 1.5 | 0.4 | 4.7 | 0.1 | 5.8 | 0.2 | 321 |

**Table 7.** Metric means and standard deviations for plankton sampled in 2012 and 2014 at 4 Lake Michigan Areas of Concern in Wisconsin and 6 non-Area of Concern comparison sites in Wisconsin and Michigan.—Continued

[Richness was computed as the number of unique taxa in the sample. Diversity is Shannon diversity, calculated as $\log_e$. MENI, Lower Menominee River; SD, standard deviation; ESCA, Escanaba River, Mich.; OCON, Oconto River; GREE, Lower Green Bay; FOXR, Fox River near Allouez (GREE and FOXR are Lower Green Bay and Fox River subsites); AHNA, Ahnapee River; KEWA, Kewaunee River; SHEB, Sheboygan River; MANI, Manitowoc River; MILR, Milwaukee River; MENO, Menomonee River; ROOT, Root River; MILH, Milwaukee Harbor (MILR, MENO, and MILH are Milwaukee Estuary subsites)]

| Statistic | Year | Zooplankton[1] | | | Soft algae | | Diatoms | | Combined phytoplankton[2] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Richness | Diversity | Density[3] | Richness | Diversity | Richness | Diversity | Richness | Diversity | Density |
| All non-Area of Concern comparison sites | | | | | | | | | | | |
| Mean | 2012 | 20.4 | 1.7 | 96,218 | 13.2 | 1.4 | 25.9 | 2.5 | 39.2 | 2.6 | 14,956 |
| SD | 2012 | 3.9 | 0.3 | 54,986 | 2.4 | 0.1 | 2.3 | 0.1 | 0.7 | 0.0 | 6,761 |
| Mean | 2014 | 28.8 | 2.0 | 248,956 | 10.1 | 1.2 | 70.6 | 3.3 | 80.7 | 3.0 | 8,627 |
| SD | 2014 | 3.5 | 0.1 | 189,852 | 2.9 | 0.2 | 11.2 | 0.3 | 8.5 | 0.1 | 1,647 |

[1]For zooplankton in 2012, high algal counts precluded identification of rotifers other than *Asplanchna priodonta* in all Fox River samples and in summer samples for Ahnapee River.

[2]Richness and diversity of combined phytoplankton were calculated for combined soft algae and diatoms; density values were from the soft algae analyses, which also included densities for diatoms. Density is in cells per milliliter.

[3]Density of zooplankton was total density, including nauplii, in number per cubic meter.

## Lower Menominee River Area of Concern

For zooplankton at MENI, metrics did not differ between either the mean of all non-AOCs or the mean of the two non-AOC comparison sites, ESCA and OCON (fig. 4, table 8). This finding was similar to 2012 when no differences were found. Lastly, no differences were found between 2012 and 2014 metrics for zooplankton at MENI.

There were no differences in the assemblages of zooplankton at MENI, ESCA, and OCON in 2014, based on results of the ANOSIM, with all three sites plotting adjacent to each other in a tight grouping within the MDS ordination plot when seasons were combined (fig. 5*A*). With seasons separate, the spring assemblage at MENI also had higher similarity to the spring assemblage at OCON than to the spring assemblage at ESCA (fig. 5*B*). Yet SIMPER results indicated that MENI and its two non-AOC comparison sites were 43 percent dissimilar, based mostly on the relative abundances of zebra mussel veligers, as well as rotifers *Lecane tenuiseta* and the bdelloid rotifer *Philodina*. Zebra mussel veligers were absent from all three sites in the spring and were present in the fall at low abundances; abundances in summer were much higher at MENI and ESCA than at OCON. The rotifer *L. tenuiseta* was in higher abundance at MENI compared to ESCA and OCON. Although abundances of *Philodina* were similar seasonally at MENI and OCON, abundances at ESCA were much lower overall. *Philodina* is commonly found in the benthos near river mouths in the Great Lakes (Stemberger, 1979), but this taxon and other bdelloid rotifers are the least well known of all the rotifer groups because they are fragile and can be damaged with some collection methods (National Oceanic and Atmospheric Administration, 2018). Rotifers in the genus *Lecane* are common in shallow areas as well as eutrophic areas such as river mouths and Great Lakes harbors in late spring through fall (Stemberger, 1979).

Metrics for combined phytoplankton at MENI did not differ from either the mean of all non-AOCs or the mean of the two non-AOC comparison sites (fig. 6, table 9). Richness was higher in 2014 than in 2012 (table 7), and this was because the diatom richness was higher in 2014 ($p<0.01$). Diversity and total density of combined phytoplankton did not differ between years even though diatom diversity was higher in 2014.

As was found in multivariate analyses for zooplankton, the assemblage of combined phytoplankton at MENI did not differ from ESCA and OCON, based on the results of the ANOSIM. The assemblage for MENI was more similar to OCON and both sites plotted close together in the MDS ordination plot (fig. 7*A*), whereas ESCA plotted distant from these two sites and all other sampled sites, underscoring the distinct assemblage at ESCA. When examined with seasons separate, samples in all seasons at OCON were similar to those at MENI, whereas those at ESCA differed from both sites (fig. 7*B*). SIMPER results indicated that MENI, ESCA, and OCON were 54 percent dissimilar, based mostly on the presence of *Microcystis aeruginosa*, *Thalassiosira pseudonana*, and *Klebsormidium*. The toxin-forming cyanobacterium *Microcystis aeruginosa* was not found at MENI but was found at ESCA and OCON in the summer and (or) the fall at low to moderate abundances. The centric diatom *T. pseudonana* was common at MENI in summer and otherwise was absent or at low abundance in other seasons; in all seasons, this diatom was absent at ESCA and at low abundance at OCON. This chain-forming diatom was thought to be a marine or brackish water species before being found in high densities in areas of the Great Lakes Basin beginning several decades ago (Lowe and Busch, 1975). Transport by ballast water from Europe to the Great Lakes is suspected for the occurrence of *T. pseudonana* in the region (Mills and others, 1993). In other parts of the world, this taxon is indicative of polluted waters where there are high nutrient concentrations and a resultant high chemical oxygen demand (Weckström and Juggins, 2006; U.S. Geological Survey, 2018). The filamentous green alga *Klebsormidium*, a cosmopolitan genus, was common in summer samples at MENI but absent from ESCA and OCON and from spring and fall samples at MENI. It is a cosmopolitan genus but identification to species has historically been difficult, and its presence in a wide variety of habitats seems to have hampered assignment of any pollution tolerance (Rindi and others, 2008).

For dominance of zooplankton, rotifers had the highest relative abundance during all seasons at MENI in 2014, ranging from 93 percent in the spring to 66 percent in the summer and back to 81 percent in the fall. Second in abundance in the summer were zebra mussel veligers; summer abundances of zebra mussel veligers ranged from 25 to 45 percent at MENI and ESCA, respectively, but comprised only 2.5 percent at OCON. For combined phytoplankton, cryptophytes were the dominant algal group in the spring and fall at MENI with more than a 42-percent abundance, and green algae were the dominant group in the summer with a 49-percent abundance. Diatoms were second in percent abundance in the spring and fall, and cryptophytes were second in percent abundance in the summer. Diatoms and cryptophytes have generally high food value for aquatic organisms (Stewart and Wetzel, 1986).

## Lower Green Bay and Fox River Area of Concern

Metrics for zooplankton did not differ between FOXR and the mean of all non-AOCs in 2014. Only the density of zooplankton differed between FOXR and the mean of the two non-AOC comparison sites, AHNA and KEWA in 2014 (fig. 4, table 8); FOXR had lower density, which indicates that density was degraded at FOXR relative to the two non-AOC comparison sites. Notably, densities in fall 2014 were higher at KEWA than at FOXR (fig. 4), primarily because of high densities of *Bosmina longirostris* that were several times higher at KEWA than at FOXR (230,000 and 4,050 individuals per cubic meter [$m^3$], respectively). The total density of zooplankton at FOXR, with nauplii included, averaged 83,012±62,916 individuals/$m^3$ but actually may have been higher (fig. 4, table 7) because large amounts of cyanobacteria made concentrating

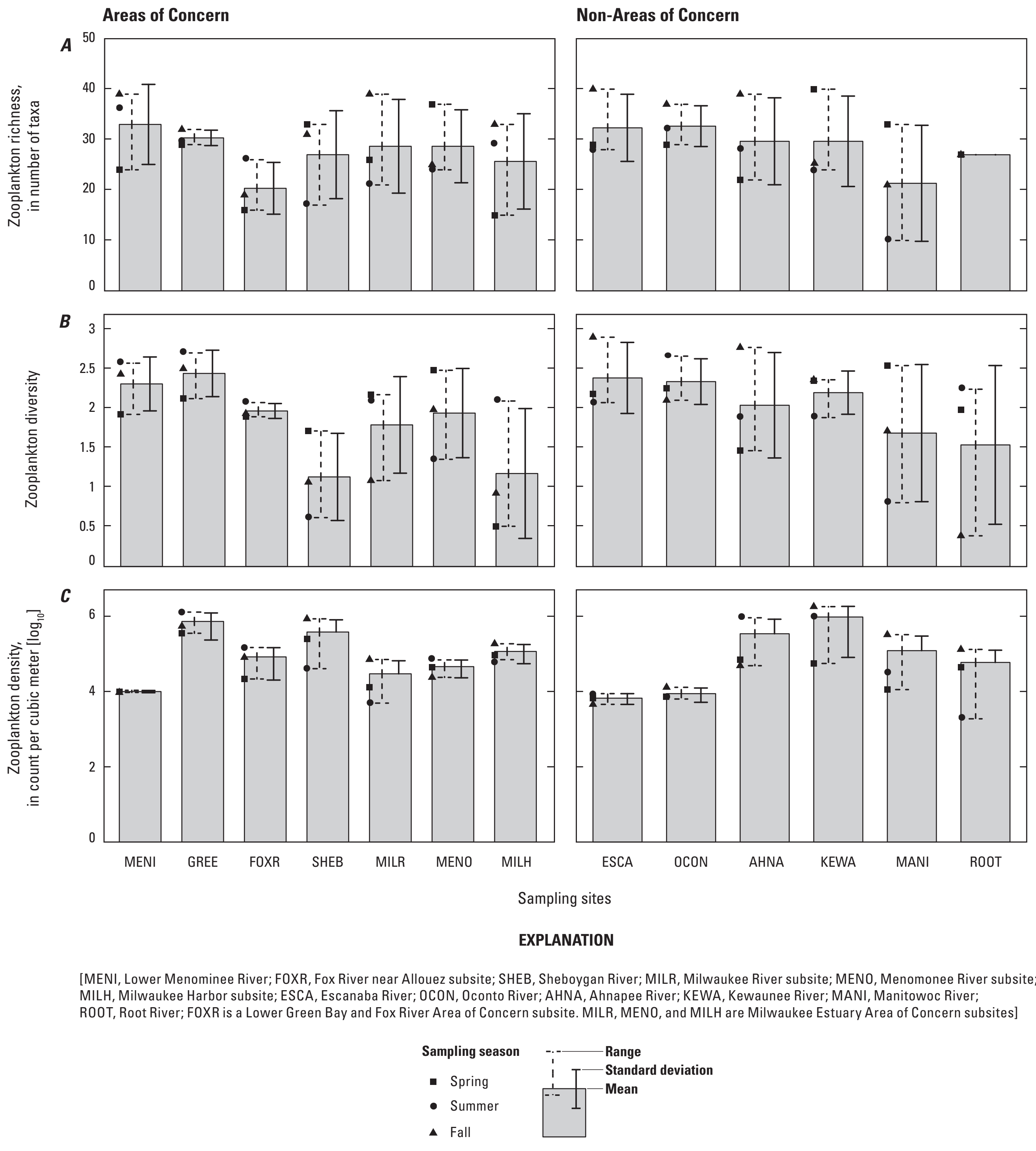



**Figure 4.** Metrics for zooplankton at 4 Lake Michigan Areas of Concern and 6 non-Area of Concern comparison sites. *A*, Zooplankton richness; *B*, zooplankton diversity; and *C*, zooplankton density.

**Table 8.** Probability values for significance in paired *t*-tests comparing metrics for zooplankton at Areas of Concern (AOCs) with the mean of all non-AOCs or the mean of the two non-AOC comparison sites.

[For zooplankton in 2012, high algal counts precluded identification of rotifers other than *Asplanchna priodonta* in summer samples for Ahnapee River and all Fox River samples; therefore, comparisons for these sites excluded other rotifers. Density comparisons are for log-10 transformed data. Values in bold italics indicate the AOC metrics were significantly lower than non-AOCs compared; the number of samples is 3 in all comparisons. MENI, Lower Menominee River; FOXR, Fox River near Allouez (Lower Green Bay and Fox River subsite); SHEB, Sheboygan River; MILR, Milwaukee River; MENO, Menomonee River (MILR and MENO are Milwaukee Estuary subsites)]

| Metric | 2012 | | 2014 | |
|---|---|---|---|---|
| | AOC: non-AOC group | AOC: non-AOC pair | AOC: non-AOC group | AOC: non-AOC pair |
| MENI site | | | | |
| Richness | 0.249 | 0.225 | 0.503 | 0.889 |
| Diversity | 0.366 | 0.854 | 0.391 | 0.733 |
| Density | 0.092 | 0.131 | 0.072 | 0.107 |
| FOXR subsite | | | | |
| Richness | 0.508 | 0.362 | 0.223 | 0.186 |
| Diversity | 0.354 | 0.924 | 0.620 | 0.594 |
| Density | 0.341 | 0.818 | 0.112 | ***0.046*** |
| SHEB site | | | | |
| Richness | 0.964 | 0.900 | 0.635 | 0.703 |
| Diversity | 0.460 | 0.432 | 0.074 | ***0.0099*** |
| Density | 0.477 | 0.428 | 0.861 | 0.863 |
| MILR subsite | | | | |
| Richness | 0.984 | 0.974 | 0.981 | 0.504 |
| Diversity | 0.144 | 0.178 | 0.570 | 0.488 |
| Density | ***0.010*** | 0.159 | 0.148 | ***0.016*** |
| MENO subsite | | | | |
| Richness | 0.585 | 0.721 | 0.982 | 0.130 |
| Diversity | 0.055 | 0.105 | 0.759 | 0.417 |
| Density | 0.123 | 0.532 | 0.275 | 0.929 |

the sample difficult for the laboratory. In 2012, cyanobacterial cells impeded the identification and counting of rotifers when the only rotifer quantified was the large-sized *Asplanchna priodonta*. For this reason, comparisons with non-AOCs and between years at FOXR excluded rotifers except *A. priodonta*. The total density of zooplankton was higher in 2012 than in 2014 at FOXR if nauplii were excluded ($p$<0.01) but not if nauplii were included; richness and diversity did not differ between 2012 and 2014 at FOXR. Metrics for combined phytoplankton did not differ between FOXR and either the mean of all non-AOCs or the mean of the two non-AOC comparison sites (fig. 6, table 9). Although richness for combined phytoplankton at FOXR in 2014 did not differ from non-AOCs, richness in 2012 was higher than the mean of all non-AOCs. Lastly, metrics for combined phytoplankton did not differ between 2012 and 2014 at FOXR.

For multivariate analyses of zooplankton, the FOXR assemblage in 2014 plotted most closely to AHNA and KEWA but separately from other sites in the MDS ordination plot with seasons combined (fig. 5*A*). Based on the ANOSIM, FOXR did not differ from its two non-AOC comparison sites (AHNA and KEWA), as shown by the MDS ordination plot with seasons separate (fig. 5*B*). This result may have been because of high seasonal variability at all three sites. Still, a SIMPER test indicated that assemblages of zooplankton at FOXR, AHNA, and KEWA were 59 percent dissimilar, primarily because of differences in the abundances of rotifers *Brachionus calyciflorus*, *Keratella crassa*, and *Conochilus unicornis*. *Brachionus calyciflorus* was more abundant at AHNA and KEWA, was detected at less than a 1-percent abundance in the spring and was otherwise absent. *Keratella crassa* was more abundant at FOXR in all seasons, especially in the spring with a 36-percent relative abundance; *C. unicornis* was also more abundant in the spring and summer at FOXR but was absent from AHNA and was in low abundance in the spring only at KEWA. Rotifers in the genus *Brachionus* as well as *K. crassa*

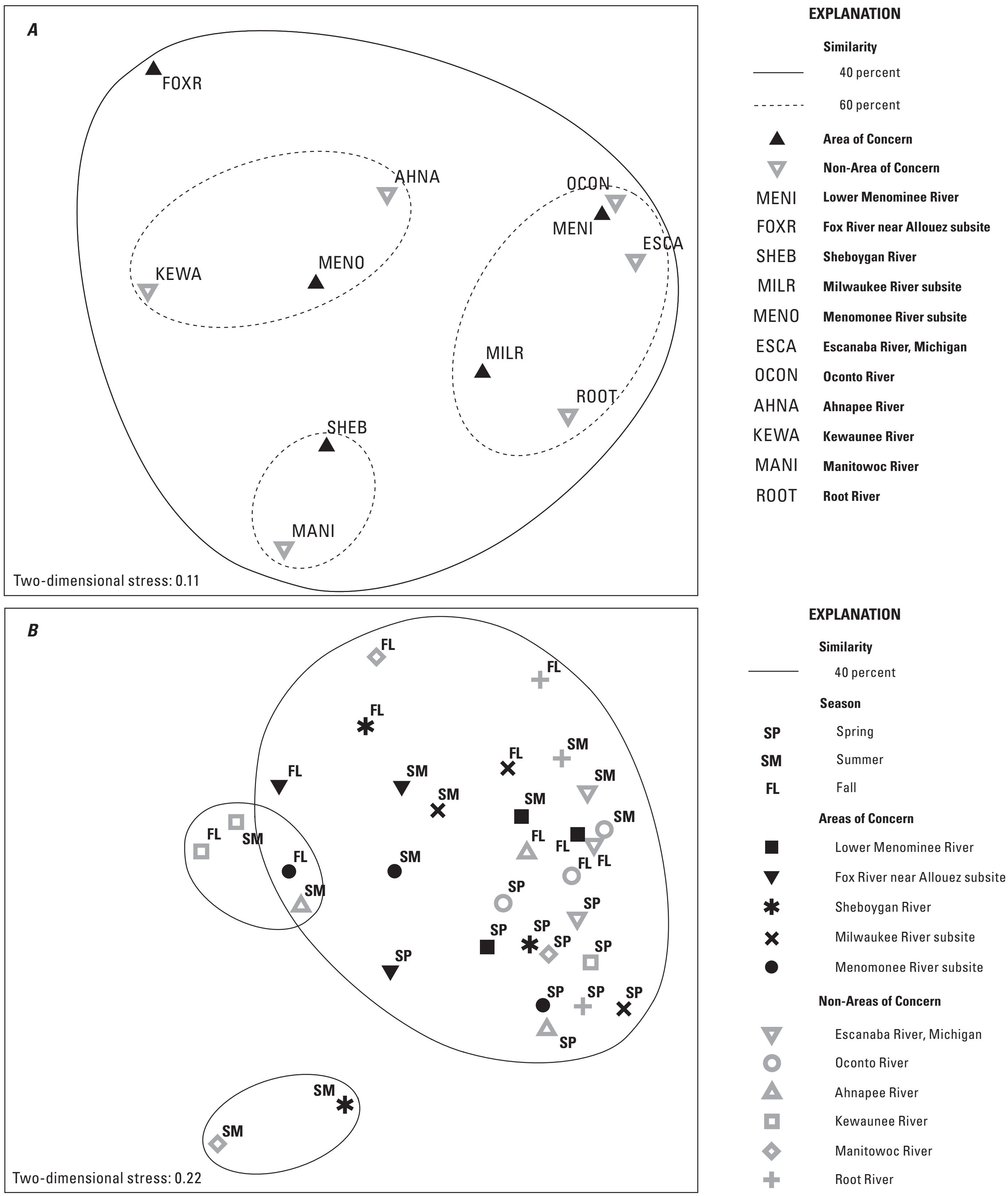



**Figure 5.** Multidimensional scaling ordination plots for zooplankton at 4 Lake Michigan Areas of Concern and 6 non-Area of Concern comparison sites, based on relative abundance (fourth-root transformed) with no rare or ambiguous taxa. *A*, Seasons combined; and *B*, seasons separate. [The Fox River near Allouez is a subsite of the Green Bay and Fox River Area of Concern. The Milwaukee River and Menomonee River are subsites of the Milwaukee Estuary Area of Concern]

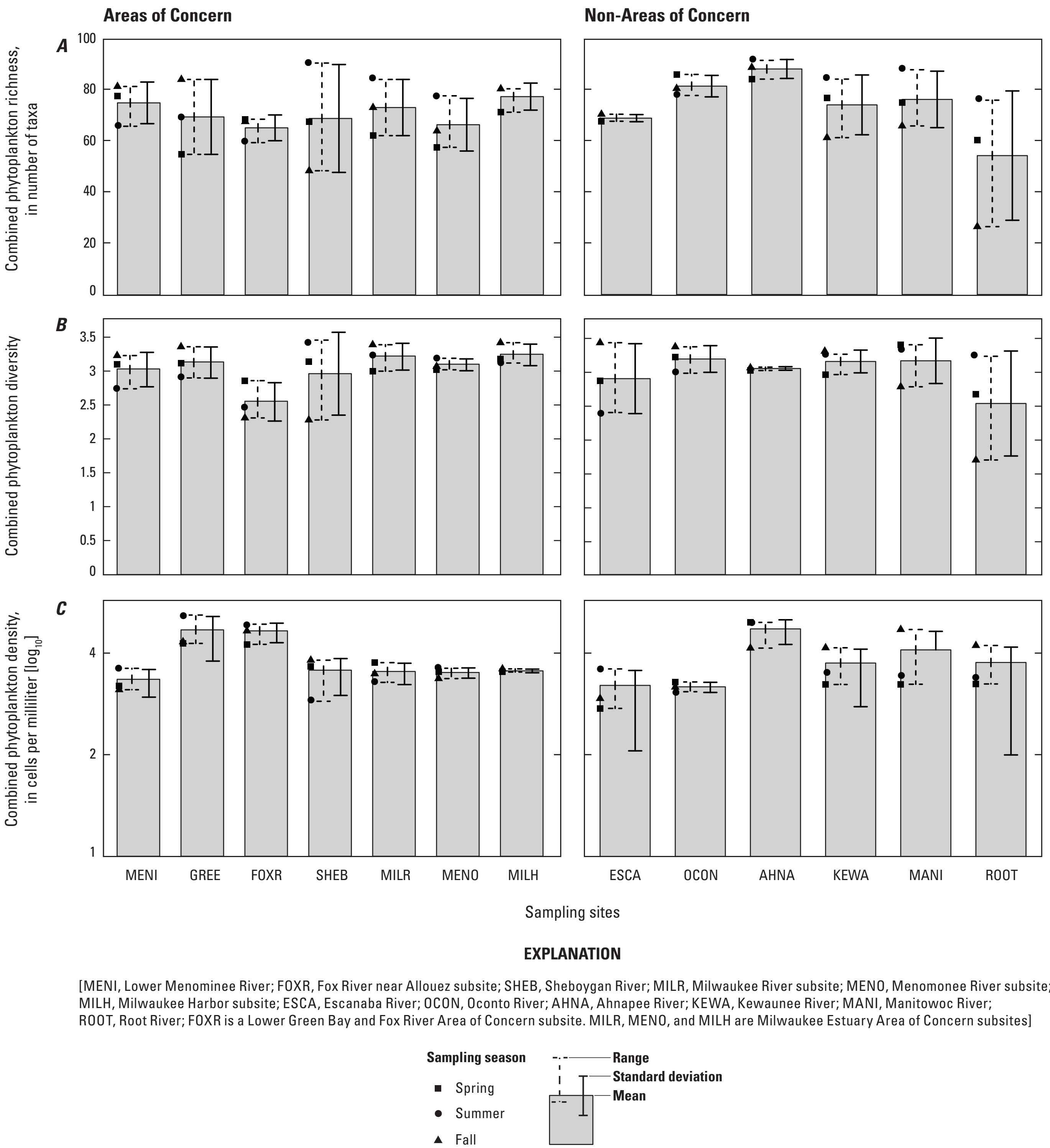



**Figure 6.** Metrics for combined (soft algae and diatoms) at 4 Lake Michigan Areas of Concern and 6 non-Area of Concern comparison sites. *A*, Combined phytoplankton richness; *B*, combined phytoplankton diversity; and *C*, combined phytoplankton density.

**Table 9.** Probability values for significance in paired *t*-tests comparing metrics for combined phytoplankton (soft algae and diatoms combined) at each Area of Concern (AOC) with the mean of all non-AOCs or the mean of the two non-AOC comparison sites.

[Values in bold italics indicate the AOC metrics were significantly lower than non-AOCs compared and, therefore, there were no such outcomes; the number of samples is 3 in all comparisons. Density comparisons are for log-10 transformed data. MENI, Lower Menominee River; FOXR, Fox River near Allouez (Lower Green Bay and Fox River subsite); SHEB, Sheboygan River; MILR, Milwaukee River; MENO, Menomonee River (MILR and MENO are Milwaukee Estuary subsites)]

| Metric | 2012 | | 2014 | |
|---|---|---|---|---|
| | AOC: non-AOC group | AOC: non-AOC pair | AOC: non-AOC group | AOC: non-AOC pair |
| | | MENI site | | |
| Richness | 0.285 | 0.782 | 0.909 | 0.972 |
| Diversity | 0.664 | 0.608 | 0.827 | 0.968 |
| Density | 0.033 | 0.687 | 0.075 | 0.090 |
| | | FOXR subsite | | |
| Richness | 0.027 | 0.110 | 0.339 | 0.131 |
| Diversity | 0.555 | 0.401 | 0.093 | 0.134 |
| Density | 0.346 | 0.988 | 0.059 | 0.430 |
| | | SHEB site | | |
| Richness | 0.225 | 0.082 | 0.591 | 0.391 |
| Diversity | 0.849 | 0.238 | 0.940 | 0.565 |
| Density | 0.337 | 0.422 | 0.204 | 0.535 |
| | | MILR subsite | | |
| Richness | 0.188 | 0.407 | 0.981 | 0.469[1] |
| Diversity | 0.223 | 0.047 | 0.241 | 0.434[1] |
| Density | 0.336 | 0.071 | 0.104 | 0.441 |
| | | MENO subsite | | |
| Richness | 0.678 | 0.908 | 0.265[2] | 0.989[2] |
| Diversity | 0.065 | 0.278 | 0.163[1] | 0.498[1] |
| Density | 0.091 | 0.390 | 0.067 | 0.733 |

[1]Double-squared-transformed data ($X^4$).

[2]Squared-transformed data ($X^2$).

were categorized as indicators of highly eutrophic conditions by Gannon and Stemberger (1978). *Keratella* may be the most common genus of freshwater limnetic rotifer and at least three species often cooccur in the Great Lakes (Stemberger, 1979). *Conochilus unicornis* prefers cooler water temperatures, and it can be found in moderately eutrophic to oligotrophic conditions (Gannon and Stemberger, 1978).

As was seen with the zooplankton, combined phytoplankton at FOXR plotted nearest to AHNA and KEWA but away from all other sites in the MDS ordination plot (fig. 7*A*). Examining seasons separately, the summer and fall samples for FOXR plotted away from AHNA and KEWA samples with the exception of the fall KEWA sample (fig. 7*B*). The ANOSIM indicated that only the assemblage at FOXR, out of all four AOCs, differed from its non-AOC comparison sites, AHNA and KEWA ($p$=0.012). The SIMPER test indicated that FOXR was 61 percent dissimilar, primarily because of the presence of the cyanobacterium *Microcystis aeruginosa*, the green alga *Scenedesmus* sp., and the diatom *Staurosira construens*, and these three taxa contributed to most of the dissimilarity between the subsite and its non-AOCs. *Microcystis aeruginosa* was detected at FOXR but not at AHNA or KEWA. *Scenedesmus* was present in a much lower abundance at FOXR and KEWA than at AHNA, where it was relatively abundant in all seasons. The genus *Scenedesmus* is common worldwide and some species are tolerant of waters with high inorganic nitrogen (Wehr and Sheath, 2003; Porter, 2008). *Staurosira construens*, although found in low abundance at AHNA and KEWA, was absent from FOXR. This diatom is sensitive to eutrophic conditions (Porter, 2008), which explains its absence from FOXR where conditions range from eutrophic to hypereutrophic.

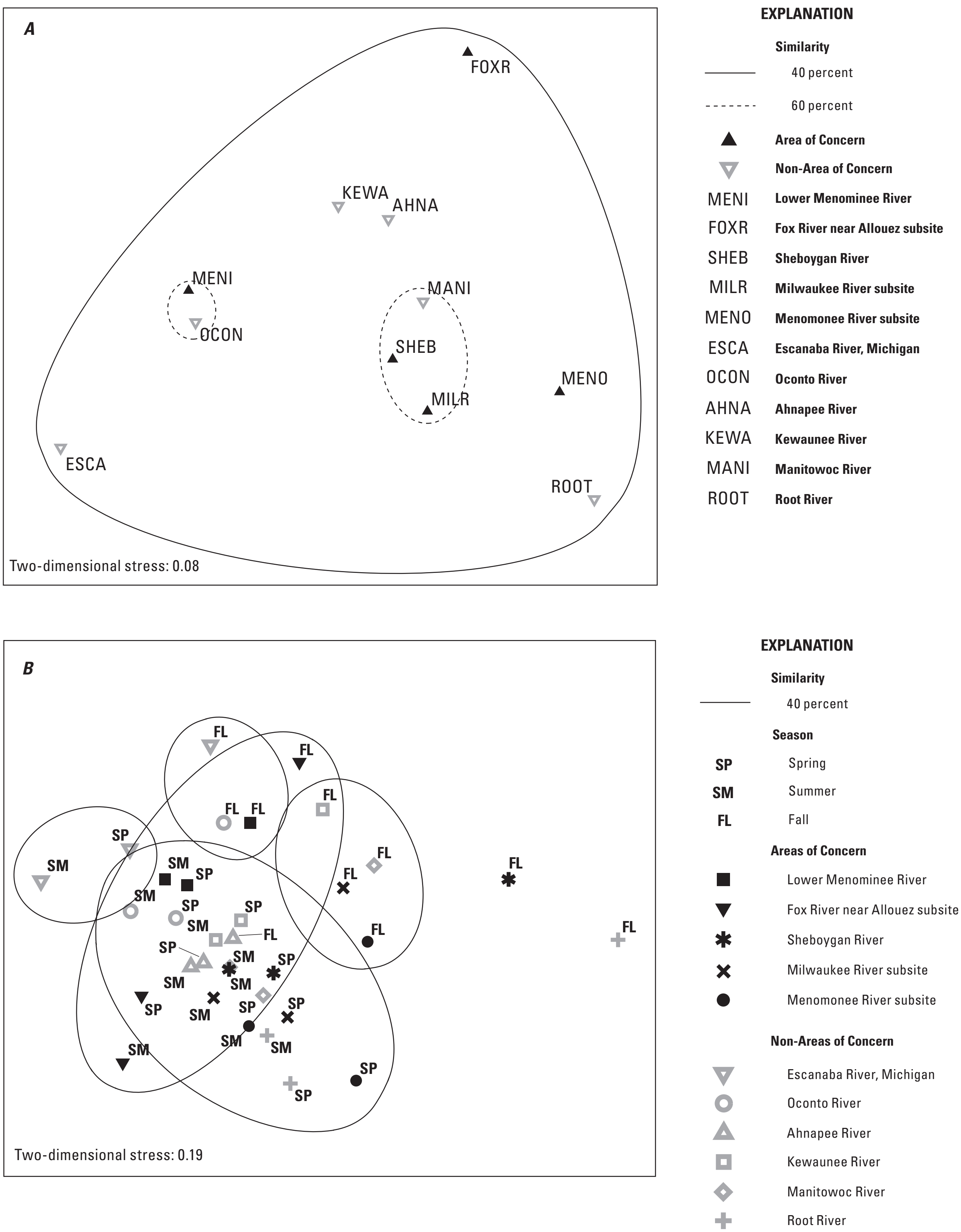



**Figure 7.** Multidimensional scaling ordination plots for combined phytoplankton (soft algae and diatoms) at 4 Lake Michigan Areas of Concern and 6 non-Area of Concern comparison sites, based on relative abundance (fourth-root transformed) with no rare or ambiguous taxa. *A*, Seasons combined; and *B*, seasons separate. [The Fox River near Allouez is a subsite of the Green Bay and Fox River Area of Concern. The Milwaukee River and Menomonee River are subsites of the Milwaukee Estuary Area of Concern]

Rotifers were the dominant taxonomic group in the zooplankton at FOXR in 2014 (81- to 87-percent relative abundance). Second in abundance were microcrustaceans: copepods (16 percent), zebra mussels (12 percent), and cladocerans (8 percent) in the spring, summer, and fall, respectively. Cyanobacteria were the dominant group of phytoplankton at FOXR in all seasons in 2014, with more than 70 percent of the relative abundance. In eutrophic conditions, cyanobacteria tend to dominate. Spring cyanobacteria were mostly the toxin producers *Anabaena* and *Microcystis aeruginosa* (36 and 27 percent, respectively). *Anabaena* is a filamentous alga and the genus is found worldwide (Wehr and Sheath, 2003). *Microcystis aeruginosa* was the dominant cyanobacterium in summer and fall 2014 with more than 80 percent of the total algal abundance. It is a coccoid and colonial organism, and it is an indicator of eutrophic conditions (Porter, 2008). Diatoms were second in abundance to cyanobacteria, and the highest diatom abundances were in the spring at 21 percent, after which abundances were 13 percent in the summer and fall samples.

## Sheboygan River Area of Concern

Metrics for zooplankton did not differ between SHEB and the mean of all non-AOCs in 2014 (fig. 4, table 8). Only diversity differed between SHEB and its two non-AOC comparison sites (KEWA and MANI at $p<0.01$) in 2014, so SHEB was rated as degraded for diversity (fig. 4, table 8). Diversity did not differ in 2012. In addition, diversity in 2014 did not differ between primary and replicate samples from the Sheboygan River AOC (Scudder Eikenberry and others, 2016a) and it averaged relatively low at 1.1±0.6 (table 7). No metrics for combined phytoplankton differed between the mean of all non-AOCs or the mean of the two non-AOC comparison sites in 2014 (fig. 6, table 9). There was no difference between 2012 and 2014 at SHEB for metrics with either zooplankton or combined phytoplankton.

For multivariate analyses with 2014 zooplankton abundances, an ANOSIM indicated the assemblage at SHEB did not differ from KEWA and MANI. In the MDS ordination plot, spring samples for SHEB, KEWA, and MANI showed their similarity by plotting close to each other; however, differences in the communities were in the summer and fall samples at KEWA, which plotted away from SHEB and MANI (fig. 5*A* and *B*). The assemblages of zooplankton at KEWA and MANI averaged a 65-percent dissimilarity to each other, and the zooplankton at SHEB was 61 percent dissimilar to the two non-AOC comparison sites. The dissimilarity between SHEB and its two non-AOC comparison sites was mostly because of the rotifer *Synchaeta*, followed by zebra mussel veligers and the rotifer *Euchlanis dilatata*. *Synchaeta* was minor in abundance in the spring at MANI and gradually diminished, it was abundant in the spring only at KEWA, and it was higher in abundance in the summer at SHEB than at the other two sites. Zebra mussel veligers were present only in the fall at SHEB and MANI, were absent at KEWA, and were nearly twice as abundant at SHEB. *Euchlanis dilatata*, a rotifer present only in spring, was more than twice as abundant at SHEB when compared to the two non-AOC comparison sites. *Synchaeta* is common in the Great Lakes and is tolerant to pollution; most species have a higher abundance in the fall through the spring when temperatures are cooler (Gannon and Stemberger, 1978; Stemberger, 1979).

An ANOSIM with combined phytoplankton found that the assemblage at SHEB did not differ from the two non-AOC comparison sites, KEWA and MANI. In the MDS ordination plot with seasons combined, the assemblage at SHEB was only 40 percent or less dissimilar to MANI but it was more dissimilar to KEWA (fig. 7*A*). In the MDS ordination plot with seasons separate, it was the fall SHEB sample that was distinct, and the spring and summer samples for SHEB and its two non-AOC comparison sites were similar (fig. 7*B*). SIMPER results indicated a 58-percent dissimilarity between SHEB and the two non-AOC comparison sites, mostly because of differences in the abundances of two taxa in the fall samples. The diatom *Aulacoseira muzzanensis* accounted for 38 percent of density in the fall for combined phytoplankton at SHEB. Otherwise, this taxon was absent or in low abundance at SHEB, similar to the taxon's distribution at KEWA and MANI. This centric diatom is an indicator of high total phosphorus (Porter, 2008). The green alga *Klebsormidium* was absent from SHEB in all seasons but found at a 34-percent relative density at MANI in the fall.

Rotifers dominated abundance in the spring and summer 2014 samples of zooplankton in the Sheboygan River AOC (96 and 94 percent, respectively). Zebra mussel veligers dominated abundance in the fall 2014 samples (73 percent). Diatoms were the dominant taxonomic group of phytoplankton at SHEB in 2014 (42, 59, and 62 percent, respectively). Second in dominance in all seasons was green algae, with abundance highest in the spring at 38 percent, nearly as high as that for the diatoms. *Scenedesmus* was the green algal taxon with the highest abundance; it is common worldwide and some species are tolerant of high inorganic nitrogen (Wehr and Sheath, 2003; Porter, 2008).

## Milwaukee Estuary Area of Concern

Comparisons with non-AOCs were made for the Milwaukee Estuary AOC with respect to only MILR and MENO and not MILH. The assemblages of plankton at MILH are discussed later in a separate section. The two non-AOC comparison sites for MILR and MENO were MANI and ROOT.

For zooplankton at MILR and MENO in 2014, no metrics differed between MILR and the mean of all non-AOCs (table 8). Only the density of zooplankton differed between MILR and the two non-AOC comparison sites; total density in 2014 was lower at MILR, so MILR was rated as degraded for density of zooplankton (fig. 4, table 8). Mean values for richness and diversity of zooplankton in 2014 were similar between MILR and MENO, with a mean richness of 28.7 at both and a slightly higher diversity at MENO. Metrics did not differ between MENO and the mean of all non-AOCs or the mean of the two non-AOC comparison sites in 2014. For combined phytoplankton, no difference was found between richness, diversity, or total density for MILR or MENO in 2014 (fig. 6, table 9) when compared to non-AOCs. Values for mean richness were 80.0±12.0 at MILR compared to 72.7±11.2 at MENO, and average diversity was the same at both (table 7). There were no differences between 2012 and 2014 metrics for combined phytoplankton at MILR or MENO.

In ordinations of zooplankton at MILR and MENO for 2014, the ANOSIM indicated no differences from MANI and ROOT. In the MDS ordination plot with seasons combined, MILR and ROOT plotted near each other but MENO and MANI plotted distant and less similar (fig. 5*A*). In the MDS ordination plot with seasons separate, spring samples for MILR and MENO were similar to each other and plotted near MANI and ROOT spring samples, with ROOT closer to MILR and MENO (fig. 5*B*). MILR and ROOT also plotted near each other in the summer and fall but MANI plotted away, especially in the summer. ROOT is closer to MILR and MENO in latitude, compared to MANI, which is much farther north, and differences in water temperatures could be a contributing factor. Overall in 2014, water temperatures at MILR were higher than at MANI at 22.3±0.3 degrees Celsius (ºC) for MILR compared to 21.3±1.0 ºC for MANI; water temperatures at MENO were higher than at MANI and ROOT ($p$<0.01) with 24.1±1.8 ºC for MENO compared to 21.3±1.0 ºC for MANI and 20.6±2.6 ºC for ROOT (table 2). A SIMPER test indicated that a 57-percent difference between assemblages at MILR and the two non-AOC comparison sites was mostly because of zebra mussel veligers and the rotifers *Euchlanis dilatata* and *Proales*. The spring-only rotifer, *E. dilatata*, was in higher abundance at MANI and ROOT, and nearly twice as high at ROOT than at MANI. Oddly, though zebra mussel veligers were abundant in fall 2014 at MILR, MANI, and ROOT, they were absent from all 2014 samples at MENO. Though zebra mussel veligers and *E. dilatata* also were among the top three taxa contributing to the 60-percent dissimilarity between MENO and the two non-AOC comparison sites, *Conochilus unicornis* was the primary taxon contributing to the dissimilarity for MENO. Although *C. unicornis* was detected in low abundance at the non-AOCs, it comprised more than two-thirds of the relative abundance in summer at MENO. *C. unicornis* prefers cooler water temperatures, and it can be found in moderately eutrophic to oligotrophic conditions (Gannon and Stemberger, 1978).

The ANOSIM with combined phytoplankton also indicated no differences between MILR or MENO and the two non-AOC comparison sites for 2014. In the MDS ordination plot with seasons combined, MILR and MANI plotted near each other with at least a 60-percent similarity overall between their assemblages (fig. 7*A*). MENO and ROOT plotted distant from MILR and MANI but near each other. With seasons separate, fall samples were distinct and the fall sample for ROOT was most different, plotting distant from all other samples (fig. 7*B*). Spring and summer samples for all four sites were more similar despite the spring samples for MENO and ROOT segregating slightly. MILR and MENO were 58 and 60 percent dissimilar, respectively, from the two non-AOC comparison sites. For MILR, the diatom *Cyclostephanos invisitatus* comprised nearly 10 percent of the relative abundance, but this taxon was only 2 percent or less at the two non-AOC comparison sites. This centric diatom is an indicator of eutrophic conditions resulting from high nitrogen and high phosphorus (Porter, 2008). In the fall, the cyanobacterium *Merismopedia* was present at ROOT at a relative abundance nearly six times higher than MILR or MANI. This genus is also an indicator of eutrophic conditions (Porter, 2008). The third taxon contributing most to the dissimilarity between MILR and its two non-AOC comparison sites was the diatom *Thalassiosira pseudonana*, which was detected at a 7-percent relative abundance in the spring at MILR. For MENO, the diatoms *Nitzschia inconspicua*, *T. pseudonana*, and *Thalassiosira weissflogii* contributed most to its dissimilarity with the two non-AOC comparison sites. *Nitzschia inconspicua* was at a higher, but still low, abundance at MENO compared to the two non-AOC comparison sites. *Thalassiosira weissflogii* comprised 43 percent of the relative abundance in the fall at ROOT but was absent or in low abundance at the other sites. All three diatom taxa are indicators of hypereutrophic conditions (high total nitrogen and phosphorus) and moderately high salinity (500–1,000 milligrams per liter chloride; Porter, 2008).

With respect to the dominance of various taxa at MILR and MENO in 2014, rotifers were dominant at both sites in the spring and summer with more than a 52-percent abundance at MILR and more than a 73-percent abundance at MENO; zebra mussel veligers comprised more than 78 percent of the density in fall zooplankton at MILR but were absent from MENO. Instead, copepods were the dominant taxonomic group in the fall at MENO (41 percent), with rotifers second. Diatoms were the dominant taxonomic group in the phytoplankton during all seasons at MILR in 2014 (41, 60, and 59 percent, respectively). Diatoms were the dominant taxonomic group at MENO in spring and fall 2014 (57 and 32 percent), but cryptophytes were the dominant group in summer 2014 (32 percent). Both have generally high food value for aquatic organisms (Stewart and Wetzel, 1986).

Out of all four AOCs assessed for plankton, only the assemblages for zooplankton at the Fox River near Allouez (a subsite in the Lower Green Bay AOC) and the Milwaukee River differed from the two non-AOC comparison sites; density of zooplankton was lower at both AOCs. Metrics for combined benthos and combined phytoplankton (diatoms and soft algae) at the Sheboygan River AOC did not differ from the two non-AOC comparison sites; however, the diversity of zooplankton in 2014 was lower at the Sheboygan River AOC than at the two non-AOC comparison sites (table 10).

**Table 10.** Summary of metric comparisons for benthos and plankton collected by the U.S. Geological Survey at Areas of Concern (AOCs) and non-AOC comparison sites in 2014, indicating where AOC metrics were significantly lower than non-AOC metrics.

[Metrics for benthos are for combined (dredge and Hester-Dendy) data except for the index of biotic integrity (IBI), which was computed for Hester-Dendy samples only. Metrics for phytoplankton are for combined (soft algae and diatom) data; the number of samples is 3 in all comparisons. Density comparisons are for log-10 transformed data. MENI, Lower Menominee River; EPT, Ephemeroptera-Plecoptera-Trichoptera; FOXR, Fox River near Allouez (Lower Green Bay and Fox River subsite); SHEB, Sheboygan River; MILR, Milwaukee River; MENO, Menomonee River (MILR and MENO are Milwaukee Estuary subsites)]

| Metric | 2012 | | 2014 | |
|---|---|---|---|---|
| | AOC: non-AOC group | AOC: non-AOC pair | AOC: non-AOC group | AOC: non-AOC pair |
| Benthos | | | | |
| Richness | None | None | None | None |
| Diversity | MENO | None | MENO | None |
| Total density | MENI | None | MENI | None |
| EPT density | None | MENI | MENI | MENI |
| EPT percent | None | FOXR | None | None |
| EPT richness | FOXR, SHEB, MILR, MENO | MILR | SHEB, MENO | MENI |
| IBI | SHEB, MENO | None | None | None |
| Zooplankton[1] | | | | |
| Richness | None | None | None | None |
| Diversity | None | None | None | SHEB |
| Total density | MILR | None | None | FOXR, MILR |
| Combined phytoplankton | | | | |
| Richness | None | None | None | None |
| Diversity | None | None | None | None |
| Total density | None | None | None | None |

[1]For zooplankton in 2012, high algal counts precluded identification of rotifers other than *Asplanchna priodonta* in summer samples for Ahnapee River and all Fox River samples; therefore, the comparisons for these sites excluded other rotifers.

## Overview of Benthos and Plankton in Lower Green Bay and Milwaukee Harbor

Although subsites in lower Green Bay (GREE, Green Bay Historical Subsite 3–1 [hereafter referred to as “GB03”], Green Bay Historical Subsite 5 [hereafter referred to as “GB05”], Green Bay Historical Subsite 8 [hereafter referred to as “GB08”], Green Bay Historical Subsite 16 [hereafter referred to as “GB16”], and Green Bay Historical Subsite 17 [hereafter referred to as “GB17”]) and the Milwaukee Harbor (MILH) were not included in direct comparisons with non-AOC comparison sites, results of this study provide an ecological assessment of the benthos and plankton that can be used for BUI evaluations and comparison to historical studies at the AOCs.

### Lower Green Bay

Within the Lower Green Bay and Fox River AOC, samples for benthos (dredge only) and plankton were collected from Green Bay at one subsite (GREE) near Long Tail Point in all three seasons in 2012 and 2014. In 2014 only, dredge samples for benthos were collected at an additional five subsites in Green Bay in all three seasons. Assemblages of benthos and plankton were compared among the other subsites sampled in the AOC. On average, GB03 had the highest richness and diversity and GB17 had the lowest of these two measures among the Lower Green Bay sites (table 11). The FOXR subsite had mean richness and diversity values that were near the median values when compared to all Green Bay subsites. An MDS ordination plot indicated that the benthic assemblages collected from GB17 during all three seasons grouped further away from the rest of the samples collected in Green Bay and the Fox River (fig. 8*A* and *B*). GB17 was east of the dredging channel on a shoal west of Point Au Sable, and its substrate material was dominated by sand. Although most samples at Green Bay subsites were dominated by oligochaetes, GB17 was dominated by midges in the spring and summer (more than 61 percent) and by zebra mussels in the fall (58 percent). GB05 was also dominated by zebra mussels in the fall, and GB03 was dominated by *Pisidium* pea clams in the fall. The ANOSIM indicated that there were differences between the benthic assemblages collected at GB17 in comparison to all

**Table 11.** Richness, diversity, and density values for benthos collected by dredge at Green Bay subsites in 2014.

[Benthic samples were not collected in 2012 and only dredge samples were collected in 2014. GREE, Lower Green Bay subsite; GB03, Green Bay Historical Subsite 3–1; GB05, Green Bay Historical Subsite 5; GB08, Green Bay Historical Subsite 8; GB16, Green Bay Historical Subsite 16; GB17, Green Bay Historical Subsite 17]

| Season | Richness[1] | Diversity[2] | Density[3] |
|---|---|---|---|
| GREE subsite | | | |
| Spring | 21 | 1.22 | 15,740 |
| Summer | 15 | 1.72 | 14,082 |
| Fall | 22 | 1.81 | 10,115 |
| GB03 subsite | | | |
| Spring | 23 | 2.23 | 9,165 |
| Summer | 26 | 2.18 | 10,510 |
| Fall | 26 | 1.92 | 8,546 |
| GB05 subsite | | | |
| Spring | 24 | 2.23 | 7,653 |
| Summer | 18 | 2.07 | 13,316 |
| Fall | 17 | 1.77 | 12,105 |
| GB08 subsite | | | |
| Spring | 9 | 1.30 | 8,903 |
| Summer | 11 | 0.96 | 12,015 |
| Fall | 11 | 0.94 | 9,388 |
| GB16 subsite | | | |
| Spring | 14 | 1.52 | 8,852 |
| Summer | 12 | 1.61 | 5,370 |
| Fall | 13 | 1.08 | 7,003 |
| GB17 subsite | | | |
| Spring | 7 | 0.30 | 5,772 |
| Summer | 7 | 1.36 | 1,594 |
| Fall | 9 | 1.48 | 427 |

[1]Richness was computed as the number of unique taxa in the sample.

[2]Shannon diversity index, calculated as $\log_e$.

[3]Density values are in count per square meter.

other Green Bay and Fox River sites. Mean dissimilarity between assemblages in GB17 and the other Green Bay and Fox River sites ranged from 76 percent (GB03) to 88 percent (GB08) according to a SIMPER test. Midge species of the genus *Cladotanytarsus* accounted for the most dissimilarity among all sites, explaining 5.9 to 11 percent of total dissimilarity. Relative abundances of zebra mussels explained 5.2 to 8.4 percent of dissimilarities between assemblages in GB17 and all other sites. Dissimilarities in these assemblages were also commonly due to differences in the abundances of several midge taxa (*Procladius* and *Chironomus*) and oligochaete taxa (immature Tubificinae, *Aulodrilus limnobius*, and *Limnodrilus hoffmeisteri*). *Aulodrilus limnobius* is an indicator of moderately eutrophic conditions and it is tolerant of moderate levels of pollution. *Limnodrilus hoffmeisteri* has a worldwide distribution; it can be locally abundant and dominant because of its adaptable nature and high tolerance to pollution, salinity, and highly eutrophic or "hypereutrophic" conditions (Bode and others, 2002; Rodriguez and Reynoldson, 2011). Based on ANOSIM and SIMPER results, the remaining 5 Green Bay sites can be placed into 2 general groupings: GB03, GB05, and GREE had similar assemblages, and GB08 and GB16 had similar assemblages (fig. 8*A* and *B*). The benthic assemblage in the Fox River was most similar to GREE and GB05 and moderately similar to GB03. The benthic assemblage at FOXR was most different from GB16 and GB17. Differences between FOXR and GB16 were mainly due the oligochaetes *Branchiura sowerbyi* and *Aulodrilus pigueti* and the midge species of the genus *Cryptochironomus*. All three taxa are highly tolerant of pollution (Barbour and others, 1999; Bode and others, 2002; Rodriguez and Reynoldson, 2011). Differences between FOXR and GB17 were mainly due to *Cladotanytarsus*, zebra mussels, and immature Tubificinae. *Cladotanytarsus* is moderately pollution tolerant and immature Tubificinae are considered to be highly tolerant (Barbour and others, 1999). Samples for benthos were not collected in Green Bay in 2012, so comparisons could not be made between years.

At the only Green Bay site where planktonic assemblages were sampled (GREE), neither the richness nor the diversity of zooplankton differed between 2012 and 2014 but the total density was higher in 2014. In 2014, the dominant group was rotifers (52 to 78 percent) with copepods second in dominance overall. The rotifer *Synchaeta* was dominant in spring 2014 (36 percent), followed by the rotifer *Polyarthra vulgaris* in summer 2014 (17 percent), and copepod nauplii in fall 2014 (23 percent). The rotifer *Keratella crassa* was second in dominance in spring and fall 2014.

The richness, diversity, and total density of combined phytoplankton at GREE did not differ between 2012 and 2014, but the total density was quite variable between seasons each year. In 2014, the dominant group was cyanobacteria (50 to 86 percent) with the highest abundance in the summer. Diatoms were second in abundance (8 to 22 percent) in all seasons. The cyanobacterium *Planktolyngbya* was dominant in spring and fall 2014 (35 and 28 percent, respectively), and *Aphanocapsa* was dominant in summer 2014 (62 percent). Second in dominance in summer and fall 2014 was the toxin producer *Microcystis aeruginosa* (21 to 24 percent), and the toxin producer Anabaena made up 6 percent of the total algal density in spring 2014. Also, in fall 2014, two other toxin-producing algae were present at GREE at a 3-percent relative abundance for *Aphanizomenon issatschenkoi* and *Planktothrix*. These results underscore the highly eutrophic character of Green Bay with the added concern of potentially toxic algal blooms. Much higher concentrations of *Anabaena* and *Microcystis aeruginosa* during all seasons in 2014 at FOXR implicate the Fox River as a potential source of these cyanobacteria to Green Bay. As an additional indicator of nutrients in the

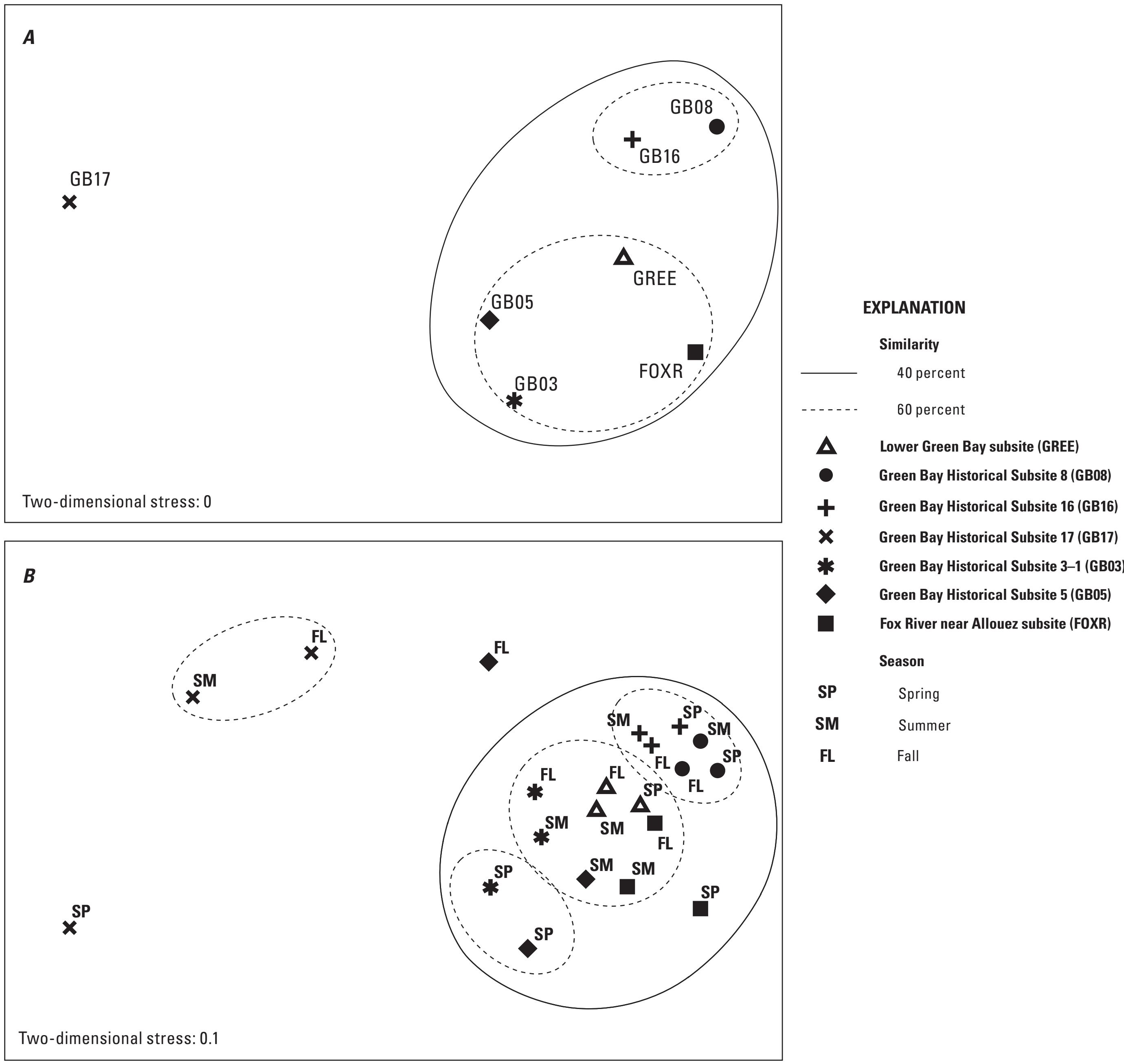



**Figure 8.** Multidimensional scaling ordination plots for the benthos collected by dredge at the Green Bay and Lower Fox River Area of Concern, based on relative abundance (fourth-root transformed) with no rare or ambiguous taxa. *A*, Seasons combined; and *B*, seasons separate.

Fox River and Green Bay, the mean chlorophyll-*a* concentration was 56 µg/L in Green Bay, compared to 150 µg/L in the fall at the Fox River subsite FOXR. Excess nutrients from the watershed have been a decades-long concern for the AOC and the watershed.

### Milwaukee Harbor

Benthos and plankton in Milwaukee Harbor were sampled at one site near the mouth by the USGS streamgage Milwaukee River at Mouth at Milwaukee, Wis., on Jones Island (USGS station 04087170). For benthos, the total richness, diversity, and density of combined benthos, as well as the IBI, did not differ between 2012 and 2014 (table 5). The mean IBI across years was 22.5±7.6 and this score is in the "poor" category. For dominance in combined benthos, oligochaetes had the highest percentages of relative abundance (87, 97, and 69 percent in the spring, summer, and fall, respectively), which were mostly due to immature Tubificinae. Zebra mussels were 29 percent of the abundance in the fall. Midges comprised less than 10 percent of the total abundance. The most common midges at MILH in 2012 and 2014 were *Dicrotendipes*, *Paratendipes*, and *Cricotopus/Orthocladius*, genera that are moderately to highly tolerant of pollution (Barbour and others, 1999). Silt was dominant in sediment at MILH, which varied by season and year somewhat, but overall, the substrate was a mix of sand and silt with a moderate amount of clay (42, 38, and 20 percent, respectively). The organic carbon content, as estimated by VOI samples was 12 percent, which is moderate relative to other sampled sites.

For zooplankton, there were no differences between 2012 and 2014 for richness, diversity, or density at MILH. For 2014 only, although rotifers dominated the assemblage in the spring and summer (76 and 98 percent), zebra mussel veligers dominated in the fall (78 percent), which followed a similar pattern to MILR that year. The most abundant rotifer at MILH in spring 2014 was *Synchaeta* (90 percent) followed by other rotifers, and less than 1 percent consisted of nonrotifer taxa. The rotifer *Keratella crassa* was dominant in summer 2014 (35 percent) with *Synchaeta* second (20 percent). *Synchaeta* was also dominant in spring 2012 at the site but zebra mussel veligers were nearly as abundant, and this relation was opposite in the summer with zebra mussel veligers being the most abundant. *Keratella crassa* was dominant in fall 2012 and zebra mussel veligers comprised nearly a quarter of the overall abundance. *Synchaeta* is a pollution-tolerant rotifer that is common in the Great Lakes and has higher abundances in the fall through the spring when water temperatures are cooler; *Keratella* is a common rotifer and several species can cooccur in the Great Lakes (Gannon and Stemberger, 1978; Stemberger, 1979).

The richness of combined phytoplankton at MILH was higher in 2014 than in 2012 because of higher diatom richness in 2014; however, laboratory processing problems with the 2012 diatom samples from MILH may have contributed to this difference. Also, specific conductance at MILH was higher in 2014 than in 2012, possibly reflecting the effects of the drought in 2012. The richness of diatoms at MILH was low in 2012, with an average of 12.7±8.7 (compared to an average richness of 77.3±4.7 in 2014). In contrast, the richness of soft algae was not different between years. The diversity and density of combined phytoplankton were not different between years. In 2014, diatoms were dominant in the spring (42 percent). Green algae became dominant in the summer (44 percent), followed by diatoms and then cryptophytes. Diatoms became dominant again in the fall (39 percent), followed by green algae. Although absent in spring and summer 2014, cyanobacteria became common in the fall. *Diatoma tenuis* was the most common diatom in the spring, and it is commonly associated with moderately eutrophic conditions (Porter, 2008). *Cyclostephanos invisitatus* was the most common diatom in the fall, and this centric taxon is an indicator of high nutrient conditions (Porter, 2008). The dominant green alga in the summer (39 percent) was the filamentous taxon *Klebsormidium* sp., and it was still important in the fall (20 percent).

## Comparison to Historical Data

Although many studies of benthos and plankton have been done in Lake Michigan, few have been done at river mouths and harbors, and most of those studies do not conform to the standards required for quantitative comparison. Taxonomic resolution and changes in taxonomic classifications over time—especially for the phytoplankton—pose large problems with using historical data. Even when site locations are relatively close, field collection methods can vary greatly between studies, and quality assurance and quality control procedures are not always reported; however, comparisons between the current study and some historical data can be made, and these comparisons are addressed for each AOC in order, with one exception. Data comparisons with Weigel and Dimick (2011) are discussed last because multiple AOCs were included.

### Benthic Assemblage Comparisons to Other Studies

In the current study, the predominant benthic taxa in bottom sediment at all sampled sites, AOCs and non-AOCs, were oligochaetes and midges. The richness, diversity, and density as well as the pollution tolerances of taxa present varied among sites. Multiple independent studies during the 1970s and 1980s of the Lower Menominee River AOC characterized the benthos as predominantly pollution-tolerant oligochaetes and midges, which were low in abundance or lacking in areas with high sediment chemical concentrations and poor substrate (Wisconsin Department of Natural Resources, 1996; Elwin Evans, unpub. data, July 1980, as cited in Wisconsin Department of Natural Resources and Michigan Department of Natural Resources, 1990). In the current study, the substrate was poor at MENI and organism densities were lower than at all non-AOCs in 2012 and 2014. Although many taxa were pollution tolerant, the dominance by taxa other than oligochaetes and the common presence of the clam *Pisidium* in all seasons in 2014 are good results for MENI and may indicate that conditions are improving.

Benthic invertebrates of Green Bay and the Fox River have shown improvements with time and water- and sediment-remediation efforts but remain generally poor quality. Historical studies of Green Bay indicated that when first assessed in the fall and winter 1938–9, the benthos of the southern bay had few populations of oligochaetes and midges except near the mouth of the Fox River (Wisconsin State Committee on Water Pollution and others, 1939). In the early 1950s, Surber and Cooley (1952) found a large increase in the abundance of these two groups of invertebrates (Surber and Cooley, 1952); however, Bertrand and others (1976) indicated that seasonal differences may have added to the differences in abundance between the two studies (Bertrand and others, 1976), which was also found in the current study. Previous studies of the Lower Green Bay and Fox River AOC found the benthos to be low in diversity and predominantly composed of tolerant Tubificinae oligochaete worms and midges (Ankley and others, 1992; Balch and others, 1956; Federal Water Pollution Control Administration, 1968; Howmiller and Beeton, 1971; Integrated Paper Services, Inc., 2000; Surber and Cooley, 1952; Wisconsin Department of Natural Resources, 1993; Wisconsin State Committee on Water Pollution and others, 1939). The change from rocky to soft, silty bottom substrates along with increases in toxins and increases in low oxygen events in the lower Fox River and into lower Green Bay near the river's mouth was accompanied by a change in the benthos from a mix of tolerant and intolerant taxa, to mostly tolerant taxa, to a lack of even tolerant taxa (Balch and others, 1956). The results of the current study still showed primarily oligochaetes and secondarily midges except at the lower Green Bay subsite, GB17, a sandy (94–97 percent; Scudder Eikenberry and others, 2016b) site where midges were dominant and either oligochaetes or pea clams were subdominant in spring and summer 2014. Burrowing mayfly larvae (*Hexagenia*), which are referred to as "fish flies" or "Green Bay flies" when adults, were once abundant in the region but declined with increasing pollution (Surber and Cooley, 1952). In 1938 and 1939, *Hexagenia* larvae were found in low densities in dredge samples of Lower Green Bay (Wisconsin State Committee on Water Pollution and others, 1939). These mayflies were also collected at 16 of 51 stations in surveys of Green Bay by Balch and others (1956) but were only rarely collected in later years (Ball and others, 1985; Wisconsin Department of Natural Resources, 2013). In the current study, *Hexagenia* were found in 2012 only in dredge samples from MENI and its two non-AOC comparison sites, ESCA and OCON, and this taxon was found in 2014 in only three samples: in summer HD samples from the Manitowoc River (MANI sampling site) and the Sheboygan River (SHEB sampling site) and in a fall dredge sample from MENI; no samples for benthos were collected in Green Bay in 2012 and no *Hexagenia* were found in Green Bay samples in 2014. A return of this species would signal improvement to the benthos of the Green Bay and Fox River AOC.

Comparisons across years for benthic assemblages in the Sheboygan River AOC are difficult because few studies have been done (Wisconsin Department of Natural Resources, 2012). A study in 1997 using dredge samples found immature Tubificinae oligochaetes made up more than 90 percent of the benthic assemblage at most Sheboygan River sites sampled, and analyses of a subset of these sites determined that there were just two species present: *Limnodrilus hoffmeisteri* and *Limnodrilus cervix* (EVS Environment Consultants, Inc., and National Oceanic and Atmospheric Administration, 1998). In the current study, immature Tubificinae oligochaetes made up more than 80 percent of the benthic invertebrates in dredge samples at SHEB. The remaining oligochaetes were primarily the tolerant species *L. hoffmeisteri* and *L. cervix*. In 2014, highly tolerant immature Tubificinae oligochaetes were 58, 67, and 88 percent of the benthos in the spring, summer, and fall, respectively, and the highly tolerant *L. hoffmeisteri* was again the dominant oligochaete found. However, metrics for combined benthos did not differ from the two non-AOC comparison sites in 2014, and the benthic assemblage is expected to improve with time because sediment remediation was completed in 2013.

For the Milwaukee Estuary AOC, benthic assemblages do not seem to have improved in recent decades; however, sediment remediation is still in progress. Benthic studies in the late 1970s and early 1980s found low diversity and a dominance of pollution-tolerant taxa—primarily oligochaetes—in the Milwaukee and Menomonee Rivers that was related to sediment contaminants, poor substrate and water-quality conditions, and inadequate food resources (Wisconsin Department of Natural Resources, 1991, 1994). Benthos in the inner harbor of the estuary also must contend with high sedimentation rates and low dissolved oxygen concentrations (Wisconsin Department of Natural Resources, 2014). In the current study, even though diversity was low but not lower than the two non-AOC comparison sites, almost complete dominance (86 to 99 percent) by oligochaetes was found in dredge samples from sites in the Milwaukee River (MILR), Menomonee River (MENO), and the Milwaukee Harbor (MILH). Highly tolerant oligochaete taxa were dominant in these samples (75 to 96 percent), indicating that the status of these assemblages has changed little over recent decades.

At several AOCs, the HD data for benthos in the current study were compared quantitatively to historical HD data from the WDNR (Brian Weigel [WDNR] and Jeffrey Dimick [Aquatic Biomonitoring Laboratory–University of Wisconsin at Stevens Point], unpub. data, 2013). Values for eight invertebrate metrics from HD sampler data collected in 2012 and 2014 as part of the current study were compared with historical study values for HD relative abundance data and metrics collected by Weigel and Dimick (2011) using similar methods near the same AOC locations in the summer or fall of 2003 and (or) 2005. Methods using HD samplers in the current study were based on methods described in Weigel and Dimick (2011), and the same laboratory processed both sets of samples. ANOSIM tests did not indicate any differences in benthic assemblages between summer and fall samples for the current study and this historical dataset, and little difference was found between the two studies for metrics. For the Lower Menominee River AOC, the Weigel and Dimick (2011) summer IBI score was 45 (fair) in 2005. In the current study, IBI scores at MENI were 15 (very poor) in spring and 20 (poor) in summer and fall in 2012; IBI scores in 2014 were 30 (poor) in spring and summer and 15 (very poor) in fall. At the Sheboygan River AOC, the percentage of EPT individuals was 2.6 in summer 2003, compared with summer and fall 2012 and fall 2014 when values were less than 1.0 percent; the percentage of EPT individuals was 2.0 percent in summer 2014. The percentage of insects, primarily gatherer-type insects, was 95 percent in 2003, compared with summer and fall 2014 when values were 28 to 34 percent and with values in 2012 that were lower. Lastly, IBIs for 2014 at the Sheboygan River AOC were higher than for 2003 but still very poor at 10 and 15 for summer and fall 2014, respectively, compared to 5 in 2003. Metric values were similar between 2005 and 2012 at MILR; however, the IBI for summer 2014 was 45 (fair), apparently because of higher richness from insects. Weigel and Dimick (2011) state that their nonwadable river IBI may not be comparable to an IBI determined at upstream wadable riverine locations because the IBI tends to underrate sites with semilacustrine flows, such as those found downstream at river mouths, and rate them lower. IBI values within these ranges would be rated as poor for a large river system (poor rating ranges from 20 to 39); however, a large river IBI may not be able to accurately rate them. A benthic IBI for river mouths and harbors may be more valuable with the addition of functional and tolerance information for oligochaetes given their importance in these ecosystems and the range in environmental preferences. The large river IBI used in the current study includes oligochaetes, because they contribute to the proportion of noninsects, but not with regard to tolerance or functional roles.

## Planktonic Assemblage Comparisons to Other Studies

Historical studies in the 1980s and 1990s in the lower Menominee River did not indicate impairment of the planktonic assemblage in the AOC with respect to contaminants, except for zooplankton in the turning basin and the 8th Street slip, where toxic effects in bioassays were found in 1989 by the WDNR (Wisconsin Department of Natural Resources and Michigan Department of Natural Resources, unpub. data, 1990). More recent studies of plankton in the Lower Menominee River were not found.

In the Lower Green Bay and Fox River AOC, the plankton assemblage still reflects the effects of decades of pollution but now also is troubled by invasive species. Historical studies in 1938 and 1939 found zooplankton such as rotifers and microcrustaceans were usually present in low numbers (Wisconsin State Committee on Water Pollution and others, 1939). Later studies in the 1980s found rotifer abundance higher than that of other microcrustaceans in the lower eutrophic part of Green Bay (Richman and others, 1984a; Richman and others, 1984b). In a study of Green Bay and near the mouth of the Fox River, the phytoplankton found in 1938 and 1939 (Wisconsin State Committee on Water Pollution and others, 1939) included mostly diatoms and cyanobacteria, with blooms of the toxin producer *Aphanizomenon*. Later surveys found the plankton to be dominated by cyanobacteria and small crustaceans, both with little food value to consumer organisms. Studies of the plankton during the 1980s found green algae dominant (as much as 80 percent) in the lower eutrophic part of Green Bay (Richman and others, 1984a; Richman and others, 1984b). Zebra mussels were first found in Green Bay in 1992 and became abundant (De Stasio and Richman, 1998). Their high densities and ability to filter large volumes of water in the bay correlated with a change in dominance from green algae to cyanobacteria, with large increases in the abundance of cyanobacteria *Anabaena* and *Microcystis* and an increase in the biovolume and chlorophyll of phytoplankton (De Stasio and others, 2014). In the current study at the Green Bay subsite GREE, the cyanobacterium *Microcystis aeruginosa* comprised 21 and 24 percent of the total density of phytoplankton in summer and fall 2014, respectively. *Microcystis* is known to thrive in high nutrient conditions. Other potentially toxic cyanobacteria including *Aphanizomenon issatschenkoi*, *Anabaena*, and *Planktothrix* also contributed 3 to 6 percent of the density in 2014 at GREE.

The WDNR stated in 1989 that there was no information on planktonic assemblages in the Sheboygan River AOC and no later publications have been found other than USGS research completed as part of the current study and a study by Olds and others (2017), which was done as a followup to the current study using the same methods. Olds and others (2017) found only the diversity of the zooplankton was lower at SHEB than at the two non-AOC comparison sites, KEWA and MANI, just as was found for 2014 in the current study.

The 2012 and 2014 data for plankton from the Milwaukee Estuary AOC were compared to data for plankton from the Milwaukee Metropolitan Sewerage District (MMSD; Eric Waldmer, MMSD, electronic files provided April 22, 2013). The MMSD collected zooplankton and phytoplankton periodically from 1980 through 1997 in the Milwaukee Estuary using methods fairly similar to those used in the current study. Specifically, the MMSD collected zooplankton using an 80-µm mesh plankton net (compared to the 63-µm mesh in the current study) with vertical hauls from 1 m off the bottom to the surface; phytoplankton were collected using a whole-water sampler but depth was not specified. Most MMSD sites were in the outer harbor and nearshore areas of Lake Michigan near Milwaukee, but one site, NS 28 (also called OH 1), was near MILH, which was sampled in 2012 and 2014 for the current study. At NS 28, rotifers and copepods were the dominant zooplankton present in samples during 1980–97. Rotifers were the dominant (59 to 75 percent) zooplankton in all seasons at the Milwaukee Harbor subsite in 2012; however, zebra mussel veligers were subdominant in 2012, and copepods and cladocerans were only minor components of the assemblage. In 2014, rotifers were also the dominant zooplankton in the spring and summer but zebra mussel veligers were the dominant (78 percent) zooplankton in the fall. With regard to specific rotifer taxa, *Filinia longiseta* was dominant during 1980–85, with species of *Synchaeta*, *Keratella*, and *Brachionus* subdominant; however, during 1988–97, *F. longiseta* was no longer a dominant rotifer and the previously subdominant taxa became more abundant. At MILH, *Synchaeta oblonga* was the dominant rotifer in spring and summer 2012 and in spring 2014; *Keratella crassa* was dominant in fall 2012 and summer 2014, and together these two taxa were the next most common zooplankton to the dominant zebra mussel veligers in fall 2014 (totaling 15 percent). At NS 28, the dominant copepod taxa during 1980–94 were cyclopoid copepods and unidentified immature copepods—nauplii and copepodids or copepodites; during 1995–97, the copepods were predominantly nauplii and the taxon *Diacyclops thomasi*, a cyclopoid copepod. The copepod taxa in 2012 were grossly similar to 1995–7, with nauplii and cyclopoid copepodites dominant and calanoid copepodites subdominant. Unidentified immature copepods (nauplii) were the dominant copepod life stages in 2014 and cyclopoid copepodites were subdominant in spring and fall; however, adult females of the cyclopoid copepod *Eucyclops elegans* and the calanoid copepod *Eurytemora affinis* were subdominant in summer 2014. Harpacticoid copepods, a benthic taxon, were first reported in the 1997 sample in low abundance, and these copepods were present at MILR in 2012 and 2014 in low abundance. Within the cladocerans, *Bosmina longirostris* was the dominant taxon in all MMSD samples as well as all seasons in 2012 and spring and summer in 2014. *Ceriodaphnia lacustris* and *Diaphanosoma birgei* were subdominant in the summer and fall 2012 samples, respectively, whereas subdominant taxa were distributed fairly evenly across all four taxa in the fall of 2014.

In the MMSD samples of phytoplankton collected near MILH, diatoms and green algae were generally the dominant algal group, followed by cyanobacteria and (or) cryptophytes, depending on the season. In 2012, diatoms were the dominant group (58 percent) in the spring, cryptophytes were dominant (50 percent) in the summer, and green algae (37 percent) and cyanobacteria (36 percent) were codominant in the fall. In 2014, diatoms were the dominant group in the spring and fall (42 and 39 percent, respectively), green algae were dominant (44 percent) in the summer (primarily *Klebsormidium*), and cryptophytes decreased from 30 percent in the spring to only 16 percent in the fall. Cyanobacteria were not found in 2014 samples. Diatom taxa were identified in about one-third of the MMSD samples and, in those samples, dominant taxa varied by season and year, so comparisons with specific diatom taxa are difficult and were not attempted here.

# Summary and Conclusions

The benthos (benthic invertebrates) and plankton (zooplankton and phytoplankton) at Wisconsin's 4 Areas of Concern (AOCs) on Lake Michigan were evaluated by collecting samples at the AOCs and 6 less-degraded comparison sites (hereafter referred to as "non-AOCs") in 2012 and 2014. This was followed by an assessment of the relative abundance and distribution of taxa as well as computed metrics representing the health of aquatic communities in those samples. Except for Green Bay and the Milwaukee Harbor, results for combined benthos (dredge and artificial substrate samples), zooplankton, and combined phytoplankton (soft algae and diatoms combined) were compared statistically between each AOC and the means of all non-AOCs and between each AOC and the means of two non-AOC comparison sites.

The status of assemblages of benthos and plankton at the AOC sites and subsites may be summarized as follows for 2014:

## Lower Menominee River AOC site (MENI)

### Benthos

- Only Ephemeroptera-Plecoptera-Trichoptera (EPT) density and EPT richness of combined benthos differed from the mean of the two non-AOC comparison sites (the Escanaba River, Michigan, non-AOC comparison site [ESCA] and the Oconto River non-AOC comparison site [OCON]). Both metrics at MENI were lower than the mean of the two non-AOC comparison sites and were therefore rated as degraded; however, this study did not investigate the benthos at MENI after remediation was completed in late 2014 and so results of the current study may not reflect the status of the postremediation assemblage.
- No benthic metrics differed between 2012 and 2014 at MENI.
- Midges were the dominant taxonomic group in spring and summer 2014 at MENI but, in fall 2014, pea clams were dominant with midges second in dominance.

### Plankton

- No metrics for zooplankton or combined phytoplankton differed between MENI and the two non-AOC comparison sites in 2014.
- Only the richness of combined phytoplankton differed between 2012 and 2014 at MENI; richness was higher in 2014.

- In the zooplankton, rotifers were the dominant taxonomic group during all seasons in 2014 at MENI.
- In the phytoplankton, dominance varied by season at MENI; the highest abundances for cryptophytes were detected in the spring and fall, and the highest abundances for green algae were detected in the summer.

## Lower Green Bay and Fox River AOC—Fox River near Allouez subsite (FOXR)

### Benthos

- For 2014, only the EPT richness of combined benthos differed between FOXR and the mean of the two non-AOC comparison sites (the Ahnapee River non-AOC comparison site [AHNA] and the Kewaunee River non-AOC comparison site [KEWA]); EPT richness at FOXR was higher. The higher EPT richness seemed to be from the presence of two caddisfly taxa, including a highly tolerant taxon and a moderately tolerant taxon.
- EPT richness was higher at FOXR in 2014 than in 2012.
- Multivariate analyses indicated that the 2014 combined benthos at FOXR differed from the two non-AOC comparison sites, mostly because of higher relative abundances of three pollution-tolerant oligochaete taxa.
- Oligochaetes were by far the dominant taxonomic group at FOXR in 2014, and sediment remediation was ongoing during sampling.

### Plankton

- For zooplankton in 2014, only density differed between FOXR and the mean of the two non-AOC comparison sites; FOXR was lower and this result indicates that the assemblage of zooplankton at FOXR was degraded relative to the non-AOCs.
- For zooplankton in 2014, rotifers were the dominant taxonomic group in all seasons at FOXR.
- Metrics for combined phytoplankton did not differ between FOXR and the two non-AOC comparison sites.
- The combined phytoplankton assemblage at FOXR differed from its two non-AOC comparison sites. Out of all four AOCs examined, this was the only one in which this was true.
- For phytoplankton in 2014, cyanobacteria were the dominant taxa at FOXR in all seasons in 2014. Spring cyanobacteria were mostly the toxin producers *Anabaena* and *Microcystis aeruginosa*, and *M. aeruginosa* was the dominant cyanobacterium in summer and fall 2014 with more than 80 percent of the total algal abundance. The dominance of harmful algae underscores the highly eutrophic nature of the Fox River and is a symptom of larger watershed concerns for high concentrations of nutrients.

## Sheboygan River AOC site (SHEB)

### Benthos

- No metrics for combined benthos differed from the two non-AOC comparison sites (the Kewaunee River non-AOC comparison site [KEWA] and the Manitowoc River non-AOC comparison site [MANI]) in 2014.
- No metrics for combined benthos differed between 2012 and 2014 at SHEB.
- Highly tolerant immature Tubificinae oligochaetes were dominant at SHEB and the highly tolerant *Limnodrilus hoffmeisteri* was the dominant mature oligochaete found.
- The benthic assemblage at SHEB differed from the two non-AOC comparison sites. This was mostly because the highly tolerant oligochaete *Paranais* and the zebra mussel were abundant at SHEB but were uncommon or absent at the two non-AOC comparison sites, and the highly tolerant midge *Glyptotendipes* was absent or nearly so at SHEB but was uncommon to abundant at the non-AOC comparison sites.

### Plankton

- For zooplankton in 2014, only diversity differed between SHEB and the mean of the two non-AOC comparison sites; diversity was lower at SHEB and was rated as degraded.
- Rotifers dominated abundance of zooplankton in spring and summer 2014 samples of zooplankton at SHEB; zebra mussel veligers dominated abundance in fall 2014.
- For combined phytoplankton in 2014, no metrics differed between SHEB and the mean of the two non-AOC comparison sites.
- Diatoms were the dominant algal group in the phytoplankton at SHEB in 2014.

### Milwaukee Estuary AOC—Milwaukee River subsite (MILR) and Menomonee River subsite (MENO)

#### Benthos

- At MILR in 2014, only EPT density for combined benthos differed from the mean of the two non-AOC comparison sites (MANI and the Root River non-AOC comparison site [ROOT]), and MILR was higher (less degraded); however, the higher EPT density at MILR may have been because of high densities of a pollution-tolerant caddisfly at MILR.
- At MENO in 2014, only the total density of combined benthos differed from the mean of the two non-AOC comparison sites, and it was higher (less degraded) at MENO. The higher total density at MENO was because of higher densities for oligochaetes, especially some taxa that have a high pollution tolerance.
- The benthic assemblages at MILR and MENO differed from the two non-AOC comparison sites because of differences in the relative abundances of several taxa. Pea clams, a tolerant oligochaete, and a tolerant caddisfly were found in higher abundance at MILR; a tolerant oligochaete was found in higher abundance at MENO but another oligochaete and a midge were absent from MENO.
- There was no difference in metrics between 2012 and 2014 for combined benthos at MILR or MENO.

#### Plankton

- The total density of zooplankton in 2014 was lower at MILR than the mean of the two non-AOC comparison sites, so MILR was rated as degraded for density.
- No metrics for zooplankton at MENO differed from the two non-AOC comparison sites.
- For zooplankton in 2014, rotifers were dominant at MILR and MENO in the spring and summer; zebra mussel veligers were dominant in the fall at MILR but were absent from MENO. Copepods (nauplii) were the dominant taxonomic group in the fall at MENO.
- For combined phytoplankton in 2014, metrics did not differ for MILR or MENO from the mean of the two non-AOC comparison sites.
- At MILR in 2014, diatoms were the dominant taxonomic group in all seasons.
- At MENO in 2014, diatoms were the dominant taxonomic group in spring, cyanobacteria were dominant in summer, and green algae were dominant in fall.

In summary for benthos, only the Lower Menominee River AOC differed from its two non-AOC comparison sites; the density and richness of taxa in insect orders Ephemeroptera-Plecoptera-Trichoptera (mayflies, stoneflies, and caddisflies) in combined benthos (dredge and artificial substrate samples) were lower at the AOC. For plankton, the assemblages for zooplankton at the Fox River near Allouez (a subsite in the Lower Green Bay AOC) and the Milwaukee River differed from their two non-AOC comparison sites; density of zooplankton was lower at both AOCs. Metrics for combined benthos and combined phytoplankton (soft algae and diatoms) at the Sheboygan River AOC did not differ from the two non-AOC comparison sites; however, the diversity of zooplankton in 2014 was lower at the Sheboygan River AOC than at the two non-AOC comparison sites.

In assessments of ecological status, it is important to consider the effect that an invasive species such as the zebra mussel can have on the benthic and planktonic assemblages included in the current study. Though seldom a component of the benthos in soft sediment, zebra mussels were numerous on the Hester-Dendy samplers, and their immature forms were a large component of the plankton in the fall at the Sheboygan River AOC and at the Milwaukee River subsite in the Milwaukee Estuary AOC. Other studies have also indicated their effect in the Green Bay and Fox River AOC. Depending on the magnitude of effect that an invasive species has, it could reduce values for metrics such as richness, diversity, density, and index of biotic integrity (IBI) at sites. The adverse effects of invasive species would be separate from the effects of sediment contamination or remediation and could hinder or even prevent the ability of ecosystems to recover after remediation efforts.

The non-AOCs selected as comparison sites in this study were selected because (a) they were thought to have similar physical characteristics (land use, surficial geology, latitude, and climate) to the AOCs, (b) they are on the western shoreline of Lake Michigan where the AOCs are, and (c) they are not AOCs and are therefore presumed to be less degraded. However, there is a great deal of complexity in these comparisons. A finding of no statistical difference between a metric at an AOC site or subsite and the two non-AOC comparison sites does not mean that the benthic or planktonic assemblage at an AOC is not degraded in some aspect. However, where a metric for an AOC site or subsite was lower and therefore more degraded than at the non-AOC comparison sites, whether or not the two non-AOC comparison sites have some degradation themselves, this potentially supports the finding of degradation at an AOC site. Unfortunately, the low number of samples made it harder to discern that an AOC site differed from non-AOCs; however, the weight of evidence across multiple metrics representing the assemblages adds confidence to the overall assessment in this study. For multivariate comparisons, large differences between AOC and non-AOC assemblages may indicate that the AOC was not meeting expectations. Lastly, there are likely physical, chemical, and biological factors influencing the assemblages that are beyond the scope of this report as well as beyond the scope of AOC designations.

It is critical to consider a variety of measures when comparing assemblages at an AOC with one or more less-degraded sites because some measures address only a single aspect of the assemblage. Use of structural measures that relate to the relative numbers of different organisms (for example, richness, diversity, and relative abundance) and functional measures that relate to the role or preferences of different organisms (for example, environmental tolerances) is important in any complete assessment of ecological status. An aquatic assemblage can change in many ways without a significant change in richness or structural diversity, such as when more tolerant taxa replace less tolerant taxa or when green algae or cyanobacteria replace diatoms. An IBI is a multimetric that combines structural and functional measures and may therefore be a more effective measure to use for defining differences or change. The benthic IBI for river mouths and harbors may be more valuable with the addition of functional and tolerance information for oligochaetes because of their importance in these ecosystems and the range in environmental preferences for this large and diverse group of organisms. At present, there are no planktonic IBIs for use in river mouths or harbors.

These assessments at Wisconsin's four AOCs along the western shoreline of Lake Michigan provide a way to evaluate the current status of assemblages of benthos and plankton in relation to other rivers and harbors along the same shoreline. Assessments using a combination of standard statistics with computed biological metrics as well as multivariate analyses with assemblage abundance data indicated whether or not the aquatic assemblage at each AOC was different from the comparison sites. Methods and results for the current study should have application to evaluations of benthic and planktonic assemblages in other Great Lakes river mouths and harbors.

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# Appendix C – Sediment Toxicity Assessment in Two Wisconsin Areas of Concern and Selected Lake Michigan Tributaries

# Sediment Toxicity Assessment in Two Wisconsin Areas of Concern and Selected Lake Michigan Tributaries

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## Background

Contaminated sediment is the most common cause for some river and harbor areas around the Great Lakes, Areas of Concern (AOCs), to be deemed environmentally degraded. Because of close contact with contaminated sediment, the Beneficial Use Impairment (BUI) for degraded benthos or bottom-dwelling organisms is one of the most widespread BUIs at the AOCs. In Wisconsin, sediment remediation for PCBs was complete at the Sheboygan River AOC in 2013 and remediation for PCBs and other chemicals is ongoing in the Milwaukee Estuary AOC. We conducted an assessment to provide toxicity data in a regional context and build upon benthos community studies that the USGS completed at the AOCs and two non-AOCs in 2014.

## Goal and Objectives

### GOAL:

- Provide data on sediment toxicity to benthos to inform decisions by the U.S. Environmental Protection Agency and Wisconsin Department of Natural Resources regarding possible removal of the "Degradation of Benthos" BUI at the Sheboygan River AOC and the Milwaukee Estuary AOC

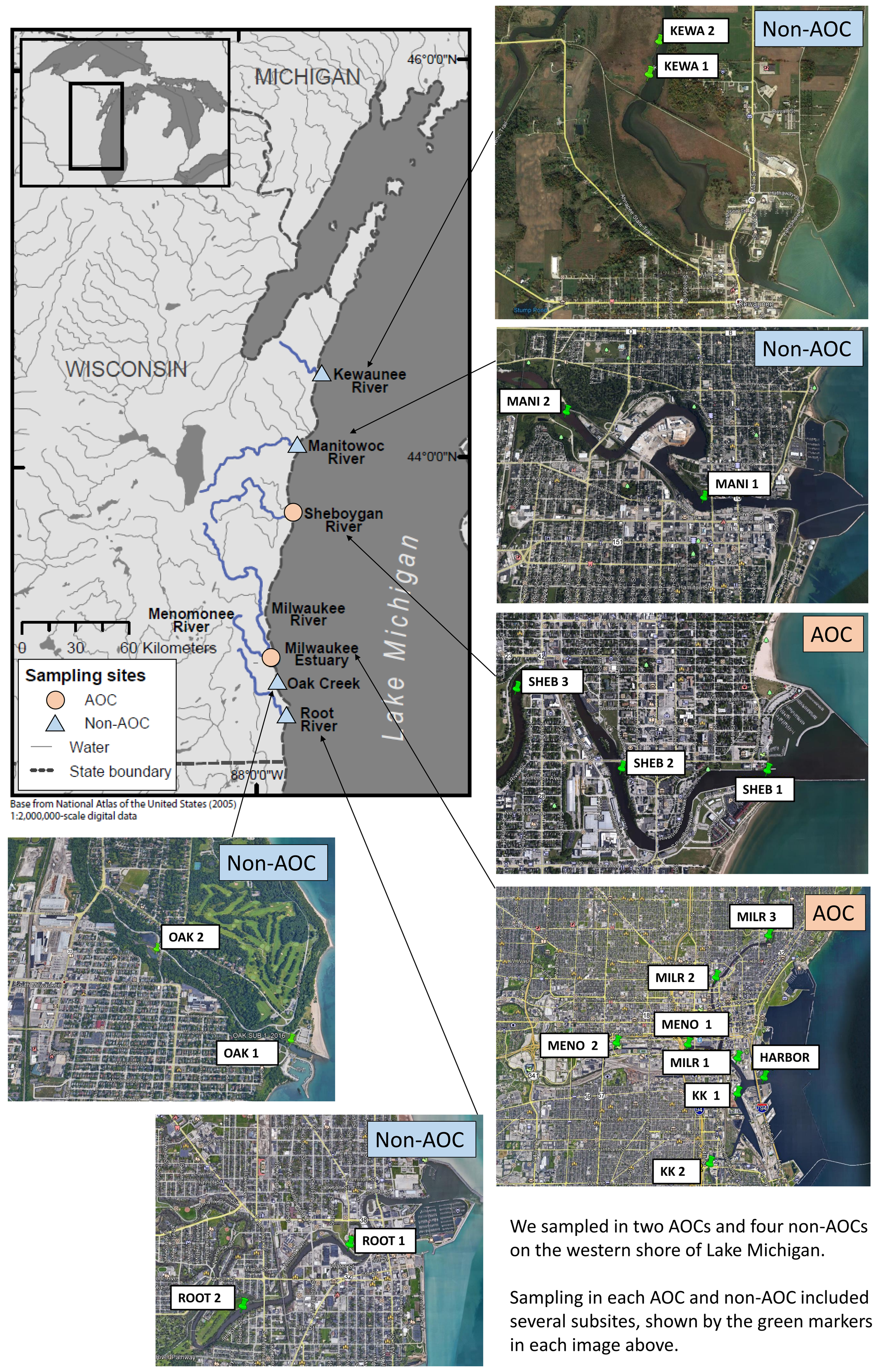
### OBJECTIVES:

- Characterize sediment toxicity and relationships between toxicity and contaminant concentrations
- Compare results to previous studies of sediment contamination, toxicity, and benthic communities at the AOCs and at two non-AOC comparison study areas: the Manitowoc River and Root River
- Compare upstream and downstream results in two additional non-AOC study areas: the Kewaunee River and Oak Creek

## Approach

In October 2016, we collected bottom sediment from two AOCs along the Lake Michigan shoreline and presumptively less-degraded study areas that are not AOCs (non-AOCs). The two AOCs are the **Sheboygan River AOC** and the **Milwaukee Estuary AOC** (Kinnickinnic River, Menomonee River, Milwaukee River, and Milwaukee Harbor) and the non-AOCs are the Kewaunee River, Manitowoc River, Oak Creek, and Root River. Sites are listed upstream to downstream in each study area.

Sediment collected was used for 1) short-term and long-term sediment toxicity tests with midges and amphipods, 2) chemical tests of ammonia, PCBs, PAHs, and selected metals, and 3) ancillary measures to determine whether these chemicals were present at toxic concentrations. At a subset of sites, we compared toxicity and chemical data with benthos community data that were collected in 2014 as part of an earlier USGS study.




We sampled in two AOCs and four non-AOCs on the western shore of Lake Michigan.

Sampling in each AOC and non-AOC included several subsites, shown by the green markers in each image above.

## Field Collection Methods

A sonde was used at each site to collect beginning and ending measurements for:

- Water temperature
- pH
- Dissolved oxygen
- Specific conductance

At each site, a Ponar dredge and winch was used to collect from 3 to 15 sediment grab that were composited in a clean plastic cooler

A subsample was collected for:

- **Total mercury**

The sample was homogenized. Subsamples were collected for:

- **PCBs and Arochlors**
- **PAHs**
- **Metals - Total and Simultaneously-Extracted (SEM)**
- **Organic carbon and loss-on-ignition**
- **Particle size**

## Preliminary Results

### STATUS:

All laboratory analyses and data reviews are complete. We are analyzing the results. A final report is in preparation and planned for completion in 2018.

### PRELIMINARY RESULTS:

***This information is preliminary and is subject to revision. It is being provided to meet the need for timely best science. The information is provided on the condition that neither the U.S. Geological Survey nor the U.S. Government shall be held liable for any damages resulting from the authorized or unauthorized use of the information.***

- Midge toxicity showed low severity (1 endpoint) in both AOC and non-AOC samples
- Amphipod toxicity was more severe; restricted to AOC samples
- Trends in sediment contaminants (organics, metals) corresponded to severity of amphipod toxicity

Results are shown for the following AOCs and non-AOC comparison study areas with one or more subsites, listed upstream to downstream:

**AOCs:**

- Sheboygan River AOC (SHEB)
- Milwaukee Estuary AOC subsites:
  - Kinnickinnic River (KK)
  - Menomonee River (MENO)
  - Milwaukee River (MILR)
  - Milwaukee Harbor (MILH)

**Non-AOCs:**

- Kewaunee River (KEWA)
- Manitowoc River (MANI)
- Oak Creek (OAK)
- Root River (ROOT)

### TOXICITY TESTING:

Summary of toxicity hazards with Probable Effects Quotients (PEQs) from sediments collected from Lake Michigan tributaries and harbors in Wisconsin, October 2016. [ --, not detected/computed]

| Site # | SITE | TOXICITY TESTS (1) | | ORGANIC ANALYSES (2) | | METAL ANALYSES (2,3) | | SUMMARY SCORE (4) |
|---|---|---|---|---|---|---|---|---|
| | | Midge | Amphipod | PAH-PEQ | PCB-PEQ | Metals-PEQ | SEM-AVS foc | |
| | **AOC STUDY AREAS** | | | | | | | |
| 3 | SHEB 3 | 0 | 0 | 0.08 | 1.0 | 1.1 | -60.1 | 2 |
| 2 | SHEB 2 | 0 | 0 | 0.15 | 1.0 | 1.4 | -32.7 | 2 |
| 1 | SHEB 1 (Ref) | 0 | 0 | 0.08 | 0.31 | 0.51 | -366.5 | 0 |
| 10 | KK 2 | 0 | 4 | 3.1 | 1.0 | 1.9 | -297.5 | 8 |
| 9 | KK 1 | 0 | 3 | 0.98 | 1.1 | 4.3 | -49.9 | 7 |
| 8 | MENO 2 | 1 | 5 | 1.7 | 0.21 | 2.7 | -268.9 | 9 |
| 7 | MENO 1 | 0 | 5 | 2.1 | 0.35 | 3.4 | -29.7 | 9 |
| 6 | MILR 3 | 1 | 0 | 0.73 | 1.4 | 0.86 | -87.4 | 2 |
| 5 | MILR 2 | 0 | 1 | 0.63 | 0.72 | 1.8 | -70.8 | 3 |
| 4 | MILR 1 | 1 | 0 | 1.1 | 1.1 | 2.8 | -23.7 | 5 |
| 11 | HARBOR | 0 | 1 | 1.1 | 1.1 | 2.3 | 85.4 | 5 |
| | **NON-AOC STUDY AREAS** | | | | | | | |
| 17 | KEWA 2 | 1 | 0 | 0 | 0.01 | 1.1 | -53.4 | 2 |
| 16 | KEWA 1 | 0 | 0 | 0.004 | 0.01 | 1.2 | -- | 1 |
| 13 | MANI 2 (Ref) | 0 | 0 | 0.04 | 0.03 | 0.34 | -- | 0 |
| 12 | MANI 1 | 0 | 0 | 0.28 | 0.14 | 1.6 | -101.0 | 2 |
| 19 | OAK 2 | 0 | 0 | 0.40 | 0.05 | 0.88 | -- | 0 |
| 18 | OAK 1 | 0 | 0 | 0.68 | 3.21 | 2.1 | -439.1 | 6 |
| 15 | ROOT 2 | 0 | 0 | 0.55 | 0.03 | 0.86 | -310.9 | 0 |
| 14 | ROOT 1 | 0 | 0 | 0.39 | 0.13 | 2.0 | -124.0 | 3 |

(1) Sediment toxicity scores are the numbers of endpoints affected by each sediment, relative to controls and reference sites

(2) Toxicity hazards for total PAH, total PCB, and 6 metals estimated from Probable Effect Quotients (PEQ). PEQs for chemicals were computed by dividing the dry weight sediment concentration of each chemical by its respective consensus-based Probable Effect Concentration (PEC; MacDonald *et al.*, 2000, Arch. Environm. Contam. Toxicol. 39: 20-31)

(3) Metal bioavailability scores based on sediment quality benchmarks based on Simultaneously-Extracted Metals (SEM) normalized by Acid-Volatile Sulfide (AVS) and the fraction of organic carbon in the sediment ([SEM-AVS]/foc; USEPA, 2005)

(4) Summary scores (sums of scores for toxicity, organics, and metals) were used to categorize sediment quality at each site

| Component score | Ranges of component values | | | Summary scores | Sediment quality group |
|---|---|---|---|---|---|
| | Toxicity | Sum-PEQ | SEM-AVS/foc | | |
| 0 | 0 | <1.0 | <0.0 | 0 - 1 | **Reference** |
| 1 | 1 | 1.0 - 1.5 | 0.0 - 130 | 2 - 4 | **Low hazard** |
| 2 | 2 | 1.5 - 2.0 | 130 - 3000 | 4 - 6 | **Intermediate hazard** |
| 3 | 3+ | >2.0 | >3000 | 7+ | **High hazard** |

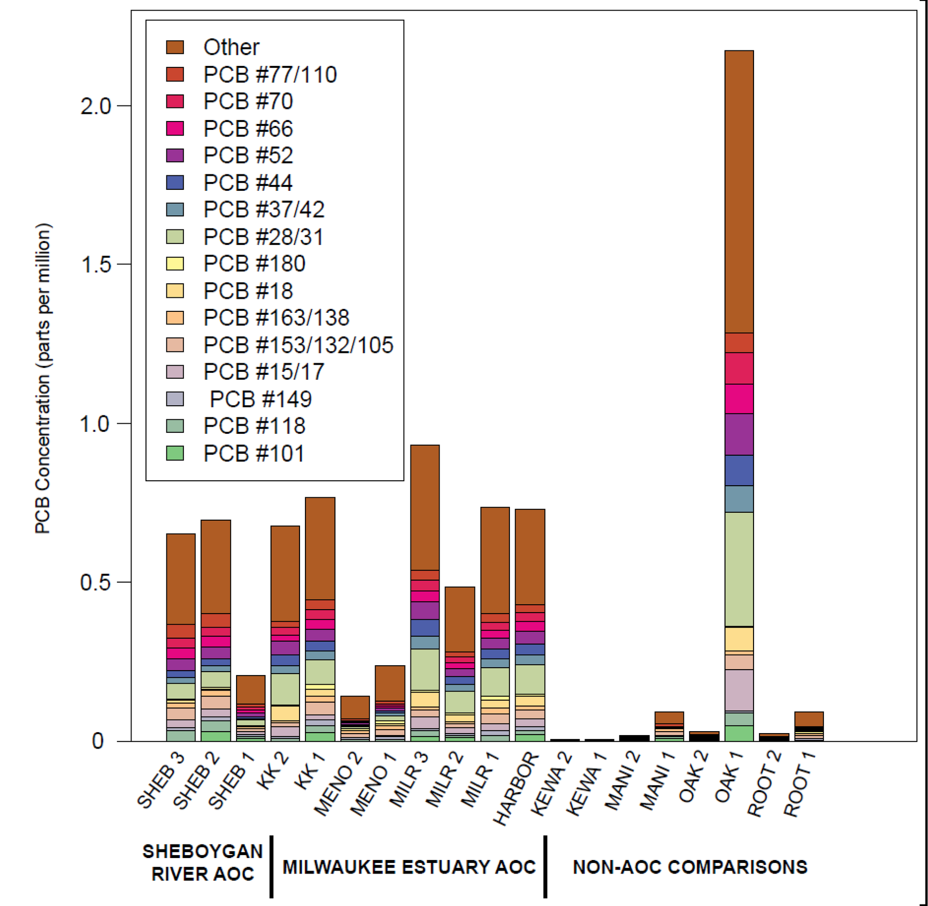
## Preliminary Results (continued)

### CHEMICAL CONCENTRATIONS IN SEDIMENT:

#### PCBs:

Most subsites in the Sheboygan River AOC and Milwaukee Estuary AOC had >10x higher total PCBs than all non-AOCs, except for the non-AOC OAK 1 (Oak Creek downstream at mouth on Lake Michigan).

PCB concentrations at AOCs were below 1 ppm but concentrations were above 2 ppm at OAK 1.

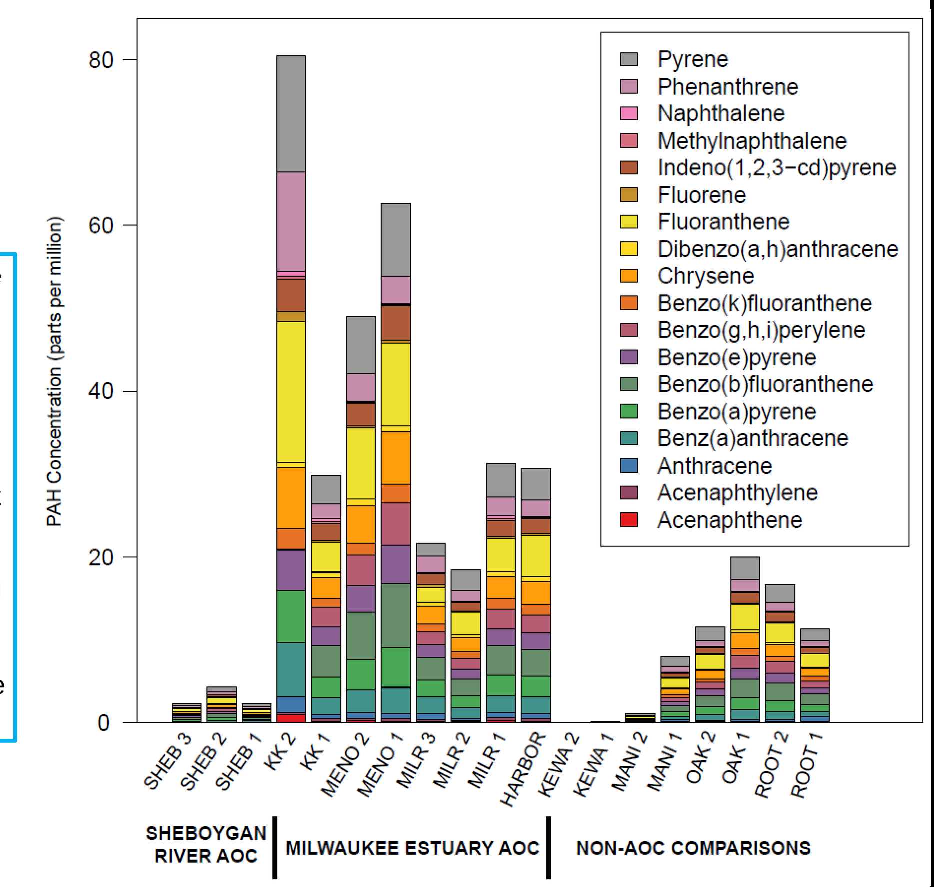



#### PAHs:

The highest PAH concentrations were found at subsites in the Milwaukee Estuary AOC at KK 2 (Kinnickinnic River) and at MENO 2 (Menomonee River upstream).

Lowest PAH concentrations were found in the Sheboygan River AOC at SHEB 1 (downstream at mouth) and at two non-AOCs, KEWA 1 and KEWA 2 (Kewaunee River) and MANI 2 (Manitowoc River upstream).

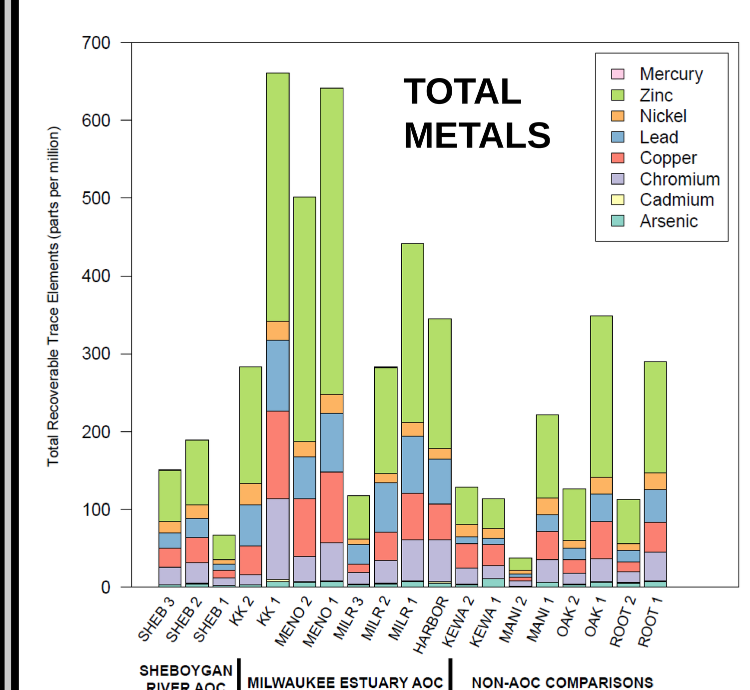
Analyses are in progress to determine source categories of PAHs.

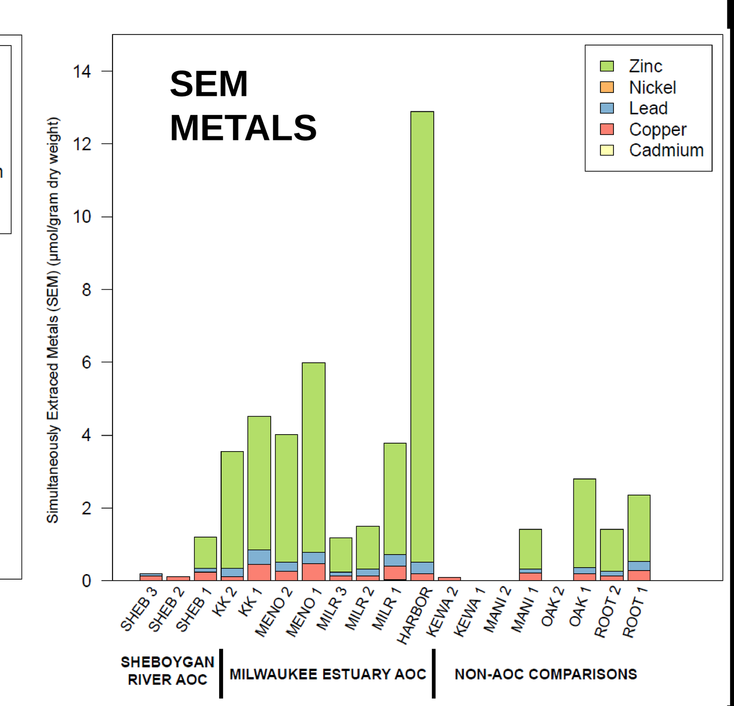



#### METALS:

Although the highest concentrations of total metals were found at KK 1 (Kinnickinnic River downstream) and MENO 1 (Menomonee River downstream), SEM and not total metals are more reflective of concentrations bioavailable to the benthos.

The highest overall concentrations of simultaneously-extracted metals (SEM) were found in the Milwaukee Estuary AOC, especially at the Milwaukee Harbor (HARBOR) because of high zinc concentrations.

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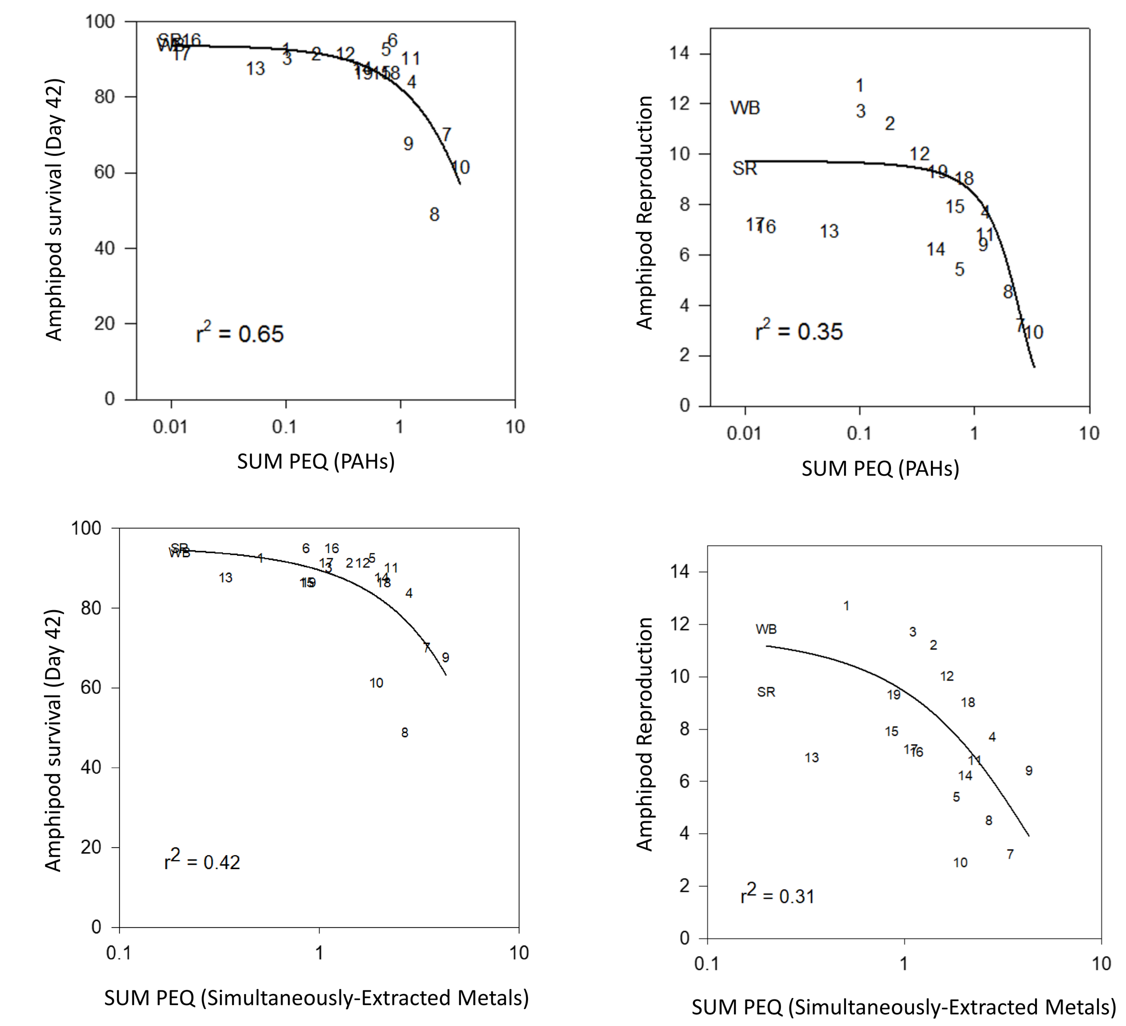
## Preliminary Results (continued)

### AMPHIPOD TOXICITY AND SEDIMENT CHEMISTRY:

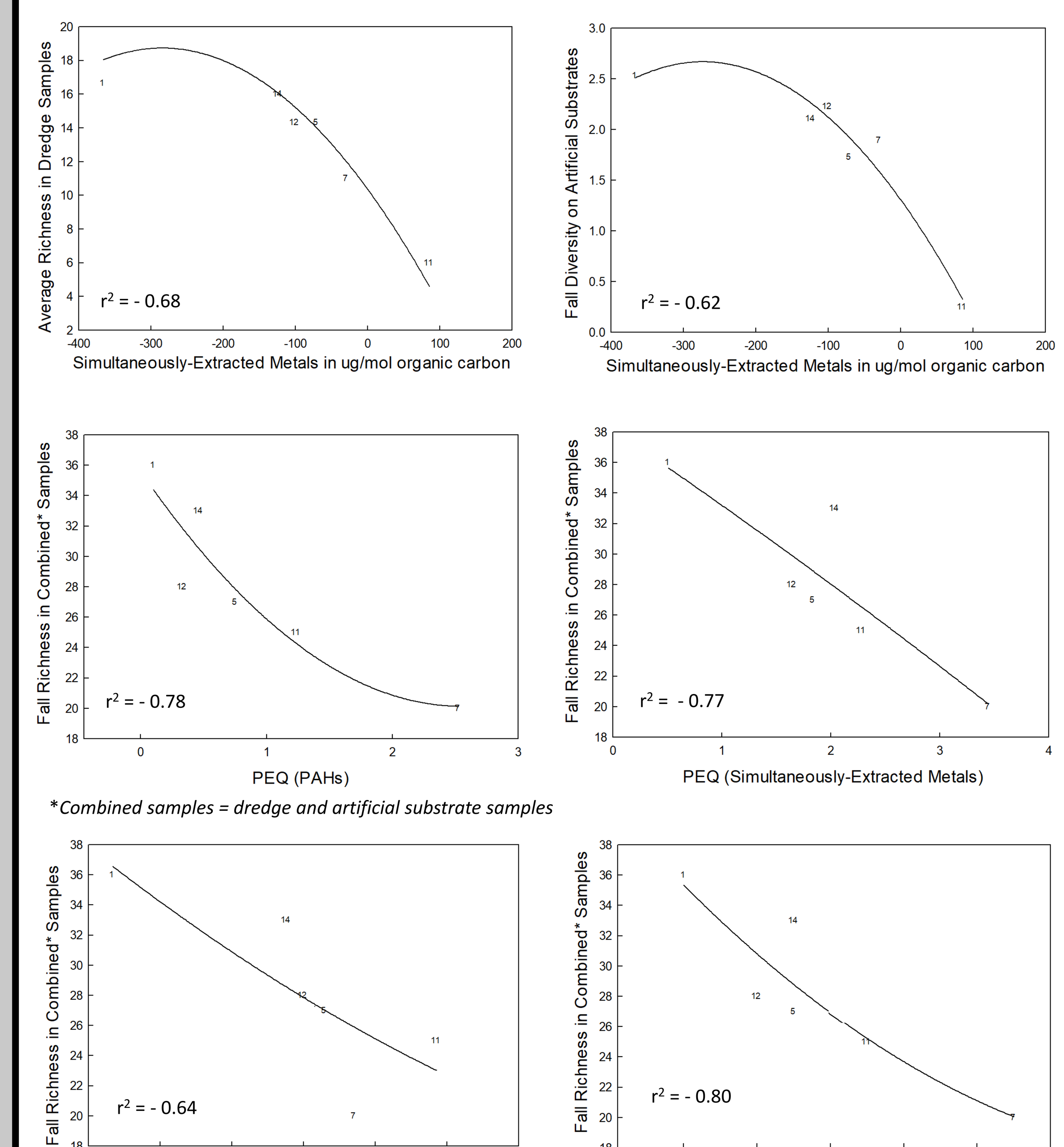
Amphipod survival and reproduction endpoints had strong associations with concentrations of total PAHs and simultaneously-extracted metals in sediments (site numbers* shown).




** WB and SR are reference sediments for comparison*

### BENTHOS, TOXICITY, AND CHEMISTRY:

Biological metrics for benthos sampled in 2014 correlated with metrics for toxicity and chemistry of sediment collected in 2016 (site numbers shown).




**Combined samples = dredge and artificial substrate samples*

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## Acknowledgments

This study was done in cooperation with the Wisconsin Department of Natural Resources, with Great Lakes Restoration Initiative funding through the Great Lakes National Program Office of the U.S. Environmental Protection Agency. Marsha Burzynski, Cheryl Bougie, Stacy Hron, James Killian, and Victor Pappas of the WDNR assisted with site selection.

# Appendix D – Verification Monitoring of Biological Communities and Physical Habitat in Select Streams within the Sheboygan River Area of Concern 2014-2016

# Verification Monitoring of Biological Communities and Physical Habitat in Select Streams within the Sheboygan River Area of Concern 2014-2016

John Masterson
Wisconsin Department of Natural Resources
Plymouth Service Center

August 2018

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## ABSTRACT

Aquatic surveys of the Sheboygan River Area of Concern (AOC), as well as tributaries within its project boundaries, were done in 2010 and 2011, prior to restoration work, to establish a baseline for biological and physical characteristics of these waters. Removal of contaminated sediment and habitat improvement projects within the Sheboygan River AOC were done in 2012 and 2013. Subsequently, verification monitoring was conducted in 2014 through 2016 to determine if dredging and habitat projects improved the water quality and biological integrity of the streams. Improvements to the biological community would be expected after removal of contaminated sediments and habitat restoration. Surveys included benthic macroinvertebrate, macrophyte communities, and stream habitat. Data derived from these surveys provide valuable information on the physical, chemical, and biological condition of streams. Aquatic plant surveys were done at one location to determine the potential to support northern pike spawning. Overall, the stream sites rated fair to excellent for invertebrate communities and stream habitat. There were a few sites that rated poor for invertebrate communities. These "poor" ratings may be attributed to degraded habitat. Aquatic plant surveys had low abundance and diversity. Overall, there were no significant changes among individual sites when comparing baseline and verification monitoring data (i.e. before and after restoration activities).

## INTRODUCTION

The Sheboygan River Area of Concern (AOC) encompasses the lower 14-miles of the Sheboygan River, downstream from the Sheboygan Falls Dam including the entire harbor and nearshore Lake Michigan. Areas of Concern (AOCs) are severely degraded geographic areas within the Great Lakes. These areas – 43 within the Great Lakes region – were designated as AOCs primarily due to contamination of river and harbor sediments by toxic pollutants. The Sheboygan River AOC is one of five Areas of Concern in Wisconsin.

It was designated as an AOC primarily due to polychlorinated biphenyl (PCB) and polycyclic aromatic hydrocarbon (PAH) contamination in Sheboygan River sediments. One primary source of PCBs was an industrial facility operated by Tecumseh Products Company; a primary source of PAHs was a manufactured gas plant (MGP) operated by Wisconsin Public Service Corporation (WPSC) (WDNR 2016).

Cleaning up these severely degraded areas is a first step toward restoring the chemical, physical, and biological integrity of the lakes as required by the Great Lakes Water Quality Agreement. When the areas have been cleaned up to the point where they are not more degraded than other, comparable non-AOC areas, they are "delisted" as AOCs. Since designation as an AOC, much progress has occurred to address pollutant sources.

These sources of impairment led to designation of nine of the possible fourteen beneficial use impairments (BUIs) as applicable to the AOC (WDNR 2008). Two of the nine BUIs, “degradation of fish and wildlife populations” and” loss of fish and wildlife habitat”, are being addressed through monitoring and habitat improvement projects within the AOC.

Efforts to improve the Sheboygan River accelerated in 2010 when the United States Environmental Protection Agency (US EPA) selected the Sheboygan River AOC as a focus for BUI removal. Careful planning throughout 2011 led to a great deal of activity in 2012 to remove contaminated sediments and enhance navigation through dredging, enhance habitat, and assess the status of selected BUIs.

There were four dredging projects within the Sheboygan River AOC, and by the end of 2012, over 400,000 cubic yards of contaminated sediment were removed from the river. These dredging projects included two Superfund projects, a Great Lakes Legacy Act dredging project and a navigational dredging project designed by the Army Corps of Engineers. Approximately $5.7 million has been invested in habitat projects. Habitat projects were completed in 2015 and included in-stream structures for fish cover, vegetated buffer areas, shoreline stabilization, invasive species control, and wetland restoration (WDNR 2016a).

Monitoring efforts by Wisconsin Department of Natural Resources (WDNR) staff to assess fish and macroinvertebrate communities, aquatic macrophytes, and stream habitat were completed in 2016. Other monitoring efforts for aquatic and wildlife populations are ongoing. Macroinvertebrate data can be used in a variety of ways for making bioassessments (Ohio EPA 1987) (WDNR 2003) (Weigel 2003) (Weigel and Dimick 2011). Stream habitat surveys can provide valuable information indicating the support or cover for macroinvertebrates and fish (WDNR 2002) (WDNR 2007) (Simonson, et al. 1993).

Assessing the status of biological and physical conditions of the Sheboygan River AOC helped determine the current health of the ecosystem and aided in choosing habitat improvement projects that were best suited to improve the aquatic resource. Benthic macroinvertebrate assemblages and stream habitat were assessed to in 2010 and 2011 to determine baseline ecosystem health of select streams (WDNR 2013).

Stream assessments were redone in 2014, 2015 and 2016 to determine if removal of contaminated sediment and implementation of habitat improvement projects improved stream habitat and the biological community. Fish community surveys were done by Travis Motl, WDNR Fish Biologist, and results are in a separate report.

## MATERIALS AND METHODS

### Site Selection

During the site selection process in 2010 the exact locations of stream dredging and habitat improvement projects were not known. Site selection for pre-implementation monitoring was done to maximize spatial coverage of streams within the AOC area and include tributaries where fish passage existed. Four individual water bodies were chosen for the study and included the lower 14-miles of the Sheboygan River, from the confluence with Lake Michigan upstream to the Sheboygan Falls Dam; Willow Creek; Weeden Creek; and the Onion River, from the confluence with the Sheboygan River upstream to the Village of Hingham Dam. Sixteen individual sites were monitored for benthic macroinvertebrates and stream habitat, and one of these sites (SR 02) included a survey of the aquatic plant community (Figure 1 and Table 1).

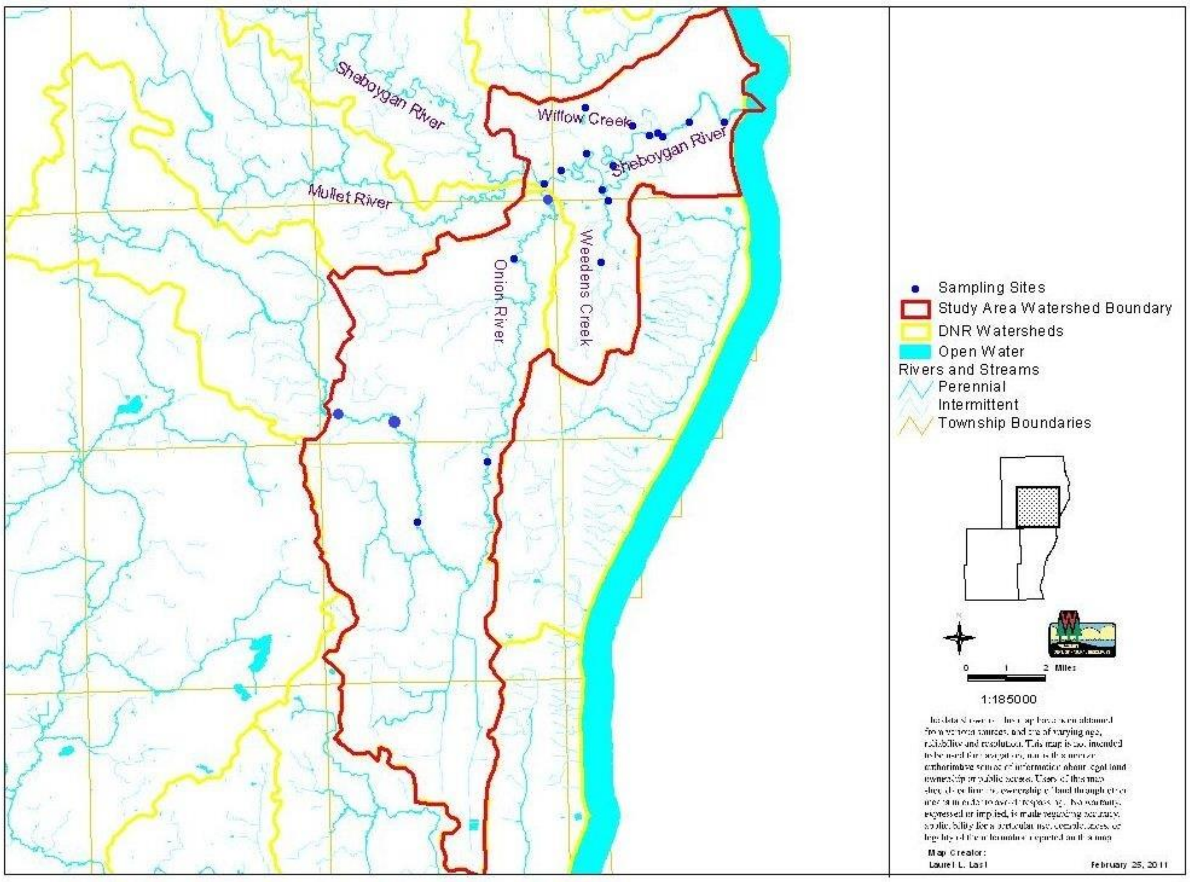


Figure 1. Sample site locations for benthic macroinvertebrates, stream habitat, and aquatic macrophyte surveys.

The Sheboygan River Watershed is the largest and possibly the most diverse watershed in the Sheboygan River basin, covering about 260 square miles. The Sheboygan River originates in east-central Fond du Lac County and flows generally southeastward into the City of Sheboygan where it enters Lake Michigan. The major tributaries to the Sheboygan River are the Onion and Mullet Rivers. There are approximately 10 dams in the watershed, which include Waelderhaus and Riverbend dams that are located within the Village of Kohler and the Sheboygan River AOC. Land use in the watershed is primarily agriculture, but the downstream most reaches are entirely urbanized.

Water quality is good in the headwaters and fair to poor in the lower reaches. Water and habitat quality were historically affected by contaminated sediments in the lower 14 miles of the river, agricultural and urban runoff, industrial and municipal wastewater treatment plant discharges, stream channelization, dams, and construction site erosion. These pollution sources lead to contaminated fish and wildlife populations (in the lower 14 miles), high stream turbidity, excess sediment, flashy flows, excess nutrients and nuisance algae, dissolved oxygen fluctuations, and fish migration barriers (WDNR 2001a).

The Weeden Creek Watershed originates in a large wetland and flows north through agricultural land interspersed with a few small woodlots before it enters the Sheboygan River within the Blackwolf Run golf course north of State Highway 28 in the Village of Kohler. Weeden Creek is 5.9 miles in length and is classified as a warm water forage fishery for its entire length (WDNR 2001). Land use in the watershed is primarily agriculture.

Factors limiting the creek's potential include fish kills, loss of wildlife habitat, loss of fish and invertebrate habitat, sedimentation, nutrients, and flashy flows. Sources include improper manure spreading, channelization, wetland drainage, cropland runoff, streambank erosion, drain tiles, and low flow. Streambank erosion and sedimentation are excessive in some areas and limits habitat for aquatic life (WDNR 2001a).

Willow Creek is located within the boundaries of the City and Township of Sheboygan, Village of Kohler and headwater areas within the Township of Sheboygan Falls. The stream is approximately 5.12 miles in length with a drainage basin of 4.22 square miles. Soil types in the watershed are glacial in origin and primarily consist of clays and hydric soils. Land use in the watershed is approximately 41% agricultural, 17% transportation, 16% open space, 15% residential, and 11% industrial/commercial.

There are portions of the headwaters that have been impacted from past development. This includes filling of wetlands, straightening of the stream channel for flood control, storm sewer discharges, thermal impacts, nutrient and sediment loading from nonpoint source runoff, and diversion of groundwater discharge to the stream. Past land use practices have degraded the water quality and biological integrity of Willow Creek. Future development in the watershed may further impact the stream. Willow Creek is classified as a Class II trout stream in the lower 1.6 miles. This section of the stream includes the areas immediately downstream of Interstate 43 to the confluence with the Sheboygan River. There is evidence of natural reproduction of coho salmon, chinook salmon and rainbow trout (WDNR 2006).

The Onion River Watershed covers 98 square miles and the river is 44 miles in length. The Onion River discharges to the Sheboygan River in Rochester Park in the City of Sheboygan Falls. Belgium Creek is the only major tributary to the Onion River. There are two dams on the Onion River, which form the Waldo and Hingham impoundments. The headwaters of the Onion River are a trout stream downstream to the top of the pool formed by the Waldo dam. The headwaters, including Ben Nutt Creek and Mill Creek, had been impacted by private fish ponds on major spring sources. Sections of these cold water reaches were restored and provide important spawning and rearing habitat for brown trout.  Land use in the watershed is primarily agricultural. The entire Village of Waldo, most of the Village of Belgium, and small portions of the Village of Cedar Grove and the City of Sheboygan Falls comprise the urban areas of the watershed.

Water quality in the Onion River Watershed ranges from excellent to good in the headwater areas to fair to poor in the lower sections. Sources of pollution degrading stream water quality are agricultural and urban runoff, and point source discharges. Streambank erosion, sedimentation and channelization limit stream habitat quality.  The upstream reaches, above the Village of Waldo impoundment, continue to exhibit excellent to good water quality, while the downstream reaches continue to be heavily affected by agricultural runoff (WDNR 2001).

Table 1.  Site locations and information for Sheboygan River AOC monitoring stations, Sheboygan County, Wisconsin.

| **Site** | **Stream** | **Location** | **Legal Description** | **Latitude Longitude*** | **Stream Order** |
|---|---|---|---|---|---|
| SR 01 | Sheboygan River | Upstream of 8$^{TH}$ Street. | T15N, R23E, Sec. 26, NE1/4 of NW1/4 | 43.74451 -87.71285 | 5 |
| SR 02 | Sheboygan River | Upstream of New Jersey Avenue. | T15N, R23E, Sec. 27, NE1/4 of NW1/4 | 43.74463 -87.73079 | 5 |
| SR 03 | Sheboygan River | Upstream of CTHY PP at Esslingen Park. | T15N, R23E, Sec. 28, SE1/4 of NW1/4 | 43.74027 -87.75094 | 5 |
| SR 04 | Sheboygan River | Upstream of Village of Kohler Municipal Garage. | T15N, R23E, Sec. 32, NE1/4 of NW1/4 | 43.72987 -87.76962 | 5 |
| SR 05 | Sheboygan River | Upstream of Weeden Creek Confluence. | T15N, R23E, Sec. 32, SW1/4 of SW1/4 | 43.72083 -87.77571 | 5 |
| SR 06 | Sheboygan River | Upstream of Walderhaus Dam. | T15N, R23E, Sec. 30, SE1/4 of SE1/4 | 43.73442 -87.78287 | 5 |
| SR 07 | Sheboygan River | Adjacent to Kohler Stables Property. | T15N, R23E, Sec. 31, NE1/4 of SW1/4 | 43.72825 -87.79589 | 5 |
| SR 08 | Sheboygan River | Upstream of Onion River Confluence. | T15N, R22E, Sec. 36, NW1/4 of SE1/4 | 43.72372 -87.80483 | 5 |
| WC 01 | Willow Creek | Upstream of confluence with Sheboygan River. | T15N, R23E, Sec.28, SW1/4 of NE1/4 | 43.74105 -87.74696 | 2 |
| WC 02 | Willow Creek | Upstream of Greendale Road. | T15N, R23E, Sec.28, NW1/4 of NW1/4 | 43.74423 -87.75937 | 1 |
| WC 03 | Willow Creek | Upstream of Woodlake Road. | T15N, R23E, Sec.19, SE1/4 of SE1/4 | 43.75103 -87.78274 | 1 |
| WE 01 | Weeden Creek | Upstream STHY 28 | T14N, R23E, Sec. 05, NE1/4 of NW1/4 | 43.71708 -87.77284 | 3 |
| WE 02 | Weeden Creek | Upstream of CTHY A | T14N, R23E, Sec. 08, NW1/4 of SW1/4 | 43.69432 -87.77714 | 3 |
| OR 01 | Onion River | Upstream of Ourtown Road. | T14N, R22E, Sec. 11, SE1/4 of SW 1/4 | 43.69667 -87.82086 | 4 |
| OR 02 | Onion River | Upstream of CTHY A | T13N, R22E, Sec. 02, NW1/4 of SW1/4 | 43.62282 -87.83698 | 4 |
| OR 03 | Onion River | Upstream of CTHY W | T14N, R22E, Sec. 32, SE1/4 of NE1/4 | 43.63817 -87.88370 | 3 |

* WGS 84 Datum

Contaminated sediment removal and habitat improvement projects were completed after baseline monitoring sites were selected and surveyed in 2010 and 2011. All dredging and habitat projects were limited to the lower 14-miles of the Sheboygan River. Verification monitoring was repeated at each of the 16 sites in 2014, 2015, and 2016, to determine if management projects improved the water quality, biological community and habitat of the Sheboygan River and select tributaries. Figure 2 shows the Sheboygan River AOC boundary and Figure 3 shows sample locations on the Sheboygan River in relation to the contaminated sediment removal and habitat improvement projects.

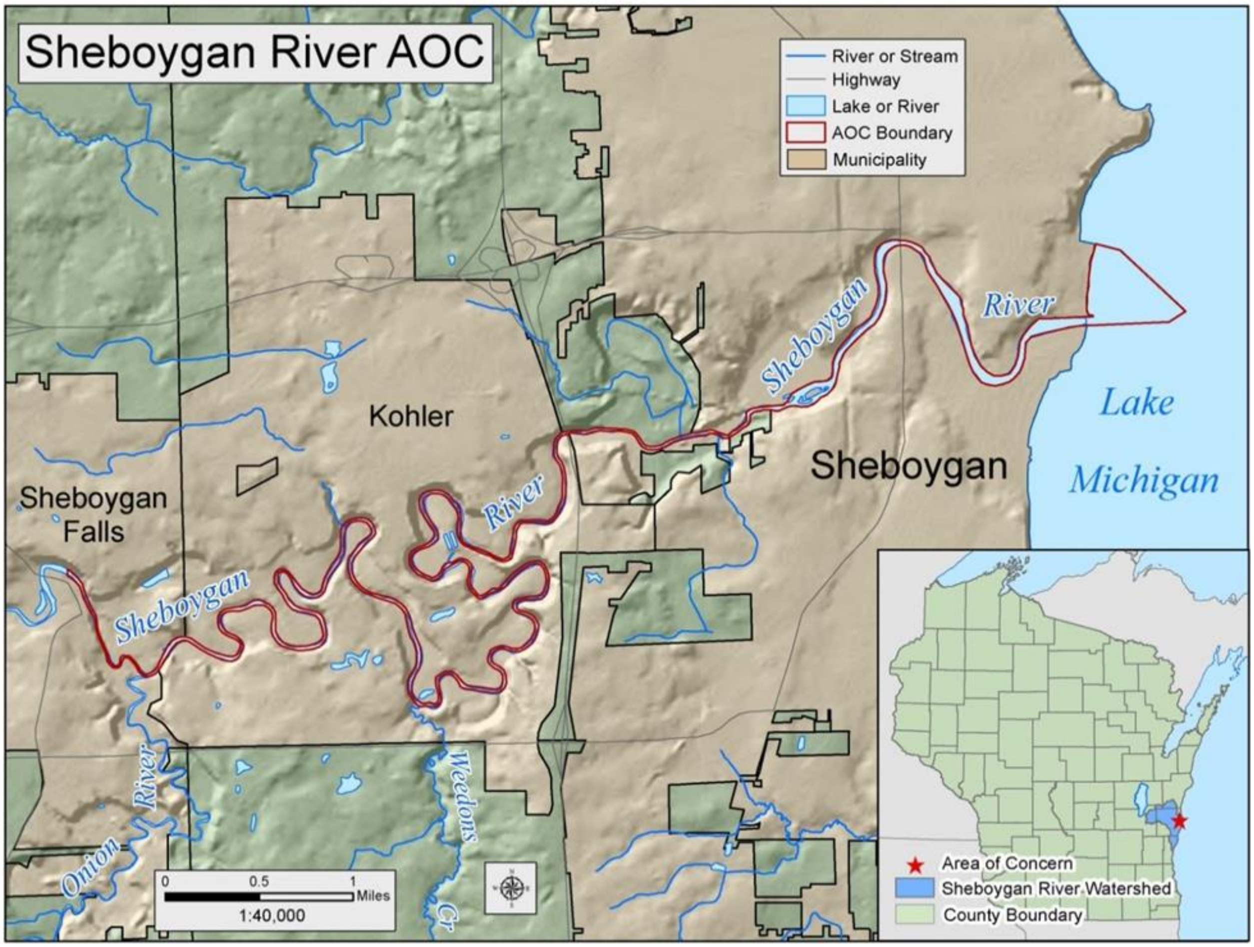


Figure 2. Location of the Sheboygan River AOC boundary, outlined in red.

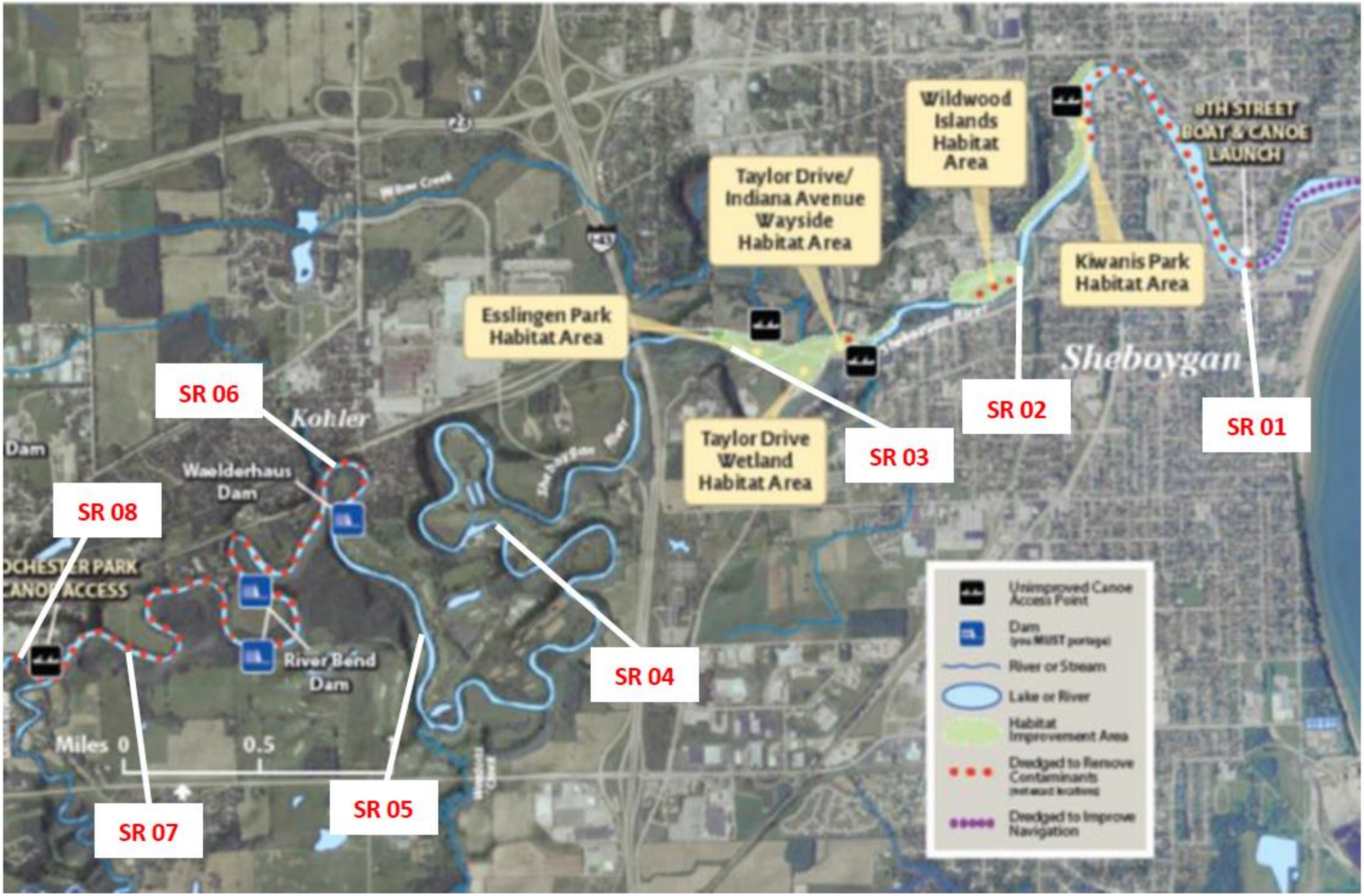


Figure 3. Location of sample sites (SR 01 – SR 08), dredging and habitat restoration projects on the Sheboygan River AOC.

**Benthic Macroinvertebrates Surveys**

Benthic macroinvertebrate communities are used as indicators of water quality. Most aquatic invertebrates are limited in mobility, so they are good indicators of localized conditions, upstream land use impacts and water quality (WDNR 2015a). Macroinvertebrates were collected using standard WDNR protocols for wadable streams (WDNR 2000). One sample was collected at each site using a D-framed kick net. Specimens were preserved in ethanol for later identification. Samples were collected during October and November of 2010, 2014, 2015 and 2016. Identification and enumeration of invertebrate taxa (generally genus and species) were done by the Benthic Invertebrate Laboratory at the University of Wisconsin – Stevens Point, Stevens Point, Wisconsin and the University of Wisconsin – Superior, Superior, Wisconsin. Taxonomic data were used to calculate several standard biotic indices.

One site (SR 01) was nonwadeable and the following sampling approach was used for this site (Weigel and Dimick 2011). We collected macroinvertebrates using modified Hester-Dendy (H-D) artificial substrate samplers during summer 2011, basing sampler construction and deployment following Ohio EPA (1987) (WDNR 2015). Each sampler used an eyebolt to hold eight 7.6 cm x 7.6 cm (3 inch x 3 inch) plates made of 3.2 mm (1/8 inch) thick masonite hardboard. Spacing between the plates allowed for colonization; spacing was 3.2 mm between each of the first three plates, 6.4 mm between each of the next three plates, and 9.6 mm between the last two plates. We fastened three samplers to an 18 kg cinder block and suspended it 1.5 m below the water surface, at low flow. The sampler was suspended by a rope off a wooden piling upstream of the bridge crossing. We avoided placement of the samplers on the bottom substrate so the device would not be inundated with sediment, for example, shifting sand or soft substrates. Velocity should be 0.09 - 0.5 m/sec. Samplers were placed to maintain 0.75 – 1.5 m of water above the sampler at low flow. Samplers were left to colonize macroinvertebrates for six-weeks within the window from mid-June through September. After six weeks, we retrieved the samplers, scraped/rinsed off the organisms, combined the sample contents, and preserved them in ethanol. All samples were delivered to the lab for identification and enumeration.

Water quality was assessed at 16 sites by examining the biological communities and their characteristics, such as number of individuals, number and types of taxa, pollution tolerance, and other traits. Computed metrics for invertebrate samples included the number of invertebrate taxa, Shannon Diversity Index, the percentage of invertebrate individuals or genera in the orders Ephemeroptera-Plecoptera-Trichoptera (EPT), (also known as mayflies, stoneflies and caddisflies) and family of Chironomidae, the Macroinvertebrate Index of Biotic Integrity (M-IBI) and Hilsenhoff's Biotic Index (HBI). Assemblage information and metrics for invertebrate samples were provided in the BUG database from the Benthic Invertebrate Laboratory at the University of Wisconsin – Stevens Point, Stevens Point, Wisconsin (Lillie et al. 2003)

The biotic indices used to assess invertebrate assemblages were the Wadeable Stream M-IBI developed by Weigel (2003) for the wadable sites, and the River M-IBI for the one nonwadeable river site (SR 01) (Weigel and Dimick 2011). Macroinvertebrate IBI values can range from 0.0 (“very poor” water quality) to 10.0 (“excellent” water quality) for the Wadeable Stream M-IBI and 0 (“poor”) to 100 (“excellent”) for the nonwadeable River M-IBI (Tables 2 & 3).

The wadeable M-IBI is composed of various metrics used to interpret macroinvertebrate sample data. The following metrics are included in the wadeable M-IBI:

o Species richness
o Ephemeroptera–Plecoptera– Trichoptera (EPT)
o Mean Pollution Tolerance Value
o Proportion of Depositional Taxa
o Proportion of Diptera
o Proportion of Chironomidae
o Proportion of Shredders
o Proportion of Scrapers
o Proportion of Gatherers
o Proportion of Isopoda
o Proportion of Amphipoda

For the nonwadeable River M-IBI, there are ten metrics that represent macroinvertebrate assemblage structure, composition, and function that constitute the IBI:

o Number of Insecta taxa
o Number of EPT taxa
o Proportion of Insecta individuals
o Proportion of intolerant EPT individuals
o Proportion of tolerant Chironomidae individuals
o Proportion of gatherer individuals
o Proportion of scraper individuals
o Proportion of individuals from the dominant 3 taxa
o Mean Pollution Tolerance Value
o Number of unique functional trait niches

The HBI is another aquatic macroinvertebrate biotic index that has been historically used by the WDNR and is still in use. It was designed to assess oxygen depletion in streams resulting from organic matter pollution (Hilsenhoff 1987). However, the HBI may also be sensitive to other types of pollution, such as from certain chemicals. The HBI represents the number of arthropod macroinvertebrates in certain genus or species, multiplied by their respective pollution tolerance score, divided by the number of arthropods in the sample. HBI values can range from 0.00 (excellent water quality) to 10.00 (very poor water quality) (Table 4).

We analyzed macroinvertebrate IBI scores by combining all sites within a river (SR, OR, WC & WE) and comparing percent change in mean IBI scores between the before and after restoration time periods. We assessed changes statistically using a two-way ANOVA comparing differences among time periods (before-after), among rivers, and if the difference among time periods depends on the river (time times river interaction effect).

Instantaneous water quality data was recorded during the collection of all benthic macroinvertebrate samples. Data was collected using a Hydrolab DS5 - Multiparameter Data Sonde. Water quality parameters included water temperature, dissolved oxygen and percent saturation, pH and conductivity. Transparency was also recorded using a clear, plastic, turbidity tube that was 120 cm in height.

Table 2. Condition category thresholds for wadeable stream Macroinvertebrate Index of Biotic Integrity (M-IBI) (Weigel, 2003).

| Wadeable Stream M-IBI Thresholds | Condition Category |
|---|---|
| > 7.5 | Excellent |
| 5.0-7.4 | Good |
| 2.5-4.9 | Fair |
| < 2.5 | Poor |

Table 3. Condition category thresholds for nonwadeable river Macroinvertebrate Index of Biotic Integrity (M-IBI) (Weigel & Dimick 2011).

| River M-IBI Thresholds | Condition Category |
|---|---|
| >75 | Excellent |
| 50-75 | Good |
| 25-49 | Fair |
| <25 | Poor |

Table 4. Water quality ratings for Hilsenhoff Biotic Index (HBI) values (Hilsenhoff, 1987).

| **HBI value** | **Water quality rating** | **Degree of organic pollution** |
|---|---|---|
| 0.00-3.50 | Excellent | No apparent organic pollution |
| 3.51-4.50 | Very Good | Possible slight organic pollution |
| 4.51-5.50 | Good | Some organic pollution |
| 5.51-6.50 | Fair | Fairly significant organic pollution |
| 6.51-7.50 | Fairly Poor | Significant organic pollution |
| 7.51-8.50 | Poor | Very significant organic pollution |
| 8.51-10.00 | Very Poor | Severe organic pollution |

## Habitat Assessment

Stream habitat was evaluated at 16 sites using qualitative procedures (WDNR 2007) during the summer or autumn of 2011, 2014, 2015 and 2016. Seven different variables for stream less than 10 meters wide are visually estimated for qualitative habitat assessment. Each habitat parameter is given a rating of excellent, good, fair, or poor, and the associated individual numeric scores are summed to provide an overall rating of stream habitat quality. Variables measured included riparian buffer width, bank erosion, pool area, width:depth ratio, riffle:riffle or bend:bend ratio, fine sediment, and cover for fish. For streams greater than 10 meters wide, variables measured included bank stability, maximum thalweg depth, riffle:riffle or bend:bend ratio, rocky substrate, and cover for fish.

## Aquatic Macrophytes Surveys

One aquatic plant survey was done in 2011, 2014, 2015 and 2016 at site SR 02 using the point-intercept (PI) method protocol (Hauxwell et al. 2010). The PI method was designed for lake surveys, so the method was slightly modified for use on this section of the Sheboygan River. Monitoring was done on 106 sample points, spaced 20 meters apart. Sample points were identified using GPS (Figure 4). Depth, substrate type, aquatic plant species, and individual species density (rake fullness) were recorded at each sample point.

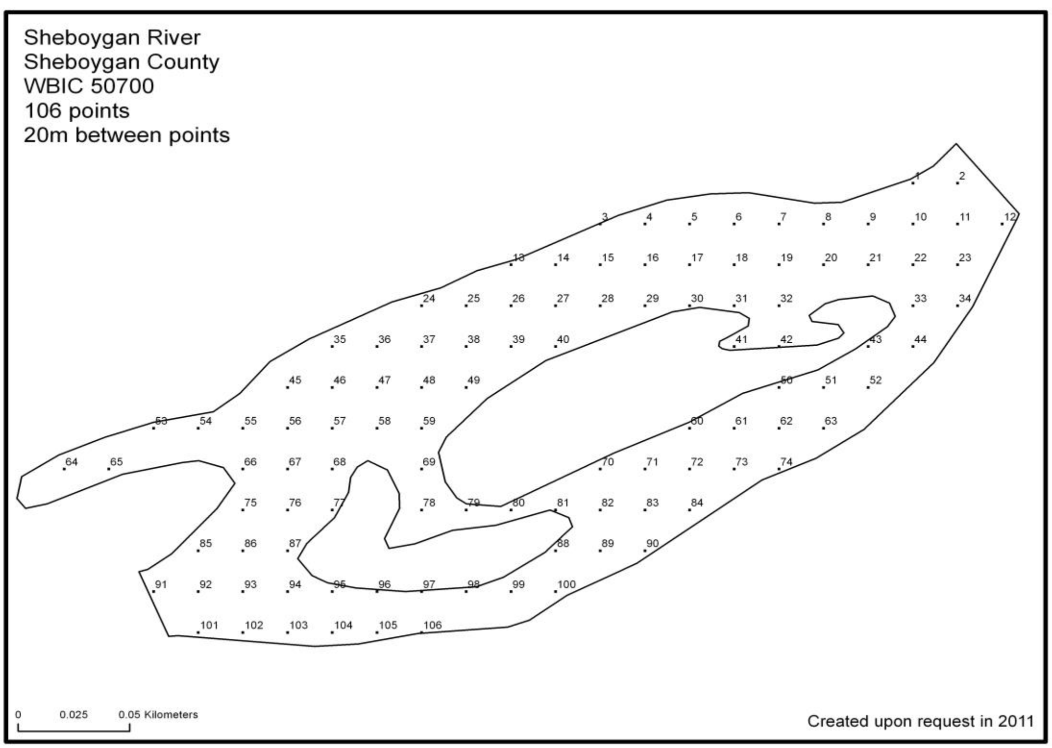


Figure 4. Aquatic plant survey sample locations, using point-intercept method, for Wildwood Island Area on the Sheboygan River (SR 02), Sheboygan, Wisconsin.

## RESULTS AND DISCUSSION

### Benthic Macroinvertebrates

M-IBI ratings for all sites ranged from “Poor” to “Excellent” (Table 5) (Figure 5). There was some annual variability among individual sites. This may be attributed to weather, time of sample collection, changes in stream habitat, and changes in the abundance and diversity of species present at the time of monitoring. The average rating of verification monitoring (2014-2016) was compared to the one year of baseline monitoring (2010 or 2011) to determine changes in M-IBI ratings before and after dredging and habitat improvements on the Sheboygan River.

The Sheboygan River site, SR 01, had a “Poor” ratings for baseline and verification monitoring. SR 02 rated “Fair” for both baseline and verification monitoring. SR 03, SR 04, SR 05, and SR 08 rated “Fair” for baseline monitoring and “Good” for the average rating for verification monitoring. SR 06 rated “Excellent” for baseline monitoring and “Good” for verification monitoring. SR 07 rated “Good” for both baseline and verification monitoring. SR 03, SR 04, SR 05, and SR 08 did show improvements in their ratings. These sites do have good to excellent habitat with significant riffle areas (Table 8). SR 06 is located between the two dams in the Village of Kohler. Some dredging did occur here, but habitat improvement projects were not done at this site. The decrease in score and rating may be due to annual variability. The “Poor” ratings for SR 01 are most likely due to poor habitat conditions. SR 01 is located near the mouth of the Sheboygan River and lacks shoreline habitat and the substrate is dominated by fine sediment.

Willow Creek sites WC 01 and WC 03 rated ‘Fair” for both baseline and verification monitoring. WC 02 rated “Good” for baseline monitoring and “Fair” for verification monitoring. The score was 5.4 for baseline monitoring and 4.87 for average of verification monitoring. This difference is score is not significant and is most likely due to annual variability.

Weeden Creek WE 01 and WE 02 rated “Fair” for both baseline and verification monitoring. Differences in scores between baseline and verification monitoring for WE 01 and WE 02 were minimal. WE 02 had a “Poor” rating in 2016 that may be associated with a manure runoff complaint that occurred approximately one week prior to sample collection.

Onion River sites OR 01, OR 02, and OR 03 all showed some improvement for their scores and ratings. OR 01 rated “Good” for baseline and verification monitoring. The score increased from 6.59 to an average of 7.10. OR 02 rated “Poor” for baseline monitoring and “Good” for verification monitoring. The score increased from 2.36 to an average of 5.20. OR 03 rated “Fair” for both baseline and verification monitoring. The score increased from 3.73 to an average of 4.30. Recent habitat or water quality improvement projects were not known to have occurred within the Onion River watershed before or during any of the monitoring for this project. Therefore, improvements in ratings for the Onion River are most likely due to annual variability.

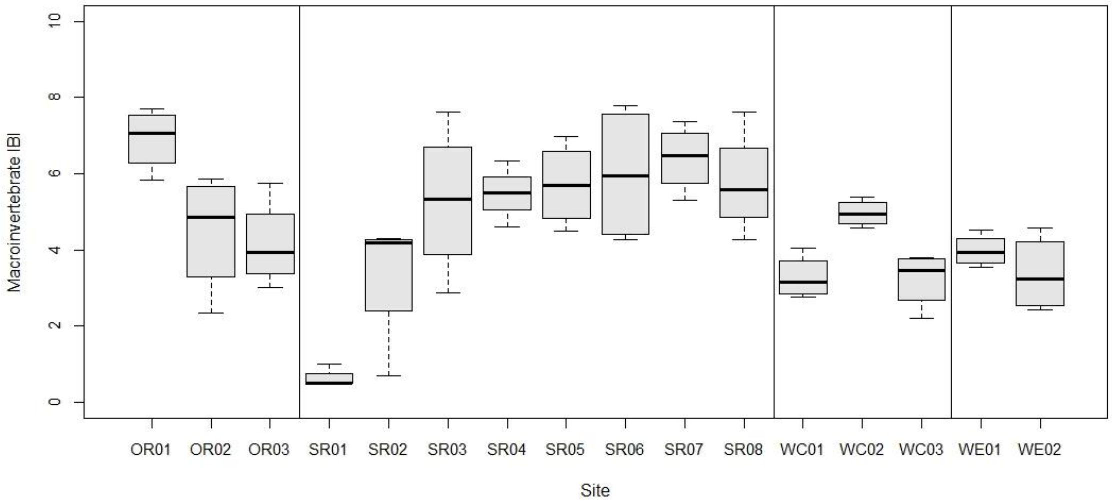


Figure 5. Macroinvertebrate IBI among all years summarized by each site, site codes are described in Table 1. SR01 (large river IBI) scores were divided by ten to standardize scale of all IBI scores.

We analyzed mean macroinvertebrate IBI scores from all sites, aggregated by river, and compared differences in the before-after datasets. Among all rivers we saw the greatest increase in mean IBI scores in the Onion River, a 21% increase (Figure 6A). The second largest increase was the Sheboygan River which increased by 17% (Figure 6B). Willow Creek and Weeden Creek each showed a slight decrease in mean macroinvertebrate IBI score between the before-after time periods, 7% and 1% decrease, respectively (Figures 6C & 6D). Although we saw an increase in IBI scores at two rivers there was no significant difference in IBI scores among time, river or time x river interaction effect (two-way ANOVA)

HBI ratings for all sites ranged from "Very Poor" to "Excellent". The Sheboygan River site (SR 01) rated "Very Poor" to "Fairly Poor" for all years sampled. The Sheboygan River site (SR 02) rated "Fairly Poor" to "Poor" for 2014, 2015, and 2016; SR 04 rated "Fairly Poor" in 2010; SR 06, Willow Creek (WC 01) and Weeden Creek (WE 02) rated "Poor" or "Fairly Poor" for all four years sampled; Weeden Creek (WE 01) rated 'Fairly Poor" in 2014; and the Onion River (OR 02) rated "Fairly Poor" in 2010. The overall poor ratings that occurred for all four sample years for SR 01, SR 02, SR 06, WC 03, and WE 02 can be associated with poor habitat conditions from stream channelization and sedimentation. Stream channelization is limited to sites WC 03 and WE 02. The one year of poor ratings for sites SR 04, WE 01, and OR 02, may be a result of annual variation among sample dates.

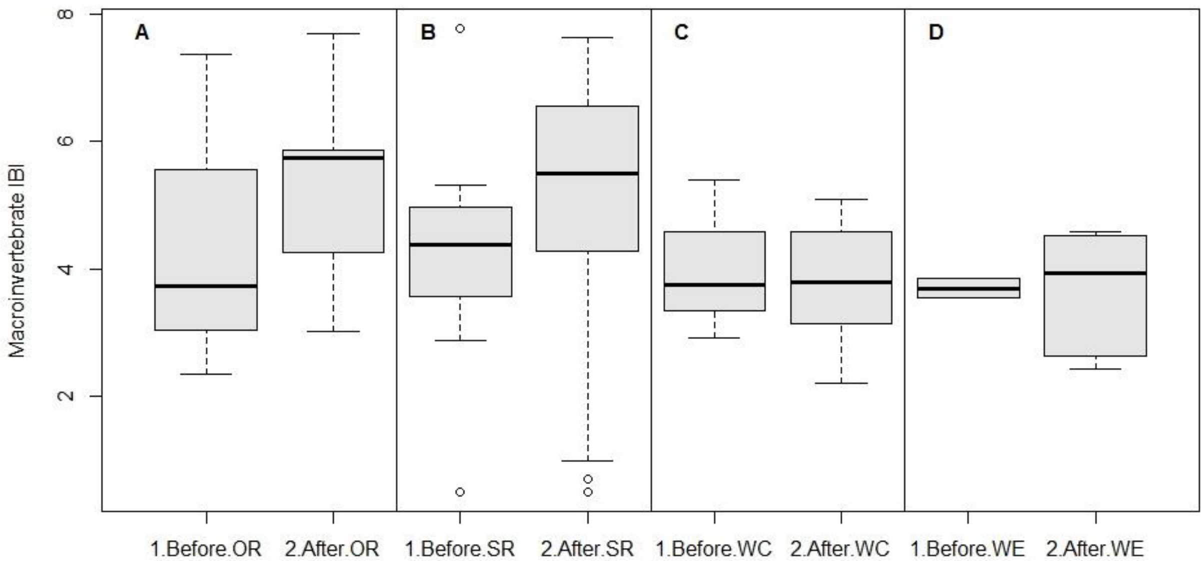


Figure 6. Macroinvertebrate IBI scores comparing before (2010 and 2011) and after (2014, 2015 & 2016) sampling time periods for each river in the study.

The number of taxa and Shannon Diversity Index (SDI) scores generally decrease with degrading water quality. For the 15 wadeable sites, the highest number of taxa (50 species and 46 genera) were found on the Onion River at OR 01 on one of the duplicate samples collected in 2010. The highest SDI was 4.93 on the Sheboygan River at SR 05 in 2016. The lowest number of taxa (13 species and 13 genera) were found on Willow Creek (WC 01) in 2015. The lowest SDI was 1.45 on Weeden Creek (WE 02) in 2010. For the one nonwadeable site (SR 01), taxa (31 species and 28 genera) and SDI (2.37) were highest in 2016. The lowest number of taxa (11 species and 11 genera) and SDI (0.47) occurred in 2014.

Higher numbers of taxa and diversity are typically found on larger streams compared to small headwater streams, if water quality and habitat conditions are in good condition on all sites. We do see this general trend for the data. Samples collected on Willow Creek (WC 01-03) and Weeden Creek (WE 01-02) had lower taxa and diversity compared to samples collected on the Sheboygan River (SR 02-08) and the Onion River (OC 01-03). Willow and Weeden Creeks are classified as headwater streams ($1^{st}$ to $3^{rd}$ order) and the Sheboygan and Onion Rivers are classified as mainstem streams or rivers ($3^{rd}$ to $5^{th}$ order).

EPT invertebrates are generally considered to be relatively intolerant of degraded water quality (Lenat 1988). Therefore, the percentages of EPT individuals tend to decrease as water quality degrades. The highest percentage of EPT taxa, 82 percent, were found on Willow Creek (WC 01) in 2010. The lowest percentage of EPT taxa were 0 percent for the Sheboygan River (SR 01) in 2011, 2014, 2015 and 2016; Sheboygan River (SR 02) in 2015; and Weeden Creek (WE 02) in 2016. The low percentages of EPT individuals for these three sites may be attributed to the fine sediments that dominate the stream substrate at SR 01 and SR 02 and the stream channelization and agricultural runoff that dominate the Weeden Creek site (WE 02). SR 02 and WE 02 did have annual variability among the sample dates, which can be expected. The Sheboygan River (SR 06) and Willow Creek (WC 03) also had low numbers of EPT individuals due to sedimentation and stream channelization.

Chironomid species are found in nearly all waterbodies, but are typically tolerant of degraded water quality (Lenat 1988). Higher percentage of Chironomidae individuals in a sample typically indicates poor water quality and habitat conditions. The Sheboygan River (SR 02) had the highest percentage, 97 percent, in 2015. Willow Creek (WC 02) had the lowest percentage, 0 percent, in 2010. Sites WC 02 and WE 01 are small headwater streams that have abundant cobble and gravel substrate, resulting in some of the lowest Chironomidae percentages for the four years of monitoring.

Values and ratings for taxa richness, diversity, M-IBIs and HBIs do vary between years that samples were collected. This can most likely be attributed to annual variability within macroinvertebrate communities as a result time of sample collection, changes in weather, water quality, and stream habitat conditions.

## Instantaneous Water Quality

Instantaneous water quality data was recorded at the same date and time as the benthic macroinvertebrate sample collection. Data was not outside the normal or standard levels for streams in the southeast region of Wisconsin (Table 6). Instantaneous values for dissolved oxygen (DO) or pH never exceeded thresholds established by WDNR (WDNR 2015a). Specific conductivity averages ~840 umhoms/cm among all sites, but was most elevated at Willow Creek with an average of 1159 umhoms/cm.

## Stream Habitat

Stream habitat is important when assessing the biological integrity of streams. The physical environment can play a key role in supporting fish and macroinvertebrate populations. Loss of fish cover and sedimentation can have severe impacts on biological communities. The fish habitat score and rating is intended to rate the ability of the physical habitat to support a diverse, healthy fish community (Simonson, et. al 1993).

All wadable sites rated “Fair” to “Excellent” (Tables 7 and 8). One site (SR 01) was nonwadeable and habitat assessment was not done because standard protocols and assessment methods are not currently available.

For most sites less than 10 meters wide, the limiting factor for habitat appears to be bank erosion, lack of pool areas, and fine sediments. Ranking for these sites were “Fair to “Good”. For stream sites that were greater than 10 meters wide, ranking ranged from “Fair” to “Excellent”. For sites that rated “Fair”, limiting factors for habitat were bank stability, riffle:riffle or bend:bend ratio, lack of rocky substrate and cover for fish.

Table 5. Benthic macroinvertebrate assemblage information from one-time surveys conducted in 2010, 2014, 2015 and 2016 at 16 stream sites within the Sheboygan River AOC. EPT, Ephemeroptera, Plecoptera, and Trichoptera; M-IBI, Macroinvertebrate Index of Biotic Integrity; HBI, Hilsenhoff Biotic Index; SR01 is a nonwadeable site with sample collected in 2011; * indicates duplicate samples for quality assurance.

| Site | Date | Species Richness | Genera Richness | % EPT Individuals | % Chironomidae Individuals | Shannon Diversity Index | M-IBI | Rating | HBI | Rating |
|---|---|---|---|---|---|---|---|---|---|---|
| SR01 | 2011-08-26 | 20 | 18 | 0 | 59 | 2.32 | 5 | Poor | 8.87 | Very Poor |
| | 2014- 10-01 | 11 | 11 | 0 | 7 | 0.47 | 5 | Poor | 8.44 | Poor |
| | 2015-10-02 | 17 | 16 | 0 | 3 | 0.24 | 10 | Poor | 7.44 | Fairly Poor |
| | 2016-09-30 | 31 | 28 | 0 | 35 | 2.37 | 5 | Poor | 8.7 | Very Poor |
| SR02 | 2010-10-28 | 37 | 36 | 41 | 39 | 4.32 | 4.28 | Fair | 5.8 | Fair |
| | 2014-11-04 | 23 | 22 | 3 | 79 | 3.34 | 4.29 | Fair | 6.7 | Fairly Poor |
| | 2015-11-06 | 16 | 16 | 0 | 97 | 2.91 | 0.7 | Poor | 7.67 | Poor |
| | 2016-11-11 | 20 | 19 | 2 | 81 | 3.07 | 4.09 | Fair | 7.08 | Fairly Poor |
| SR03 | 2010-10-28 | 19 | 18 | 81 | 17 | 2.95 | 2.88 | Fair | 5.16 | Good |
| | 2014-10-29 | 29 | 28 | 56 | 39 | 3.28 | 4.9 | Fair | 5.25 | Good |
| | 2015-11-06 | 33 | 31 | 55 | 27 | 4.30 | 5.75 | Good | 4.68 | Good |
| | 2016-11-10 | 29 | 28 | 78 | 8 | 3.38 | 7.62 | Excellent | 4.33 | Very Good |
| SR04 | 2010-10-28 | 26 | 25 | 19 | 25 | 3.78 | 4.61 | Fair | 7.05 | Fairly Poor |
| | 2014-10-30 | 34 | 33 | 79 | 12 | 3.46 | 5.49 | Good | 3.35 | Excellent |
| | 2015-11-11 | 29 | 28 | 29 | 63 | 4.13 | 6.33 | Good | 5.25 | Good |
| | 2016-11-11 | 29 | 28 | 60 | 9 | 3.77 | 5.49 | Good | 4.3 | Very Good |
| SR05 | 2010-11-10 | 39 | 37 | 66 | 24 | 3.07 | 4.49 | Fair | 5.32 | Good |
| | 2014-11-04 | 40 | 36 | 50 | 26 | 4.49 | 6.21 | Good | 4.39 | Very Good |
| | 2015-11-13 | 25 | 24 | 32 | 57 | 3.95 | 5.17 | Good | 5.2 | Good |
| | 2016-11-14 | 44 | 44 | 48 | 36 | 4.93 | 6.97 | Good | 5.07 | Good |
| SR06 | 2010-11-10 | 46 | 45 | 11 | 40 | 4.37 | 8.72 | Excellent | 7.57 | Poor |
| | 2014-10-30 | 35 | 33 | 6 | 61 | 4.08 | 7.32 | Good | 6.63 | Fairly Poor |
| | 2015-11-11 | 22 | 21 | 1 | 71 | 3.21 | 4.26 | Fair | 7.48 | Fairly Poor |
| | 2016-11-11 | 26 | 25 | 1 | 50 | 3.68 | 4.55 | Fair | 7.29 | Fairly Poor |
| SR07 | 2010-11-10 | 27 | 26 | 64 | 31 | 3.21 | 5.01 | Good | 5.05 | Good |
| | 2010-11-10* | 31 | 29 | 41 | 49 | 3.62 | 5.62 | Good | 5.49 | Good |
| | 2014-10-30 | 29 | 28 | 58 | 24 | 3.90 | 6.69 | Good | 3.77 | Very Good |
| | 2014-10-30* | 33 | 30 | 59 | 18 | 3.92 | 6.84 | Good | 4.44 | Very Good |
| | 2015-11-11 | 36 | 35 | 28 | 39 | 4.44 | 5.08 | Good | 5.03 | Good |
| | 2015-11-11* | 36 | 35 | 25 | 50 | 4.71 | 7.29 | Good | 5.08 | Good |
| | 2016-11-14 | 35 | 33 | 43 | 22 | 4.23 | 7.38 | Good | 4.6 | Good |

Table 5. Benthic macroinvertebrate assemblage information from one-time surveys conducted in 2010, 2014, 2015 and 2016 at 16 stream sites within the Sheboygan River AOC. EPT, Ephemeroptera, Plecoptera, and Trichoptera; M-IBI, Macroinvertebrate Index of Biotic Integrity; HBI, Hilsenhoff Biotic Index; SR01 is a nonwadeable site with sample collected in 2011; * indicates duplicate samples for quality assurance - Continued.

| Site | Date | Species Richness | Genera Richness | % EPT Individuals | % Chironomidae Individuals | Shannon Diversity Index | M-IBI | Rating | HBI | Rating |
|---|---|---|---|---|---|---|---|---|---|---|
| SR08 | 2010-11-10 | 27 | 25 | 40 | 56 | 2.94 | 4.27 | Fair | 5.44 | Good |
| | 2014-10-30 | 35 | 34 | 53 | 30 | 4.25 | 7.62 | Excellent | 4.89 | Good |
| | 2015-11-11 | 37 | 33 | 40 | 54 | 4.50 | 5.44 | Good | 4.85 | Good |
| | 2016-11-11 | 33 | 33 | 52 | 11 | 4.44 | 5.7 | Good | 4.88 | Good |
| WC01 | 2010-10-28 | 15 | 15 | 82 | 9 | 2.55 | 2.92 | Fair | 4.13 | Very Good |
| | 2014-10-29 | 23 | 22 | 46 | 13 | 3.01 | 3.4 | Fair | 4.3 | Very Good |
| | 2015-10-20 | 13 | 13 | 36 | 15 | 2.35 | 2.76 | Fair | 4.11 | Very Good |
| | 2016-11-10 | 35 | 31 | 14 | 45 | 3.94 | 4.05 | Fair | 5.77 | Fair |
| WC02 | 2010-10-28 | 12 | 11 | 63 | 0 | 2.52 | 5.4 | Good | 4.24 | Very Good |
| | 2014-10-29 | 14 | 13 | 50 | 2 | 2.42 | 5.36 | Good | 4.06 | Very Good |
| | 2014-10-29* | 15 | 15 | 49 | 2 | 2.41 | 4.23 | Fair | 3.89 | Very Good |
| | 2015-10-20 | 14 | 14 | 22 | 2 | 2.65 | 4.59 | Fair | 5.42 | Good |
| | 2016-11-10 | 15 | 15 | 9 | 1 | 2.28 | 5.24 | Good | 4.49 | Very Good |
| | 2016-11-10* | 17 | 17 | 13 | 4 | 2.76 | 4.94 | Fair | 5.2 | Good |
| WC03 | 2010-10-28 | 32 | 32 | 1 | 15 | 2.78 | 3.76 | Fair | 7.71 | Poor |
| | 2014-10-29 | 21 | 21 | 1 | 68 | 3.18 | 2.2 | Poor | 6.87 | Fairly Poor |
| | 2015-10-20 | 24 | 24 | 5 | 38 | 3.59 | 3.8 | Fair | 7.71 | Poor |
| | 2016-11-10 | 28 | 27 | 1 | 81 | 3.48 | 3.15 | Fair | 6.82 | Fairly Poor |
| WE01 | 2010-10-28 | 17 | 16 | 44 | 3 | 2.62 | 3.55 | Fair | 4.86 | Good |
| | 2014-10-29 | 18 | 18 | 12 | 10 | 3.18 | 4.53 | Fair | 6.51 | Fairly Poor |
| | 2015-10-20 | 18 | 18 | 17 | 6 | 2.67 | 3.63 | Fair | 4.69 | Good |
| | 2015-10-20* | 17 | 17 | 6 | 9 | 2.47 | 3.91 | Fair | 4.94 | Good |
| | 2016-11-10 | 28 | 27 | 29 | 19 | 3.80 | 4.13 | Fair | 4.61 | Good |
| | 2016-11-10* | 29 | 29 | 25 | 26 | 3.88 | 4.06 | Fair | 4.78 | Good |
| WE02 | 2010-10-28 | 15 | 15 | 3 | 1 | 1.45 | 3.85 | Fair | 7.78 | Poor |
| | 2014-10-29 | 27 | 27 | 1 | 35 | 3.41 | 4.58 | Fair | 7.11 | Fairly Poor |
| | 2015-11-06 | 19 | 19 | 2 | 76 | 3.07 | 2.63 | Fair | 7.36 | Fairly Poor |
| | 2016-11-10 | 23 | 21 | 0 | 48 | 3.42 | 2.43 | Poor | 7.3 | Fairly Poor |

Table 5. Benthic macroinvertebrate assemblage information from one-time surveys conducted in 2010, 2014, 2015 and 2016 at 16 stream sites within the Sheboygan River AOC. EPT, Ephemeroptera, Plecoptera, and Trichoptera; M-IBI, Macroinvertebrate Index of Biotic Integrity; HBI, Hilsenhoff Biotic Index; SR01 is a nonwadeable site with sample collected in 2011; * indicates duplicate samples for quality assurance - Continued.

| Site | Date | Species Richness | Genera Richness | % EPT Individuals | % Chironomidae Individuals | Shannon Diversity Index | M-IBI | Rating | HBI | Rating |
|---|---|---|---|---|---|---|---|---|---|---|
| OR01 | 2010-11-03 | 33 | 31 | 63 | 24 | 3.95 | 6.59 | Good | 5.32 | Good |
| | 2010-11-03* | 50 | 46 | 49 | 38 | 4.23 | 8.16 | Excellent | 5.3 | Good |
| | 2014-10-29 | 40 | 38 | 40 | 51 | 3.94 | 7.69 | Excellent | 5.25 | Good |
| | 2015-10-16 | 31 | 29 | 51 | 35 | 4.23 | 6.72 | Good | 4.88 | Good |
| | 2016-11-11 | 27 | 26 | 30 | 8 | 4.02 | 5.84 | Good | 4.56 | Good |
| OR02 | 2010-11-03 | 31 | 31 | 28 | 54 | 3.79 | 2.36 | Poor | 6.68 | Fairly Poor |
| | 2014-10-30 | 28 | 28 | 36 | 55 | 3.51 | 5.49 | Good | 5.72 | Fair |
| | 2015-10-16 | 28 | 28 | 28 | 59 | 3.79 | 4.25 | Fair | 5.85 | Fair |
| | 2016-11-11 | 36 | 35 | 35 | 34 | 4.02 | 5.87 | Good | 5.75 | Fair |
| OR03 | 2009-10-29 | 23 | 23 | 30 | 59 | 2.84 | 3.73 | Fair | 4.97 | Good |
| | 2014-10-30 | 26 | 26 | 13 | 62 | 3.18 | 5.74 | Good | 5.6 | Fair |
| | 2015-10-16 | 28 | 28 | 28 | 59 | 3.79 | 3.02 | Fair | 4.69 | Good |
| | 2016-11-14 | 24 | 24 | 1 | 79 | 3.22 | 4.15 | Fair | 6.42 | Fair |

Table 6. Water quality and physical data for Sheboygan River AOC baseline (2010) and verification (2014-2016) monitoring. NA means Not Available. Baseline monitoring data collected in 2011 for SR01 and 2009 for OC03.

| Site | Date | Water Temp. (C) | D.O. (mg/L) | D.O. (% sat.) | pH (su) | Conductivity (umhos/cm) | Transparency (cm) | Water Color | Measured Velocity (m/s) | Average Stream Depth (m) | Average Stream Width (m) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SR01 | 2011-08-26 | 22.37 | 12.9 | 150.9 | 8.27 | 669.6 | 31 | Turbid | 0.09 | 3 | 75 |
| | 2014-10-01 | 22.11 | 12.8 | NA | 8.81 | 703 | NA | Turbid | 0.04 | 3.5 | 75 |
| | 2015-10-02 | 15.38 | 10.1 | NA | 8.16 | 698 | 120 | Stained | 0 | 3.5 | 75 |
| | 2016-09-30 | 16.1 | 9.22 | NA | 7.92 | 680 | NA | Turbid | 0.01 | 4 | 75 |
| SR02 | 2010-10-28 | 9.01 | 10.7 | 95.3 | NA | 699.2 | NA | Turbid | NA | 0.3 | 50 |
| | 2014-11-04 | 8.03 | 13.8 | 118 | 8.46 | 740 | 120 | Stained | 0.07 | 0.5 | 50 |
| | 2015-11-06 | 12.8 | 13.2 | 127.6 | 8.32 | 760 | 120 | Clear | NA | 0.4 | 50 |
| | 2016-11-11 | 9.19 | 13.3 | 117.4 | 8.29 | 745 | 120 | Stained | 0.19 | 0.3 | 50 |
| SR03 | 2010-10-28 | 8.99 | 10.9 | 97.1 | NA | 700 | NA | Stained | 0.61 | 0.4 | 30 |
| | 2014-10-29 | 10.9 | 13.1 | 121.7 | 8.52 | 743 | 120 | Stained | 1.09 | 0.4 | 30 |
| | 2015-11-06 | 12.9 | 12.6 | 122.2 | 8.36 | 752 | 120 | Clear | 1.04 | 0.3 | 30 |
| | 2016-11-10 | 9.66 | 12.7 | 113.4 | 8.22 | 739.6 | 120 | Stained | NA | 0.35 | 30 |
| SR04 | 2010-10-28 | 8.82 | 11.4 | 100.9 | NA | 708 | NA | Stained | 0.38 | 0.5 | 30 |
| | 2014-10-30 | 9.87 | 14 | 126.1 | 8.45 | 745 | 120 | Stained | 0.87 | 0.3 | 30 |
| | 2015-11-11 | 7.56 | 16.9 | 145.1 | 8.53 | 747 | 120 | Clear | 0.94 | 0.2 | 30 |
| | 2016-11-11 | 9.21 | 12.8 | 113 | 8.25 | 743 | 120 | Stained | 0.64 | 0.25 | 30 |
| SR05 | 2010-11-10 | 6.63 | 12.7 | 104.2 | 8.51 | 768 | NA | Clear | 0.32 | 0.6 | 40 |
| | 2014-11-04 | 7.99 | 12.5 | 108.2 | 8.21 | 727 | 120 | Stained | 0.31 | 0.25 | 40 |
| | 2015-11-13 | 7.14 | 11.9 | 100.5 | 8.01 | 751 | 120 | Clear | 0.66 | 0.3 | 40 |
| | 2016-11-14 | 7.06 | 13 | 109.2 | 8.21 | 740 | 120 | Stained | 0.76 | 0.25 | 40 |
| SR06 | 2010-11-10 | 7.27 | 13.5 | 112.1 | 8.63 | 768 | NA | Clear | 0.07 | 1.5 | 35 |
| | 2014-10-30 | 9.55 | 12.7 | 113.4 | 8.25 | 739 | 120 | Stained | 0.01 | 0.5 | 35 |
| | 2015-11-11 | 8.3 | 12 | 105 | 8.16 | 763 | 120 | Stained | 0.07 | 0.6 | 35 |
| | 2016-11-11 | 9.18 | 12.3 | 108.4 | 8.15 | 745 | 98 | Stained | 0.1 | 0.5 | 35 |
| SR07 | 2010-11-10 | 7.09 | 13.6 | 112.7 | 8.65 | 766 | NA | Clear | 0.61 | 0.5 | 35 |
| | 2014-10-30 | 9.09 | 13.5 | 120 | 8.27 | 742 | 120 | Stained | 0.27 | 0.3 | 35 |
| | 2015-11-11 | 7.01 | 14.4 | 121.7 | 8.19 | 760 | 120 | Clear | 0.38 | 0.2 | 35 |
| | 2016-11-14 | 6.93 | 13.6 | 113.5 | 8.21 | 738 | 120 | Stained | 0.88 | 0.2 | 35 |

Table 6. Water quality and physical data for Sheboygan River AOC baseline (2010) and verification (2014-2016) monitoring. NA means Not Available. Baseline monitoring data collected in 2011 for SR01 and 2009 for OC03 - Continued.

| Site | Date | Water Temp. (C) | D.O. (mg/L) | D.O. (% sat.) | pH (su) | Conductivity (umhos/cm) | Transparency (cm) | Water Color | Measured Velocity (m/s) | Average Stream Depth (m) | Average Stream Width (m) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SR08 | 2010-11-10 | 7.27 | 13.6 | 113.3 | 8.7 | 769 | NA | Stained | 0.27 | 0.6 | 20 |
| | 2014-10-30 | 8.79 | 12.8 | 112.4 | 8.18 | 736 | 120 | Stained | 0.46 | 0.4 | 20 |
| | 2015-11-11 | 6.94 | 14 | 118.6 | 8.42 | 768 | 120 | Clear | 0.54 | 0.4 | 20 |
| | 2016-11-11 | 8.57 | 12.6 | 110 | 8.22 | 734 | 120 | Stained | 0.49 | 0.2 | 20 |
| WC01 | 2010-10-28 | 7.88 | 10.8 | 93.2 | NA | 1092 | NA | Clear | 0.2 | 0.2 | 6 |
| | 2014-10-29 | 8.9 | 11.6 | 103 | 7.82 | 1373 | 120 | Clear | 0.42 | 0.15 | 6 |
| | 2015-10-20 | 12.8 | 10.2 | 100.3 | 8.12 | 1393 | 120 | Clear | 0.56 | 0.2 | 5 |
| | 2016-11-10 | 9.77 | 11.8 | 106 | 7.92 | 846 | 120 | Clear | 0.2 | 0.1 | 3.5 |
| WC02 | 2010-10-28 | 8.49 | 11.4 | 100.2 | NA | 1041 | NA | Clear | 0.42 | 0.2 | 4 |
| | 2014-10-29 | 9.36 | 11.6 | 103.6 | 8.09 | 1309 | 120 | Clear | 0.31 | 0.2 | 4 |
| | 2015-10-20 | 12.7 | 10.3 | 100.8 | 8.1 | 1296 | 120 | Clear | 0.47 | 0.2 | 2.5 |
| | 2016-11-10 | 10.5 | 11.1 | 101.9 | 7.78 | 1260 | 120 | Clear | 0.4 | 0.1 | 3.5 |
| WC03 | 2010-10-28 | 8.1 | 6.41 | 55.9 | NA | 1154 | NA | Clear | NA | 0.15 | 2.5 |
| | 2014-10-29 | 9.51 | 9.2 | 82.4 | 7.79 | 1242 | 120 | Clear | 0.07 | 0.1 | 2.5 |
| | 2015-10-20 | 14 | 1.11 | 11.2 | 7.65 | 1195 | 120 | Clear | 0 | 0.1 | 2.5 |
| | 2016-11-10 | 8.98 | 10.3 | 91.2 | 7.8 | 710 | 68 | Clear | 0.11 | 0.1 | 2 |
| WE01 | 2010-10-28 | 7.85 | 10.6 | 91.9 | NA | 901 | NA | Turbid | 0.23 | 0.2 | 3.5 |
| | 2014-10-29 | 9.68 | 11.8 | 106.4 | 8.24 | 950 | 120 | Clear | 0.23 | 0.2 | 3.5 |
| | 2015-10-20 | 14.2 | 10.5 | 106.4 | 8.24 | 900 | 65 | Turbid | 0.11 | 0.15 | 3 |
| | 2016-11-10 | 8.26 | 12.3 | 106.6 | 7.9 | 887 | 72 | Stained | 0.58 | 0.1 | 2 |
| WE02 | 2010-10-28 | 6.93 | 10.6 | 89.3 | NA | 1053 | NA | Clear | 0.11 | 0.1 | 2 |
| | 2014-10-29 | 9.32 | 9.38 | 83.6 | 7.86 | 972 | 120 | Stained | 0.3 | 0.2 | 2 |
| | 2015-11-06 | 11.1 | 10.9 | 101.8 | 7.35 | 1092 | 120 | Clear | 0.28 | 0.1 | 2.5 |
| | 2016-11-10 | 7.87 | 6.84 | 58.6 | 7.31 | 886 | 55 | Stained | 0.68 | 0.1 | 2 |
| OR01 | 2010-11-03 | 7.57 | 14.8 | 126.9 | 8.67 | 734 | NA | Clear | 0.52 | 0.25 | 25 |
| | 2014-10-29 | 10.1 | 12.1 | 110.6 | 8.43 | 781 | 120 | Clear | 0.61 | 0.3 | 25 |
| | 2015-10-16 | 9.53 | 11 | 100.2 | 7.8 | 653 | 120 | Clear | 0.58 | 0.2 | 25 |
| | 2016-11-11 | 8.34 | 12.7 | 109.8 | 7.98 | 771 | 120 | Clear | 0.79 | 0.2 | 25 |

Table 6. Water quality and physical data for Sheboygan River AOC baseline (2010) and verification (2014-2016) monitoring. NA means Not Available. Baseline monitoring data collected in 2011 for SR01 and 2009 for OC03 – Continued.

| Site | Date | Water Temp. (C) | D.O. (mg/L) | D.O. (% sat.) | pH (su) | Conductivity (umhos/cm) | Transparency (cm) | Water Color | Measured Velocity (m/s) | Average Stream Depth (m) | Average Stream Width (m) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| OR02 | 2010-11-03 | 6.71 | 13.7 | 115.4 | 8.41 | 727 | NA | Clear | 0.16 | 0.4 | 15 |
| | 2014-10-30 | 8.41 | 9.73 | 84.7 | 7.61 | 779 | 85 | Stained | 0.62 | 0.3 | 15 |
| | 2015-10-16 | 9.97 | 8.91 | 81.9 | 8.02 | 657 | 70 | Clear | 0.19 | 0.25 | 15 |
| | 2016-11-11 | 8.42 | 10.1 | 87.8 | 7.71 | 775 | 59 | Turbid | 0.55 | 0.35 | 15 |
| OR03 | 2009-10-29 | 9.98 | 10.04 | 91.4 | 8.43 | 732 | NA | Stained | 0.5 | 0.3 | 9 |
| | 2014-10-30 | 8.04 | 10.8 | 92.9 | 7.84 | 725 | 57 | Turbid | 0.49 | 0.25 | 9 |
| | 2015-10-16 | 10.7 | 10.6 | 99.4 | 8.3 | 611 | 120 | Clear | 0.63 | 0.2 | 9 |
| | 2016-11-14 | 6.55 | 12.9 | 106.2 | 8.19 | 713 | 120 | Clear | 0.62 | 0.35 | 9 |

Table 7. Qualitative stream habitat scores and ratings for streams < 10 meters wide.

| Site | Date | Flow (cfs) | Riparian Buffer | Bank Erosion | Pool Area | Width Depth | Riffle: Riffle Ratio | Fine Sediments | Fish Cover | Total Habitat Score | Habitat Rating |
|---|---|---|---|---|---|---|---|---|---|---|---|
| WC01 | 2011-08-05 | 0.81 | 15 | 5 | 3 | 5 | 10 | 10 | 5 | 53 | Good |
| | 2014-09-19 | 0.32 | 15 | 5 | 3 | 5 | 10 | 5 | 5 | 48 | Fair |
| | 2015-10-20 | 0.31 | 15 | 5 | 3 | 5 | 10 | 5 | 5 | 48 | Fair |
| | 2016-11-10 | 0.86 | 15 | 5 | 3 | 5 | 10 | 5 | 10 | 53 | Good |
| WC02 | 2011-06-17 | 0.72 | 15 | 5 | 3 | 5 | 10 | 10 | 10 | 58 | Good |
| | 2014-09-19 | 0.25 | 15 | 5 | 3 | 5 | 10 | 10 | 10 | 58 | Good |
| | 2015-10-20 | 0.22 | 15 | 5 | 3 | 5 | 10 | 10 | 10 | 58 | Good |
| | 2016-11-10 | 0.59 | 15 | 5 | 7 | 5 | 15 | 10 | 15 | 72 | Good |
| WC03 | 2011-06-17 | 0.23 | 15 | 10 | 3 | 10 | 5 | 0 | 5 | 48 | Fair |
| | 2014-09-19 | 0 | 15 | 10 | 3 | 10 | 5 | 0 | 5 | 48 | Fair |
| | 2015-10-20 | 0 | 15 | 10 | 3 | 10 | 5 | 0 | 5 | 48 | Fair |
| | 2016-11-10 | 0.18 | 15 | 10 | 0 | 0 | 0 | 0 | 5 | 30 | Fair |
| WE01 | 2011-06-14 | 1.76 | 15 | 0 | 7 | 10 | 15 | 10 | 10 | 67 | Good |
| | 2014-09-18 | 0.39 | 15 | 0 | 7 | 10 | 15 | 5 | 10 | 62 | Good |
| | 2015-10-20 | 0 | 15 | 0 | 7 | 10 | 15 | 5 | 10 | 62 | Good |
| | 2016-11-10 | 1.44 | 15 | 0 | 7 | 10 | 15 | 10 | 10 | 67 | Good |
| WE02 | 2011-06-13 | 1.28 | 5 | 5 | 0 | 10 | 5 | 10 | 10 | 45 | Fair |
| | 2014-09-18 | 0.07 | 5 | 5 | 0 | 10 | 5 | 10 | 10 | 45 | Fair |
| | 2015-11-06 | 0.02 | 5 | 5 | 0 | 10 | 5 | 10 | 10 | 45 | Fair |
| | 2016-11-10 | 0.95 | 5 | 5 | 3 | 5 | 15 | 5 | 10 | 48 | Fair |
| OR03 | 2009-07-15 | 7.42 | 15 | 5 | 0 | 5 | 10 | 5 | 10 | 50 | Good |
| | 2014-09-19 | 11.95 | 15 | 5 | 0 | 5 | 10 | 5 | 10 | 50 | Good |
| | 2015-10-16 | 9.02 | 15 | 5 | 0 | 5 | 10 | 5 | 10 | 50 | Good |
| | 2016-11-14 | 22.5 | 15 | 5 | 3 | 10 | 5 | 5 | 10 | 53 | Good |
| Top Score | | | 15 | 15 | 10 | 15 | 15 | 15 | 15 | 100 | Excellent |

Qualitative Ratings: Excellent ≥ 75; Good 50 to 74; Fair 25 to 49; Poor < 25.

Table 8. Qualitative stream habitat scores and rating for stream width > 10 meters.

| Site | Date | Flow (cfs) | Bank Stability | Maximum Thalweg Depth | Riffle: Riffle Ratio | Rocky Substrate | Fish Cover | Total Habitat Score | Habitat Rating |
|---|---|---|---|---|---|---|---|---|---|
| SR02 | 2011-08-02 | 131 | 8 | 16 | 0 | 16 | 8 | 48 | Fair |
| | 2014-10-01 | 98.3 | 8 | 16 | 0 | 16 | 16 | 56 | Fair |
| | 2015-11-06 | 221 | 8 | 16 | 0 | 16 | 16 | 56 | Fair |
| | 2016-11-11 | 252 | 4 | 16 | 0 | 16 | 25 | 61 | Good |
| SR03 | 2011-09-01 | 71 | 8 | 16 | 12 | 25 | 16 | 77 | Good |
| | 2014-09-18 | 200 | 8 | 16 | 12 | 25 | 16 | 77 | Good |
| | 2015-11-06 | 202 | 8 | 16 | 12 | 25 | 16 | 77 | Good |
| | 2016-11-10 | 249 | 8 | 16 | 12 | 25 | 25 | 86 | Excellent |
| SR04 | 2011-07-26 | 55.5 | 8 | 16 | 12 | 25 | 25 | 86 | Excellent |
| | 2014-10-01 | 124 | 8 | 16 | 12 | 25 | 25 | 86 | Excellent |
| | 2015-11-11 | 135 | 8 | 16 | 12 | 25 | 25 | 86 | Excellent |
| | 2016-11-11 | NA | 12 | 25 | 12 | 25 | 16 | 90 | Excellent |
| SR05 | 2011-08-01 | 61 | 8 | 8 | 12 | 25 | 25 | 78 | Good |
| | 2014-11-04 | 148 | 8 | 8 | 12 | 25 | 25 | 78 | Good |
| | 2015-11-13 | 166 | 8 | 8 | 12 | 25 | 25 | 78 | Good |
| | 2016-11-14 | 205 | 8 | 16 | 8 | 25 | 25 | 82 | Excellent |
| SR06 | 2011-09-01 | NA | 4 | 25 | 4 | 8 | 16 | 57 | Fair |
| | 2014-10-01 | NA | 4 | 25 | 4 | 8 | 16 | 57 | Fair |
| | 2015-11-11 | NA | 4 | 25 | 4 | 8 | 16 | 57 | Fair |
| | 2016-11-11 | NA | 8 | 25 | 8 | 8 | 16 | 61 | Good |
| SR07 | 2011-08-30 | 59.9 | 4 | 8 | 8 | 16 | 25 | 61 | Good |
| | 2014-09-24 | 212 | 4 | 8 | 8 | 16 | 25 | 61 | Good |
| | 2015-11-11 | 142 | 4 | 8 | 8 | 16 | 25 | 61 | Good |
| | 2016-11-14 | 212 | 4 | 16 | 12 | 25 | 25 | 82 | Excellent |
| SR08 | 2011-09-01 | 44 | 4 | 16 | 12 | 25 | 25 | 82 | Excellent |
| | 2014-09-24 | 169 | 4 | 16 | 12 | 25 | 25 | 82 | Excellent |
| | 2015-11-11 | 102 | 4 | 16 | 12 | 25 | 25 | 82 | Excellent |
| | 2016-11-11 | NA | 8 | 16 | 4 | 16 | 16 | 60 | Good |
| OR01 | 2011-08-04 | 18.2 | 12 | 8 | 12 | 25 | 25 | 82 | Excellent |
| | 2014-09-19 | 17.5 | 12 | 8 | 12 | 25 | 25 | 82 | Excellent |
| | 2015-10-16 | 12.6 | 12 | 8 | 12 | 25 | 25 | 82 | Excellent |
| | 2016-11-11 | 26.7 | 12 | 8 | 12 | 25 | 25 | 82 | Excellent |
| OR02 | 2011-06-28 | 13.3 | 4 | 8 | 0 | 8 | 8 | 28 | Fair |
| | 2014-09-19 | 4.34 | 4 | 8 | 0 | 8 | 8 | 28 | Fair |
| | 2015-10-16 | 10.8 | 4 | 8 | 0 | 8 | 8 | 28 | Fair |
| | 2016-11-11 | 41.8 | 4 | 8 | 4 | 8 | 16 | 40 | Fair |
| | | | | | | | | | |
| Top Score | | | 12 | 25 | 12 | 25 | 25 | 99 | Excellent |

Qualitative Ratings: Excellent > 80; Good 60 to 80; Fair 20 to 60; Poor < 20.

## Aquatic Macrophytes

Aquatic macrophyte surveys were conducted in 2011, 2014, 2015 and 2016 at one location (SR 02) to determine the potential to support annual Northern Pike spawning in the spring. Tables 9 and 10 summarize select data for the SR 02 site survey. Figure 3 provides an aerial view of the site boundary showing wetland delineations. All 106 sample points were not included in the surveys because some of the sample points were in upland areas, which was due to the islands within the sample site (Figure 2).

A total of 15 species of macrophytes were recorded for all sample years combined. The range of individual species present were two to eight for each of the four years. The frequency of species occurrence throughout the site was very low, primarily 1 to 5 percent. *Cladophora* sp. was present in 38 percent of sample points in 2011, but was not present in 2014, 2015 or 2016. Rake density or fullness was low, one out of three, in almost all samples for all four years. The Floristic Quality Assessment ratings were "Low" for all four years because of low diversity.

SR 02 appears to have a macrophyte community that would not currently support northern pike spawning habitat for natural reproduction or a nursery. The main reasons that a macrophyte community cannot get established within this site is probably due to excessive stream flows, turbidity, foraging from common carp, and ice scour of the substrate during the early Spring.

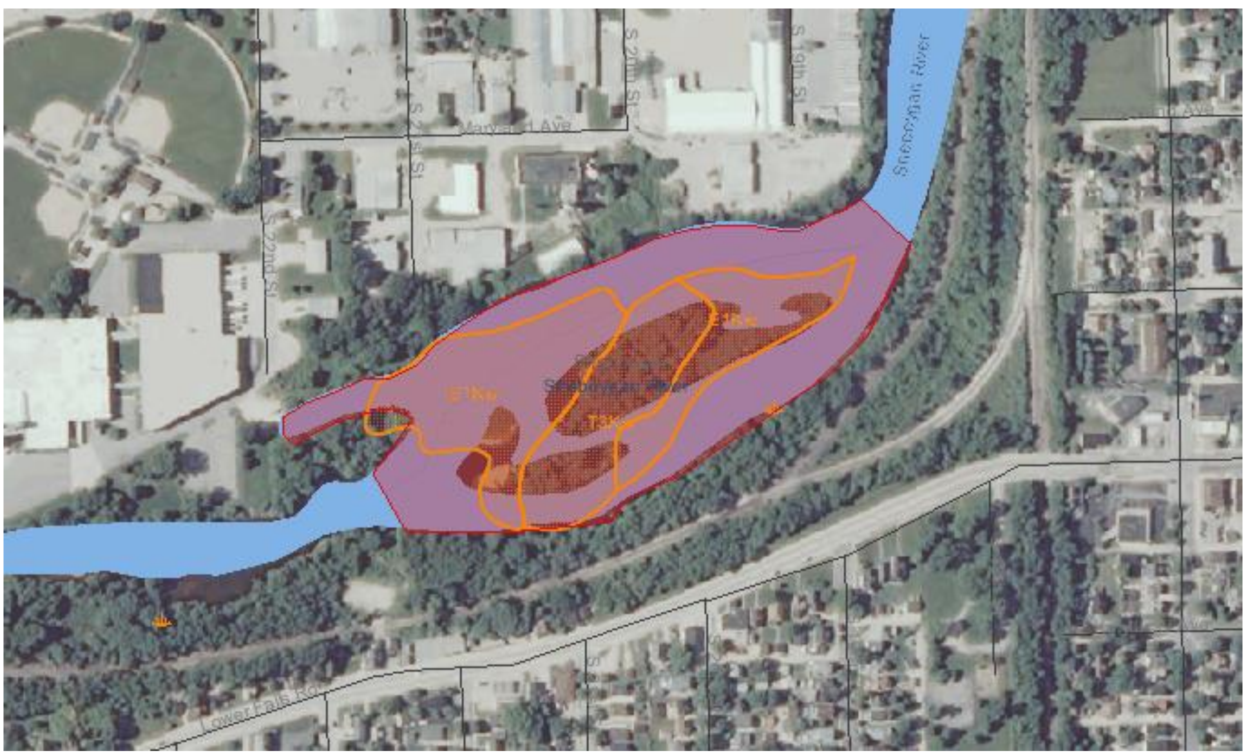


Figure 7. Aerial photo of sample site (SR 02) for aquatic macrophyte surveys. Red highlighted area is site boundary and orange lines are delineated wetlands within the site.

Table 9. Summary of aquatic plant survey data for site SR 02 on the Sheboygan River in 2011, 2014, 2015 and 2016. Sample points within upland areas were not included in survey. Data reported as presence/total sample points (percentage).

| Date | 2011-09-20 | 2014-09-17 | 2015-08-27 | 2016-08-25 |
|---|---|---|---|---|
| Total Sample Points | 106 | 106 | 106 | 106 |
| Sample Points in Upland Areas | 30/106 (28%) | 28/106 (26%) | 26/106 (25%) | 21/106 (20%) |
| Sample Points in Survey | 74 | 78 | 80 | 85 |
| Depth Range (ft) | 0.1 - 4.5 | 0.5 - 6 | 0.5 - 7.5 | 0.5 - 7.5 |
| Average Depth (ft) | 1.58 | 2.8 | 3.07 | 3.18 |
| Stream Flow (cfs) | 74.7 | 207 | 51 | 149 |
| | | | | |
| *Substrate* | | | | |
| Muck | 15/74 (20%) | 18/78 (23%) | 25/80 (31%) | 41/85 (48%) |
| Sand | 13/74 (18%) | 9/78 (12%) | 12/80 (15%) | 10/85 (12%) |
| Gravel | 48/74 (65%) | 51/78 (65%) | 43/80 (54%) | 34/85 (40%) |
| | | | | |
| *Species List* | | | | |
| Aquatic moss | | 2/78 (2.6%) | | |
| *Ceratophyllum demersum* - Coontail | | | 2/80 (2.5%) | 1/85 (1.2%) |
| *Cladophora* sp. - Filamentous Algae | 28/74 (38%) | | | |
| *Lythrum salicaria* - Purple loosestrife | | | 3/80 (3.8%) | |
| *Nuphar variegata* - Spatterdock | | | 1/80 (1.3%) | |
| *Nymphaea odorata* - White water lily | | 1/78 (1.3%) | 1/80 (1.3%) | 2/85 (2.4%) |
| *Phragmites australis* - Common reed | | | 1/80 (1.3%) | |
| *Pontederia cordata* - Pickerelweed | | | 1/80 (1.3%) | 1/85 (1.2%) |
| *Potamogeton crispus* - Sago pondweed | 1/74 (1.4%) | 1/78 (1.3%) | 1/80 (1.3%) | 4/85 (4.7%) |
| *Potamogeton friesii* - Fries' pondweed | | 2/78 (2.6%) | 2/80 (2.5%) | 2/85 (2.4%) |
| *Potamogeton nodosus* - Long-leaf pondweed | | 1/78 (1.3%) | | |
| *Potamogeton zosteriformes* - Flat Stem Pondweed | | | | 1/85 (1.2%) |
| *Schoenoplectus acutus* - Hardstem bullrush | | | | 1/85 (1.2%) |
| Other species present | None | Arrowhead, Coontail | None | None |
| Total species present | 2 | 7 | 8 | 7 |
| Floristic Quality Assessment Score & (Rating) | 3 (Low) | 12 (Low) | 14.8 (Low) | 15.1 (Low) |

Table 10. Floristic Quality Assessment (FQA) values and quality ratings for Wisconsin lake plant communities (Nichols 1998).

| FQA Value | Quality Rating |
|---|---|
| < 17 | Low |
| 17 to 24.4 | Medium |
| > 24.4 | High |

## CONCLUSIONS AND RECOMMENDATIONS

Benthic macroinvertebrates and stream habitat were sampled at 16 stream sites within the Sheboygan River AOC in Sheboygan County by the Wisconsin Department of Natural Resources in 2010/2011, 2014, 2015 and 2016. Sample collection and surveys in 2010 and 2011 were done for baseline monitoring to determine the health of select stream sites before contaminated sediment was removed and habitat restoration projects were implemented. Verification monitoring was done for three consecutive years in 2014 through 2016 to determine if removal of contaminated sediment and habitat restoration improved the water quality and biological integrity of select streams.

In 2010 and 2011 the degraded sites that had “Poor” ratings were on the lower portion of the Sheboygan River (SR 01), between the two dams on the Sheboygan River (SR 06), and the channelized headwater areas of Willow Creek (WC 03) and Weeden Creek (WE 02). Sites SR 01, SR 02 and SR 06 on the Sheboygan River, WC 03, and WE 02 had overall “Poor” ratings for the baseline and verification monitoring. The majority of the “Poor” ratings were associated with the HBI. The “Poor” ratings were most likely the result of poor stream habitat conditions from old channelization and sedimentation from nonpoint source runoff, and in some cases severe streambank erosion. The rest of the sites primarily rated “Fair” to “Excellent”. There were a few ratings of “Poor” or “Fairly Poor” scattered among sites SR 04, WE 01, and OR 02. However, these ratings occurred in only one of the four years of monitoring, and while there is some fine sediment at these sites, the change in annual IBI ratings is likely the result of annual variability within the invertebrate community. There are some differences of the ratings between the M-IBI and the HBI. This is expected because of the different variables that are used to calculate the two biotic indices.

All stream habitat surveys on the wadable sites rated “Fair” to “Excellent”. A habitat survey was not done for the one nonwadeable site (SR01) because WDNR does not have a protocol for nonwadeable sites. For most wadeable sites less than 10 meters wide, the limiting factor for habitat were bank erosion, lack of pool areas, and fine sediments. Ranking for these sites were “Fair to “Good”. For stream sites that were greater than 10 meters wide, ranking ranged from “Fair” to “Excellent”. For sites that rated “Fair”, limiting factors for habitat were bank stability, riffle:riffle or bend:bend ratio, lack of rocky substrate and cover for fish. There were no significant changes in scores or ratings before (2011) and after (2014 through 2016) contaminated sediment was removed and habitat restoration projects were implemented.

Site SR 02 appears to have a macrophyte community that would not currently support northern pike spawning habitat for natural reproduction or a nursery. Aquatic plant abundance and diversity is limited and the Floristic Quality Assessment ratings for all sample years is “Low”. There was more diversity in 2014 through 2016, compared to 2011. However, the frequency of species present is very low, ranging from 1 to 5 percent. A significant percentage of the bottom substrate consists of fine sediment (sand and muck), so there is adequate material for aquatic plants to take root. The main reasons that a macrophyte community cannot get established within this site is probably due to excessive stream flows, turbidity, foraging from common carp, and ice scour of the substrate during the early Spring.

All the sediment removal and habitat restoration projects associated with the AOC were conducted on the Sheboygan River. Surprisingly, we saw the largest increase in macroinvertebrate IBI score at the Onion River sites. The Onion River generally has good water quality along the upper portion of the watershed, but no known restoration activities took place during the study. Conversely, Weeden Creek and Willow Creek showed no overall difference among time periods, although there was some variability in IBI scores among sites. These sites reacted as expected with a river with no restoration work during the study, some sites had minor increases, some minor decreases, but within commonly observed natural variability and no overall differences when combined. Although not statistically significant, we did see an increase in IBI scores at the Sheboygan River. IBI scores in the Sheboygan River increased in most of the middle and upper reaches, SR03-SR08, except for one site (SR06), which is located between two dams in the Village of Kohler. The two lower reaches (SR02 and SR01) showed almost no change between the before-after time periods. Although we cannot tie macroinvertebrate responses to a specific restoration activity, there is some evidence that macroinvertebrates are responding to restoration activities in the waterbody/watershed. The lack of statistical significance may be related to small before restoration sample sizes, or variability in response among individual sites (SR06, SR02, and SR01) masking improvements in the entire waterbody.

Based on the baseline and verification monitoring there was not a significant change in benthic macroinvertebrate index ratings or stream habitat ratings among individual sites. The aquatic plant surveys at SR 02 did have an increase in diversity but frequencies were very low. Therefore, the macrophyte community would not support northern pike spawning and nursery habitat at this time.

At all the sites in the Sheboygan River, besides SR01, the mean macroinvertebrate IBI scores after restoration are above, and many well-above, the threshold established by WDNR for bioassessments (WDNR 2015). The results of this study can support a recommendation for delisting the “Degradation of Fish and Wildlife Populations” and “Loss of Fish and Wildlife Habitat” beneficial use impairments for the Sheboygan River AOC.

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**APPENDIX**

Benthic macroinvertebrate assemblage information from one-time surveys conducted in 2010, 2014, 2015 and 2016 at 16 stream sites within the Sheboygan River AOC. EPT, Ephemeroptera, Plecoptera, and Trichoptera; M-IBI, Macroinvertebrate Index of Biotic Integrity; HBI, Hilsenhoff Biotic Index; SR01 is a nonwadeable site with sample collected in 2011; * indicates duplicate samples for quality assurance.

| Site | Date | IBI | Rating | HBI | Rating | FBI | Rating | HBI Max 10 | Rating | Species Richness | Genera Richness | % EPT Individuals | EPT Genera Richness | % Chironomidae Individuals | Shannon's Diversity Index | % Scrapers | % Filterers | % Shredders | % Gatherers |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SR01 | 2011-08-26 | 5 | Poor | 8.87 | Very Poor | 6.87 | Poor | 7.23 | Fairly Poor | 20 | 18 | 0 | 6 | 59 | 2.32 | 0 | 36 | 1 | 58 |
| | 2014-10-01 | 5 | Poor | 8.44 | Poor | 6.93 | Poor | 8 | Poor | 11 | 11 | 0 | 9 | 7 | 0.47 | 0 | 93 | 0 | 7 |
| | 2015-10-02 | 10 | Poor | 7.44 | Fairly Poor | 6.91 | Poor | 6.56 | Fairly Poor | 17 | 16 | 0 | 0 | 3 | 0.24 | 0 | 97 | 0 | 3 |
| | 2016-09-30 | 5 | Poor | 8.7 | Very Poor | 6.82 | Poor | 7.87 | Poor | 31 | 28 | 0 | 11 | 35 | 2.37 | 0 | 41 | 0 | 54 |
| SR02 | 2010-10-28 | 4.28 | Fair | 5.8 | Fair | 4.76 | Good | 5.94 | Fair | 37 | 36 | 41 | 25 | 39 | 4.32 | 18 | 28 | 5 | 43 |
| | 2014-11-04 | 4.29 | Fair | 6.7 | Fairly Poor | 6.88 | Poor | 6.64 | Fairly Poor | 23 | 22 | 3 | 9 | 79 | 3.34 | 0 | 13 | 34 | 51 |
| | 2015-11-06 | 0.7 | Poor | 7.67 | Poor | 6.79 | Poor | 7.74 | Poor | 16 | 16 | 0 | 0 | 97 | 2.91 | 3 | 2 | 1 | 74 |
| | 2016-11-11 | 4.09 | Fair | 7.08 | Fairly Poor | 6.86 | Poor | 6.61 | Fairly Poor | 20 | 19 | 2 | 11 | 81 | 3.07 | 0 | 4 | 13 | 82 |
| SR03 | 2010-10-28 | 2.88 | Fair | 5.16 | Good | 4.14 | Very Good | 5.18 | Good | 19 | 18 | 81 | 39 | 17 | 2.95 | 18 | 65 | 7 | 10 |
| | 2014-10-29 | 4.9 | Fair | 5.25 | Good | 4.84 | Good | 5.3 | Good | 29 | 28 | 56 | 39 | 39 | 3.28 | 4 | 50 | 2 | 34 |
| | 2015-11-06 | 5.75 | Good | 4.68 | Good | 4.79 | Good | 4.64 | Good | 33 | 31 | 55 | 35 | 27 | 4.30 | 13 | 45 | 4 | 30 |
| | 2016-11-10 | 7.62 | Excellent | 4.33 | Very Good | 4.04 | Very Good | 4.37 | Very Good | 29 | 28 | 78 | 54 | 8 | 3.38 | 12 | 31 | 4 | 51 |
| SR04 | 2010-10-28 | 4.61 | Fair | 7.05 | Fairly Poor | 4.49 | Good | 7.04 | Fairly Poor | 26 | 25 | 19 | 28 | 25 | 3.78 | 3 | 15 | 24 | 56 |
| | 2014-10-30 | 5.49 | Good | 3.35 | Excellent | 2.75 | Excellent | 4.51 | Good | 34 | 33 | 79 | 39 | 12 | 3.46 | 51 | 33 | 1 | 14 |
| | 2015-11-11 | 6.33 | Good | 5.25 | Good | 5.62 | Fair | 5.18 | Good | 29 | 28 | 29 | 32 | 63 | 4.13 | 6 | 37 | 9 | 39 |
| | 2016-11-11 | 5.49 | Good | 4.3 | Very Good | 3.73 | Excellent | 4.9 | Good | 29 | 28 | 60 | 39 | 9 | 3.77 | 35 | 29 | 4 | 29 |
| SR05 | 2010-11-10 | 4.49 | Fair | 5.32 | Good | 4.38 | Good | 5.22 | Good | 39 | 37 | 66 | 38 | 24 | 3.07 | 10 | 69 | 3 | 17 |
| | 2014-11-04 | 6.21 | Good | 4.39 | Very Good | 4.5 | Good | 4.68 | Good | 40 | 36 | 50 | 33 | 26 | 4.49 | 33 | 22 | 4 | 32 |
| | 2015-11-13 | 5.17 | Good | 5.2 | Good | 5.7 | Fair | 5.1 | Good | 25 | 24 | 32 | 46 | 57 | 3.95 | 9 | 37 | 4 | 45 |
| | 2016-11-14 | 6.97 | Good | 5.07 | Good | 4.88 | Good | 5.08 | Good | 44 | 44 | 48 | 39 | 36 | 4.93 | 19 | 28 | 10 | 32 |
| SR06 | 2010-11-10 | 7.78 | Excellent | 7.57 | Poor | 4.9 | Good | 7.1 | Fairly Poor | 46 | 45 | 10 | 13 | 36 | 4.37 | 2 | 6 | 3 | 59 |
| | 2014-10-30 | 7.32 | Good | 6.63 | Fairly Poor | 6.73 | Poor | 6.69 | Fairly Poor | 35 | 33 | 6 | 12 | 61 | 4.08 | 1 | 29 | 10 | 45 |
| | 2015-11-11 | 4.26 | Fair | 7.48 | Fairly Poor | 6.86 | Poor | 7.21 | Fairly Poor | 22 | 21 | 1 | 5 | 71 | 3.21 | 1 | 12 | 6 | 57 |
| | 2016-11-11 | 4.55 | Fair | 7.29 | Fairly Poor | 6.64 | Poor | 7.18 | Fairly Poor | 26 | 25 | 1 | 4 | 50 | 3.68 | 18 | 19 | 9 | 28 |
| SR07 | 2010-11-10 | 5.01 | Good | 5.05 | Good | 4.05 | Very Good | 5.39 | Good | 27 | 26 | 64 | 38 | 31 | 3.21 | 28 | 52 | 4 | 14 |
| | 2010-11-10* | 5.62 | Good | 5.49 | Good | 4.31 | Good | 5.4 | Good | 31 | 29 | 41 | 31 | 49 | 3.62 | 19 | 51 | 10 | 17 |
| | 2014-10-30 | 6.69 | Good | 3.77 | Very Good | 3.52 | Excellent | 4.38 | Very Good | 29 | 28 | 58 | 43 | 24 | 3.90 | 34 | 33 | 5 | 22 |
| | 2014-10-30* | 6.84 | Good | 4.44 | Very Good | 4.31 | Good | 4.41 | Very Good | 33 | 30 | 59 | 37 | 18 | 3.92 | 27 | 41 | 5 | 21 |
| | 2015-11-11 | 5.08 | Good | 5.03 | Good | 5.24 | Fair | 5.04 | Good | 36 | 35 | 28 | 29 | 39 | 4.44 | 26 | 26 | 21 | 25 |
| | 2015-11-11* | 7.29 | Good | 5.08 | Good | 5.47 | Fair | 5.09 | Good | 36 | 35 | 25 | 29 | 50 | 4.71 | 23 | 27 | 16 | 28 |
| | 2016-11-14 | 7.38 | Good | 4.6 | Good | 4.3 | Good | 4.69 | Good | 35 | 33 | 43 | 36 | 22 | 4.23 | 23 | 35 | 12 | 27 |
| SR08 | 2010-11-10 | 4.27 | Fair | 5.44 | Good | 4.34 | Good | 5.05 | Good | 27 | 25 | 40 | 40 | 56 | 2.94 | 8 | 72 | 8 | 11 |
| | 2014-10-30 | 7.62 | Excellent | 4.89 | Good | 4.59 | Good | 4.86 | Good | 35 | 34 | 53 | 38 | 30 | 4.25 | 19 | 45 | 9 | 20 |
| | 2015-11-11 | 5.44 | Good | 4.85 | Good | 5.13 | Fair | 4.94 | Good | 37 | 33 | 40 | 30 | 54 | 4.50 | 8 | 31 | 15 | 40 |
| | 2016-11-11 | 5.7 | Good | 4.88 | Good | 4.38 | Good | 4.8 | Good | 33 | 33 | 52 | 33 | 11 | 4.44 | 16 | 30 | 13 | 34 |
| WC01 | 2010-10-28 | 2.92 | Fair | 4.13 | Very Good | 2.78 | Excellent | 5.03 | Good | 15 | 15 | 82 | 27 | 9 | 2.55 | 1 | 22 | 48 | 28 |
| | 2014-10-29 | 3.4 | Fair | 4.3 | Very Good | 3.4 | Excellent | 5.34 | Good | 23 | 22 | 46 | 18 | 13 | 3.01 | 27 | 9 | 40 | 21 |
| | 2015-10-20 | 2.76 | Fair | 4.11 | Very Good | 3.7 | Excellent | 4.67 | Good | 13 | 13 | 36 | 31 | 15 | 2.35 | 46 | 19 | 31 | 2 |
| | 2016-11-10 | 4.05 | Fair | 5.77 | Fair | 5.34 | Fair | 5.7 | Fair | 35 | 31 | 14 | 6 | 45 | 3.94 | 12 | 4 | 19 | 61 |

Benthic macroinvertebrate assemblage information from one-time surveys conducted in 2010, 2014, 2015 and 2016 at 16 stream sites within the Sheboygan River AOC. EPT, Ephemeroptera, Plecoptera, and Trichoptera; M-IBI, Macroinvertebrate Index of Biotic Integrity; HBI, Hilsenhoff Biotic Index; SR01 is a nonwadeable site with sample collected in 2011; * indicates duplicate samples for quality assurance - Continued.

| Site | Date | IBI | Rating | HBI | Rating | FBI | Rating | HBI Max 10 | Rating | Species Richness | Genera Richness | % EPT Individuals | EPT Genera Richness | % Chironomidae Individuals | Shannon's Diversity Index | % Scrapers | % Filterers | % Shredders | % Gatherers |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| WC02 | 2010-10-28 | 5.4 | Good | 4.24 | Very Good | 3.14 | Excellent | 4.71 | Good | 12 | 11 | 63 | 36 | 0 | 2.52 | 15 | 15 | 43 | 26 |
| | 2014-10-29 | 5.36 | Good | 4.06 | Very Good | 3.15 | Excellent | 4.83 | Good | 14 | 13 | 50 | 38 | 2 | 2.42 | 26 | 2 | 41 | 29 |
| | 2014-10-29* | 4.23 | Fair | 3.89 | Very Good | 2.97 | Excellent | 4.69 | Good | 15 | 15 | 49 | 33 | 2 | 2.41 | 33 | 7 | 42 | 14 |
| | 2015-10-20 | 4.59 | Fair | 5.42 | Good | 5.05 | Fair | 5.06 | Good | 14 | 14 | 22 | 29 | 2 | 2.65 | 30 | 18 | 4 | 46 |
| | 2016-11-10 | 5.24 | Good | 4.49 | Very Good | 4.31 | Good | 4.94 | Good | 15 | 15 | 9 | 33 | 1 | 2.28 | 51 | 3 | 6 | 39 |
| | 2016-11-10* | 4.94 | Fair | 5.2 | Good | 4.99 | Good | 5.17 | Good | 17 | 17 | 13 | 35 | 4 | 2.76 | 30 | 5 | 6 | 58 |
| WC03 | 2010-10-28 | 3.76 | Fair | 7.71 | Poor | 7.68 | Very Poor | 7 | Fairly Poor | 32 | 32 | 1 | 6 | 15 | 2.78 | 2 | 3 | 1 | 89 |
| | 2014-10-29 | 2.2 | Poor | 6.87 | Fairly Poor | 6.92 | Poor | 7.35 | Fairly Poor | 21 | 21 | 1 | 5 | 68 | 3.18 | 6 | 51 | 1 | 37 |
| | 2015-10-20 | 3.8 | Fair | 7.71 | Poor | 7.11 | Poor | 7.77 | Poor | 24 | 24 | 5 | 13 | 38 | 3.59 | 1 | 30 | 0 | 60 |
| | 2016-11-10 | 3.15 | Fair | 6.82 | Fairly Poor | 6.84 | Poor | 7.13 | Fairly Poor | 28 | 27 | 1 | 7 | 81 | 3.48 | 8 | 42 | 2 | 41 |
| WE01 | 2010-10-28 | 3.55 | Fair | 4.86 | Good | 4.13 | Very Good | 5.26 | Good | 17 | 16 | 44 | 31 | 3 | 2.62 | 39 | 44 | 1 | 16 |
| | 2014-10-29 | 4.53 | Fair | 6.51 | Fairly Poor | 6.05 | Fairly Poor | 6.03 | Fair | 18 | 18 | 12 | 22 | 10 | 3.18 | 11 | 5 | 2 | 76 |
| | 2015-10-20 | 3.63 | Fair | 4.69 | Good | 4.42 | Good | 5.52 | Fair | 18 | 18 | 17 | 22 | 6 | 2.67 | 39 | 18 | 1 | 42 |
| | 2015-10-20* | 3.91 | Fair | 4.94 | Good | 4.84 | Good | 5.67 | Fair | 17 | 17 | 6 | 24 | 9 | 2.47 | 55 | 6 | 0 | 39 |
| | 2016-11-10 | 4.13 | Fair | 4.61 | Good | 4.01 | Very Good | 5.24 | Good | 28 | 27 | 29 | 19 | 19 | 3.80 | 21 | 11 | 24 | 44 |
| | 2016-11-10* | 4.06 | Fair | 4.78 | Good | 4.24 | Very Good | 5.46 | Good | 29 | 29 | 25 | 17 | 26 | 3.88 | 25 | 6 | 20 | 48 |
| WE02 | 2010-10-28 | 3.85 | Fair | 7.78 | Poor | 7.75 | Very Poor | 6.6 | Fairly Poor | 15 | 15 | 3 | 13 | 1 | 1.45 | 1 | 4 | 0 | 94 |
| | 2014-10-29 | 4.58 | Fair | 7.11 | Fairly Poor | 6.35 | Fairly Poor | 6.77 | Fairly Poor | 27 | 27 | 1 | 7 | 35 | 3.41 | 5 | 5 | 1 | 83 |
| | 2015-11-06 | 2.63 | Fair | 7.36 | Fairly Poor | 6.83 | Poor | 7.44 | Fairly Poor | 19 | 19 | 2 | 11 | 76 | 3.07 | 5 | 2 | 2 | 88 |
| | 2016-11-10 | 2.43 | Poor | 7.3 | Fairly Poor | 6.93 | Poor | 7.16 | Fairly Poor | 23 | 21 | 0 | 0 | 48 | 3.42 | 4 | 3 | 1 | 87 |
| OR01 | 2010-11-03 | 6.59 | Good | 5.32 | Good | 4.15 | Very Good | 5.38 | Good | 33 | 31 | 63 | 42 | 24 | 3.95 | 19 | 46 | 8 | 26 |
| | 2010-11-03* | 8.16 | Excellent | 5.3 | Good | 4.09 | Very Good | 5.17 | Good | 50 | 46 | 49 | 28 | 38 | 4.23 | 14 | 52 | 8 | 25 |
| | 2014-10-29 | 7.69 | Excellent | 5.25 | Good | 5.2 | Fair | 5.22 | Good | 40 | 38 | 40 | 32 | 51 | 3.94 | 7 | 43 | 7 | 38 |
| | 2015-10-16 | 6.72 | Good | 4.88 | Good | 4.89 | Good | 5.02 | Good | 31 | 29 | 51 | 38 | 35 | 4.23 | 25 | 44 | 13 | 13 |
| | 2016-11-11 | 5.84 | Good | 4.56 | Good | 4.2 | Very Good | 4.63 | Good | 27 | 26 | 30 | 35 | 8 | 4.02 | 67 | 19 | 7 | 5 |
| OR02 | 2010-11-03 | 2.36 | Poor | 6.68 | Fairly Poor | 4.58 | Good | 6.68 | Fairly Poor | 31 | 31 | 28 | 10 | 54 | 3.79 | 5 | 20 | 3 | 68 |
| | 2014-10-30 | 5.49 | Good | 5.72 | Fair | 5.66 | Fair | 5.78 | Fair | 28 | 28 | 36 | 25 | 55 | 3.51 | 6 | 64 | 3 | 16 |
| | 2015-10-16 | 4.25 | Fair | 5.85 | Fair | 5.75 | Fair | 6.05 | Fair | 28 | 28 | 28 | 25 | 59 | 3.79 | 22 | 23 | 9 | 39 |
| | 2016-11-11 | 5.87 | Good | 5.75 | Fair | 5.15 | Fair | 5.7 | Fair | 36 | 35 | 35 | 34 | 34 | 4.02 | 22 | 34 | 2 | 37 |
| OR03 | 2009-10-29 | 3.73 | Fair | 4.97 | Good | 5.28 | Fair | 4.72 | Good | 23 | 23 | 30 | 30 | 59 | 2.84 | 19 | 61 | 13 | 6 |
| | 2014-10-30 | 5.74 | Good | 5.6 | Fair | 6.03 | Fairly Poor | 5.37 | Good | 26 | 26 | 13 | 23 | 62 | 3.18 | 13 | 54 | 16 | 7 |
| | 2015-10-16 | 3.02 | Fair | 4.69 | Good | 4.91 | Good | 4.97 | Good | 28 | 28 | 28 | 25 | 59 | 3.79 | 22 | 23 | 9 | 39 |
| | 2016-11-14 | 4.15 | Fair | 6.42 | Fair | 6.34 | Fairly Poor | 6.16 | Fair | 24 | 24 | 1 | 8 | 79 | 3.22 | 12 | 11 | 13 | 59 |

# Appendix E – Qualitative Unionid Mussel Surveys and Habitat Assessment of the Sheboygan River AOC

# Qualitative Unionid Mussel Surveys and Habitat Assessment of the Sheboygan River AOC. Sheboygan County, Wisconsin

**Survey and Report Conducted By:**
**Jason Dare, Ecologist**
**Dare Ecosystem Management, LLC**
**Sullivan, WI**
**August-October 2011**

**TABLE OF CONTENTS:**

**Abstract:**
The lower Sheboygan River and Harbor were designated a Great Lakes Area of Concern (AOC) in 1985 by the International Joint Commission (IJC). This AOC encompasses a section of river downstream from the Sheboygan Falls Dam to the entire harbor and near-shore area of Lake Michigan. It is suspected that high levels of nutrients, solids, and toxic chemicals along with land use changes have contributed to the degradation of animal and plant populations in this section of the Sheboygan River. The AOC designation has prompted the WDNR to authorize a qualitative assessment of freshwater mussels within the Sheboygan River. The resulting inventory found live mussels at 13 of the 14 sample sites with all 14 sites showing evidence of historic mussel communities. Eleven native unionid species were observed during the surveys and one exotic species the Zebra Mussel (*Dreissena polymorpha)* was observed through a recent study conducted in the harbor by the National Oceanic and Atmospheric Association .The most widely distributed species where live mussels were found were the Floater 92.3% (Pyganodon grandis), Fat Mucket 92.3% (Lampsilis siliquoidea), Creeper 92.3 % *(Strophitus undulatus*), and White Heelsplitter 84.6 %( Lasmigona complanata).
Based on observations made while conducting the surveys, siltation, possible past pollution and possible low dissolved oxygen appear to be the biggest threats to mussel survivability in the Sheboygan AOC.
Future research into mussel populations in the AOC should focus on understanding the relationships of the sediment and the rivers contaminants impact on mussels. In the lower reaches of the stream within the City of Sheboygan, studies should focus on understanding the concentration of total ammonia in the sediments and solutions to the low dissolved oxygen rates that may exist.
Preventative efforts in the AOC should concentrate on protecting the mussels from future negative perturbations such as siltation, stream bed dredging, bridge and construction projects, and invasive species. Future small scale restoration efforts should focus on overland flow, and educating the constituents about nonpoint source pollution.

**LIST OF TABLES**

**Introduction:**

The primary goals of this project were to determine the presence and distribution of native unionid mussel species (especially state listed species), develop a baseline of the determined presence and distribution throughout the survey area, identify threats to these populations and provide suggestions for habitat improvement for the mussel species of greatest conservation need.

Within the area of concern (AOC), historical data was minimal. The historical data that was available and was referenced consisted of relict shells that were found during the inventory process. The qualitative data gathered for this project provides an initial baseline for the long-term monitoring of the Sheboygan AOC's mussel communities.

The AOC for this project includes the lower 14 miles of the Sheboygan River from the City of Sheboygan Falls to the City of Sheboygan (Appendix I). Within the AOC, five separate government property managers exist; the city of Sheboygan Falls manages three parks along the river, the Village of Kohler, the State of Wisconsin owns land along the river at the UW-Sheboygan campus, Sheboygan County manages land at Esslinger Park as well as nearby lands and the city of Sheboygan manages several parks and land along the river, most notably Kiwanis Park and Wildwood Island. One notable private landowner along the river and within the AOC is the Kohler Company which owns the Black Wolf Run Golf Course and surrounding land. The majority of the highest quality terrestrial and aquatic resources exist within and along the land the Kohler Company owns.

**Methods:**

The methodology used to conduct the surveys for this project was set forth in the work plan by the WI DNR. Some additional tasks were completed to add to this methodology. First, multiple reconnaissance trips were conducted in the summer of 2011 to determine the best points to survey for mussels. Points were chosen in the AOC based on the observation that they would be good sites for possible mussel populations, had unique or different habitat compared to the majority of the river or were located near publicly held land where stakeholders could use the information and make terrestrial or aquatic management decisions to improve habitat for mussels.

First, shoreline searches were conducted to locate dead mussel shells that were on shore due to past high waters or in mammalian middens. Each terrestrial assessment lasted a minimum of 15 minutes on each side of the rivers shoreline. If a high number of shells were found or it was determined that the site showed promise to find new species, the surveys would extend to a half hour. After the shoreline search was conducted and survey points were determined, a qualitative wading/ snorkeling survey was completed at all points. Each point was surveyed until no new species had been found in a 30 minute period. All living and dead valves found were identified to species. Live mussels were immediately returned to the river, and valves were kept from dead mussels to be identified at the end of each sampling point's survey. If spots looked like suitable habitat for WI DNR species of concern, or valves were found of species like this, extra time was spent looking for individuals within the stream.

After the mussel survey was completed at each point, accompanying habitat data was collected. At each point the location was recorded using a Garmin 450 GPS unit. This is a recreational grade GPS with manufacturer specs on accuracy at 5-15 meters. In addition, water and air temperatures were recorded. Substrate type and percentages were estimated using the Wentworth scale of substrate size. Other estimates included, flow rate (no flow, low, normal, flood, high), water clarity, water color, water surface, depth (ankle, calf, knee, waist, chest) and approximate length of area searched. As the survey was conducted and while conducting the habitat assessment other notable biological observations were recorded when observed.

## DATA ANALYSIS:

Total number of points sampled: The total number of places sampled for mussels (Appendix II).
Current number of sites with unionid mussels: The number of sites where at least one live mussel was found.
Historic number of sites with unionid mussels: The number of sites where at least one live mussel OR an empty valve indicating mussels lived there in the past was found.
Historic community survival rate: The percentage of sites that had mussels in the past that still support mussels at the time of the study.
Average number of live species per site: The mean number of live native unionid species found at all sites with live mussels.
Average historical number of species per site: This mean number of live native unionid species AND species represented by empty valves found at all sites with live or relic mussels.
Total extant species richness within the AOC: The number of different live mussel species found throughout the entire AOC.
Total historic species richness within the AOC: The number of different live mussel species AND species represented by only empty valves found throughout the entire AOC.
Relative frequency: This value shows a species' frequency relative to all other species. It is expressed as a percentage, and the total of all species' relative frequency and will add up to 100%. Organizing species from highest to lowest relative frequency value (Table, 2, and Appendix VIII ) gives us an idea of which species are most important within the overall unionid community in the AOC. As the surveys were sampled quantitatively, this value is only suggestive.
Community Health: A site was ranked poor, fair, good or excellent based on apparent water quality, water flow rate, substrate suitability, historic species survivability, and evidence of reproduction. An excellent site offered all of these and demonstrated both species richness, individual density and some evidence of recruitment; a poor site offered little habitat, few individuals, and no evidence of recruitment.

## RESULTS:

Fourteen points were surveyed for mussels (Appendix II). Of these, 13 points had live mussels with one additional site having supported mussels in the past. Point 14 was taken out of some of the data analyses because its substrate was unlike most of the watershed. Point 14's substrate was primarily bedrock below the impoundment and above a waterfall, however, there was one decent buildup of gravel and silt that was thick enough to support one live creeper *(Strophitus undulates*).

Four mussel species *Fusconaia flava*, *Lasmigona costata, Venustaconcha ellipsiformis,* and *Elliptio dilatata* were only found as relict population as no live animals were found during the project.
It was determined the AOC lost an average of nearly 2.64 species/site based on relic shell analysis vs. live mussels. One of the 14 sites (site 1) that historically supported mussels showed no signs of a living population. Analysis in species richness that can be found in (Table 1) shows that seven extant mussels are in the AOC at this time and eleven have historically been there

**Table 1: General Survey Summary Statistics Sheboygan AOC, August – October, 2011**

| Summary Statistics: | Sites | Sites |
|---|---|---|
| Total number of points sampled | 14 | 13* |
| Current number of sites with live unionid mussels | 13 | 12 |
| Historic number of sites with unionid mussels | 14 | 13 |
| Historic community survival rate | 92.9 | 92.3 |
| Average number of live species per site | 4.14 | 4.38 |
| Average historic number of species per site | 6.78 | 7.23 |
| Total extant species richness within the drainage | 7 | 7 |
| Total historic species richness within the drainage | 11 | 11 |
| Percent extirpated species | 36.36 | 36.36 |
| Average species lost per site | 2.64 | 2.85 |

*this is a summary with 13 sites(taking out Site 14)

**Table 2: Current and Historic Relative Frequencies and Relative Abundance of Unionid Mussels in the Sheboygan AOC. August-October 2011.**

| Species | Common Name | State Status | Current Sites | Historic Sites | Current Rel. Freq. Site | Historic Rel. Freq. Site | Current Rel. Abundance |
|---|---|---|---|---|---|---|---|
| *Lasmigona complanata* | White Heelsplitter | | 11 | 12 | 78.6% | 85.7% | 34.8% |
| *Strophitus undulatus* | Creeper | | 12 | 13 | 85.7% | 92.9% | 20.1% |
| *Elliptio dilatata* | Spike | | 0 | 11 | 0.0% | 78.6% | 0.0% |
| *Pyganodon grandis* | Giant Floater | | 12 | 13 | 85.7% | 92.9% | 13.9% |
| *Alasmidonta marginata* | Elktoe | S.C. | 3 | 5 | 21.4% | 35.7% | 1.5% |
| *Lampsilis cardium* | Plain Pocketbook | | 6 | 11 | 42.9% | 78.6% | 7.7% |
| *Lampsilis siliquoidea* | Fat Mucket | | 12 | 13 | 85.7% | 92.9% | 20.9% |
| *Fusconaia flava* | Wabash Pigtoe | | 0 | 7 | 0.0% | 50.0% | 0.0% |
| *Lasmigona costata* | Flutedshell | | 0 | 2 | 0.0% | 14.3% | 0.0% |
| *Anodontoides ferussacianus* | Cylindrical Papershell | | 2 | 5 | 14.3% | 35.7% | 1.2% |
| *Venustaconcha ellipsiformis* | Ellipse | THR. | 0 | 3 | 0.0% | 21.4% | 0.0% |

Overall the mussel communities surveyed had some general similarities. One similarity was that juveniles were a small proportion of the living mussels seen in the stream. Fat mucket, white heelsplitters and plain pocketbooks were the only juvenile species found during this survey. Most sites were dominated by white heelsplitters, fat muckets, floaters, and creepers. The data collected revealed that the white heelsplitter was the most abundant species found within the AOC.

Site 1: Kiwanis Park- This site once supported a high amount and richness of mussels in comparison to the rest of the river. Currently, this section is slow mowing water with a decent substrate that is covered with silt. The waters are also dominated by algae and the section remained turbid for much of the 2011 field season. The areas substrate did not become visible till early October, and was so turbid and silty one did not know what and where they were stepping into in the previous months. However, this will be a good site to conduct more inventories to see if live mussels can be found. It would not be a surprise to find a live floater, white heel splitter or fat mucket in this stretch in the future. No live mussels were found during this survey. This is an easy site to look for relict shells as the sediment is covering up a massive amount of old shells. One possible explanation for the decrease in live animals is the probability of low dissolved oxygen. In this condition, old living adults can cling on for years without active colonization by juveniles occurring. Juvenile mussels are more susceptible to low dissolved oxygen rates and therefore cannot persist in this environment. If dissolved oxygen readings were taken by others conducting work in the stream, it would be useful to use this when assessing the sites suitability for mussels and creating a restoration plan for the site.

Site 2: Industrial Park. Julson Ct. Wildwoods Islands- This site had below average habitat, but still had live mussels using the habitat. It was estimated that 90% of the substrate was covered with a thin layer of silt. Even so, gravel and silt intermixed with a small percentage of sand provided some habitat for mussels. Algae blooms were also very prominent throughout this survey point. Some areas along the substrate were too hard and compacted for mussels to get established but most of the section is physically available for mussels. This site had a few juvenile white heel splitters and fat muckets which proved it was suitable for future recruitment. The site is adjacent to an industrial park and effluent is leaving the parking area via underground pipes. Consequently, this point would be a good point to monitor if the local water quality is improving, and if more uncommon unionids begin to re-colonize this rivers stretch in the future.

Site 3: Taylor Ave. The site can be a good area to use to educate people about mussels. The land adjacent to the river could be restored to collect more overland runoff. This spot had a tremendous darter population, and a good bluegill population as well. More floater and creeper individuals could have been found but more time was spent looking for other species. This point had a large amount of cobble size substrate but where silt and gravel existed live mussels were present. Overall, creepers were the dominant species.

Site 4: Esslinger Park.

The presence of a great silt and gravel mixture at this point made for decent fish and mussel habitat. There was a good johnny darter population here. Also, rainbow darters, and possibly a fantail darter were present. Having good darter populations is important for a variety of mussels including the ellipse and slippershell. Live elktoe mussels were found here and this should be monitored over time as they were uncommon within the AOC. Overall, the area is a good site and should be protected from future in-stream destructive projects, as well as monitored for any future bridge work. One easy restoration project that could be conducted is to add more buffer to the parks near stream uplands.

Site 5: UW-Sheboygan
There was a fair amount of cobble in this section and the hard substrate structure of much of the point made it hard to find mussels. The unit's survey went up to the most eastern bridge. This site should be monitored if bridgework is completed in the future because there are live mussels. The population was lower than expected, but downstream and upstream of the bridge are probably better sections to conduct future surveys.

Site 6: HWY CTH A River Wildlife Area
The water was slow in this stretch but not stagnant. It has a great structure of gravel and silt with areas of cobble. When I first entered the river I thought it would be a floater, creeper, and fat mucket only section with not much else inhabiting the site. However, that quickly changed as I moved upstream. The point's habitat diversified and it became a tremendous spot around the bend. This point should be a future survey area to monitor if a monitoring program is established. This area is also a great area to see where exposed and eroding clay /silt banks exist. The eroding banks here are an example of the makeup of much of the watersheds terrestrial soils. The erosion in this unit is not horrible and does not need to be addressed. It is only mentioned as an example. While there was a tremendous amount of clay in suspension I was able to find mussels with my hands, which has lead me to the conclusion that the site may have a much higher mussel population than observed on that day. Relict elktoes and ellipse were found here. Future inventories should look to locate live point records for these species here.

Site 7: River Wildlife Maintenance Sheds.
This site had the most diverse substrate of all the sites and supported a decent living mussel population. There were spots with sand dominated layers adjacent to silt layers, along with exposed flats of both sand and silt that supported mussels on their edges in normal and high flow periods. This area exhibited a fair amount of mussel movement as individuals responded to the stress of reduced water flow. In higher water periods, the edges of these sand, gravel and silt flats should hold mussels. There are some minor erosion issues near the golf course that are occurring. Remediating these issues could be a focus of future restoration efforts.

Site 8: River Wildlife Lodge
Upstream from this point around the bend the water slows down and white heelsplitters are the dominant species. The substrate was ideal throughout the stretch with some unsuitable cobble areas in the main part of the channel. Overall it's a good spot to find mussels. One live elktoe was found and a relict ellipse was found. This spot is not in need of any restoration and should be included in any future monitoring program for the rivers mussel community.

Site 9: Below Impoundment River Wildlife Area
High water velocity and cobble substrate made finding mussels very difficult. The salmon were quite active here spawning and mucked up the water in the lower stretch of this point while I was surveying as well. I believe further downstream the mussels populations and richness would be higher and easier to survey for.

Site 10: Above Dam impoundment River Wildlife Area.
This unit is stagnant water behind the dam. It’s not unlike most impoundments in that it has been a silt trap during the life of the dam. At this time it was hard to find dead or living mussels. Some live shells were found at the most upstream part of the point that I reached at the end of the survey. Only two species were found in total and the dead shells consisted of the same species.

Site 11: River Wildlife Area Horse crossing(stables)
During the reconnaissance phase the spot looked ideal, but it produced fewer live specimens than anticipated. There were a high number of johnny darters and the habitat looked decent for ellipse and slippershells. This general area should be monitored in the future possibly going upstream and downstream from this point to find these species of concern. The point had a fast rate of flow and coupled with some cobble and a hard substrate, the area was a less desirable point than most in the AOC.

Site 12: Rochester Park
Great mix of gravel and silt at this point. With the Onion River meeting the Sheboygan River just upstream, this point should be a future monitoring point. This point should also be an area where new species may be picked up over time. The buffers along the stream at this park are good but could be improved by leaving more areas unmowed. Living elktoes were found here and they should be monitored in any future projects that are undertaken.

Site 13:Fall View Park
Pill clams (*Sphaeriidae*) were really abundant in this stretch of stream. This stretch might be a good area for future fish surveys as darters, bluegills and multiple northern pike were observed. The site had a good diversity of substrate but the velocity of flow, hardness of much of the substrate plus some bedrock made most of the habitat unsuitable for mussels. Points nearby downstream should be chosen for future monitoring as they should be more suitable for mussels.

Site 14: Settlers Park
The whole stretch was bedrock covered in spots by a thin layer of sand, gravel, cobble and silt. One small dense silt patch had enough thickness to support one floater mussel. This unit should not be monitored in the future for mussels as most of the unit is unsuitable for mussels.

**Community Health (appendix II)**

Observations were made and information was collected and compiled to give a ranking of the point’s mussel community health. This data is located in **(appendix II).**

General Observations of Mussel Populations and Habitat.

*Low numbers of juvenile species seen (white heelsplitter, fat mucket, and plain pocketbook). Low juvenile individual numbers were collected.
*The park land beside the river has very little upland vegetated buffers.
*The whole river upstream from Taylor ave. to Sheboygan Falls has a good possibility for mussel populations
*Overall the turbidity made finding mussels hard most of the season. Moving your hand through the substrate almost always produced a cloud of sediment that unless you were in faster moving water destroyed your visibility.
* A typical wading/ snorkel survey lasted from 1.5 hours to a little over 2 hours.

**Discussion**

The Sheboygan AOC's mussel population has probably gone through similar historical threats that many of the Wisconsin's mussel populations have experienced. One of the common historical disturbances was siltation from agricultural practices. Any siltation from agricultural practices is occurring mainly upstream from of the AOC today.

Other possible common historical threats include stream channelization or ditching of tributaries, river dredging, wetland drainage and field tiling that leads to rapid water runoff, bank erosion, streambed destabilization, commercial harvesting, loss or reduced population of host fish, and water quality degradation.

The last common historical threat that needs to be mentioned is the influence of dams. Dams are often cited as a major threat to mussels and are mentioned here because there are two dams located within the AOC. Dams have fragmented river connections, formed silt-laden impoundments, increased sediment loads upstream, erode habitat downstream and restrict fish distribution. Because dams have done this and even more damage to river systems, they are often removed to restore a river's natural flow. This practice however can be a threat to the mussel that exist below the dam because toxic sediment can be distributed and released downstream. If removing a dam is a restoration goal, removal of the sediments behind the dam need to be considered.

The river has many current problems and possible future threats. A few are listed here to remind the stakeholders of basic issues that may impact mussels. Today, contaminants from urban runoff are one of the mussel population's main threats. Another common issue is predation from inflated mammalian predator populations. Not unlike most of Wisconsin, predation by mammals like raccoons and muskrats has increased as trapping by humans has declined. Also mammals have adapted to our habitat alternations and large predators have decreased increasing these mammal populations.

The introductions of invasive species are going to impact mussels and already have. Zebra mussels have been found in the harbor of the city of Sheboygan but no zebra mussels were found during this project. Other invasive species like rusty crayfish, black carp (Asian carp), and the quagga mussel could impact future native mussel populations. The influx of new parasites and diseases brought on by our increased ability to move water and organisms from all over the world to new locations may influence mussel populations negatively. Climate change is another issue that needs to be mentioned as there are endless threats and management issues regarding this change.

In the AOC, five issue stand out as having potential major impacts on the current mussel populations and they are mentioned below to bring awareness to these threats.

**The watershed soil**. The river is quite turbid from suspended fine silts and clays from the upstream and adjacent terrestrial salty clayey loams and silt loam soils. This sediment stays suspend in the water column for a significant time period after rain events and has the potential to negatively impact mussel populations. The direct burial of mussels by sediment in slow flowing water where silt can settle out probably has happened in spots along the river and any action to reduce siltation would help the AOC's mussel population. Habitat alteration was observed in multiple spots as fine silts had filled in spaces where gravel and small rocks existed. While some species can tolerate this process others species habitats are totally destroyed from this disturbance.

**Past pollution and sediments.** The degree to which past pollution is affecting mussels is unknown for the river. It's easy to understand that some mussels have died instantly because of current or past pollution events and that some may have died over time due to pollution. What is most important now is the ability to understand why some mussels are not having reproductive success or recruitment success from juveniles. If juveniles are actually in low numbers, it might be important to take into account that past and current pollution can affect the endocrine system of mussels. These disruptors may influence the reproduction of fish host and mussels alike. Very little is known about how toxins affect growth, reproduction, and behavior of mussels at sub lethal doses. Just as some of the new toxins of today may influence mussel populations, contaminants from the past like metals, PCB's, and polyaromatic hydrocarbons could impact current and future mussel populations. These pollutants concentrate higher in the sediments of aquatic system as most are minimally soluble in water. What can make these pollutants even more damaging is their tendency to stay locked up in the sediments of these systems. This could be detrimental to juvenile mussels that carry out their life living in and feeding on the sediment of the river.

**Dissolved oxygen and juvenile mussels.** As it has been stated before, there were very few juveniles observed during the survey. Of these juveniles, many were more degradation tolerant species. Unlike adult individuals, juveniles cannot tolerate low dissolved oxygen. This may be one of the many reasons why no living mussels were found or would be hard to find in a section like Point #1. If future surveys are conducted, more time should be spent searching at a spot like Point #1 because low dissolved oxygen readings are expected (review past fish or other current studies to verify this). In the lower stretches of the river algae blooms were more common and it would be easy to assume that the impacts from upstream agriculture, adjacent overland flow and even the yearly event of mass die-offs and decomposition from salmon could all contribute in the degradation of this section and decrease in the available dissolved oxygen.

**High ammonia-** This is another issue of recruitment and juveniles. Ammonia is vey toxic to mussels and it is typically found more often in sediments rather than in the water column. It is generally believed that ammonia has increased in aquatic systems over the past century. Along with low dissolved oxygen, high ammonia concentrations in the sediment may reduce or be responsible for possible recruitment failures of mussel species over time. Beside the usual culprits of excessive nitrogen loading into rivers, the Sheboygan River undergoes a major die-off event that many Wisconsin streams do not. The die-off of Lake Michigan's non native salmon and trout species may be a rather small portion of the total nitrogen make up but with more stocked fish decomposing more ammonia may build up in the sediment. This along with the effects of lower dissolved oxygen that would take place due to mass decomposition from the die offs could have an effect on juvenile mussel success in the past and future. While the fate of mussels is not directly tied to the introduction of non- native salmon it is mentioned here and should be taken into consideration when future plans or research is conducted because the die offs are an event that is rather new to the system. The role these die-off's play on overall biotic health, dissolved oxygen and ammonia should be a future research focus. While there is no proof that these die-offs impact mussels it is brought up here to draw attention to the possible increase in total ammonia in these systems due to the increase of organic matter.

**Non -point source pollution and buffers.** Non-point source pollution from overland flow is going to happen due to the location of a major city along the river. The goal here at the very least should be to create more vegetated buffers along the stream, protect the wetlands in the watershed, create rain gardens for retention of water and to foster education, and educate the public and government officials about non-point pollution.

Management Recommendations Summary:

1. Analyze, monitor, educate, and create projects that minimize non-point pollution and reduce overland flow. Examples include: buffer strips, rain gardens and no-mow zones in parks.
2. Work with agricultural stakeholders upstream from Sheboygan Falls to see if any proactive riparian and soil conservation is needed and can be conducted.
3. Collect data on dissolved oxygen throughout the AOC, and ammonia concentrations in sediments. This may lead to clues about current in-stream conditions and lead to future ideas and goals for restoration.
4. Like #3, promote future studies on sediments PCB, metal and other contaminants concentration. If recruitment and reproduction seem to be limited these sediments may have to be removed.
5. Maintain good fish diversity and population.

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Appendix II

| Site | GPS | | Community Health |
|---|---|---|---|
| Site 1: Kiwanis Park | 43.751174 | -87.725453 | poor |
| Site 2: Industrial Park. Julson Ct. Wildwoods Islands | 43.743056 | -87.735491 | Fair |
| Site 3: Taylor ave, PP | 43.740763 | -87.742572 | Good |
| Site 4: Esslinger Park | 43.740086 | -87.751087 | Good |
| Site 5: UW-Sheboygan | 43.741324 | -87.758418 | Fair |
| Site 6: HWY CTH A River Wildlife Area | 43.734578 | -87.763331 | Excellent |
| Site 7: River Wildlife Maintenance Sheds. | 43.731429 | -87.771944 | Good |
| Site 8: River Wildlife Lodge | 43.730392 | -87.76525 | Good |
| Site 9: Below Impoundment River Wildlife Area | 43.731937 | -87.782178 | fair |
| Site 10: Above Dam impoundment River Wildlife Area | 43.734489 | -87.783134 | Poor |
| Site 11: River Wildlife Area Horse crossing(stables) | 43.725708 | -87.798624 | Fair |
| Site 12: Rochester Park | 43.724049 | -87.803322 | Excellent |
| Site 13:Fall View Park | 43.727598 | -87.809798 | Fair |
| Site 14: Settlers Park | 43.730826 | -87.81166 | poor |

Community Health: A site was ranked poor, fair good or excellent based on its apparent water quality, flow rate, substrate suitability, historic species survivability, and evidence of reproduction. An excellent site offered all of these and demonstrated both species richness and some evidence of recruitment; a poor site offered little habitat, few individuals, and no evidence of recruitment.

Appendix VI
Species of Concern:
Present
*Alasmidonta viridis Elktoe S.C*
This species is a new record for Sheboygan County and the Sheboygan River watershed. It is essential to protect not only the habitat of the elktoe, but also the white sucker, northern hogsucker, shorthead redhorse, rock bass and warmouth, as they serve as hosts for the glochidia. Including the sites where it was historically and currently found, sites 3, 5, and 11 could be future sites to look for this species. It is quite possible that most of the habitat upstream from CTH A to the dam located within the Black River Gold Course is suitable for elktoe mussel.

*Historically Present*
*Venustaconcha ellipsiformis* Ellipse THR.
Relict ellipse mussels were found at 3 sites and were probably well distributed throughout the AOC in the past. Specifically, site #11 should be a focus if future work is conducted to find this species alive in the AOC. The fact that no live ellipses were found in this inventory does not prove that they are extirpated from the AOC and they should still be prioritized for future planning. Maintaining good populations of rainbow darter, johnny darter and mottled sculpin would be a key focus. High numbers of johnny darters, and high numbers of rainbow darters were observed in various points throughout the AOC.

Possible
*Ligumia recta Black Sandshell S.C*
The host fish include American eel, bluegill, largemouth bass and white crappie. There is a possible occurrence of this species, but unlikely.

*Actinonaias ligaeintina Mucket S.C.*
The host fish include killifish, various sunfish and basses. There is a possible occurrence of this species, but unlikely.

*Pleurobema sintoxia Round* Pigtoe SC
This species has been found in counties near Sheboygan, and is often found along with wabash pigtoe. Maintaining redbelly dace, spotfin shiner and bluntnose minnow populations is important. Bluntnose minnows are a common minnow species and maintaining a healthy population of this species should be possible.
.
*Lasmigona compressa* Creek Heelsplitter SC
The creek heelsplitter has been found in other tributaries to the Sheboygan River. One site to check in the future is where the Onion River joins the Sheboygan River near site #12. Maintaining crappie, spotfin shiner, and yellow perch populations is a basic management requirement.

*Utterbackia imbecillus* Paper Pondshell S.C
This Species may inhabit slower moving water resembling conditions observed at point #10, or above the Sheboygan falls dam. When this species uses a host, amphibians and multiple fish can be used so there is no need to manage for certain fish. This species has

been found upstream of the AOC in the Sheboygan River, but was not recorded in any of the points sampled in 2011 for this project.

*Alasmidonta viridis* Slippershell THR

The known host fish species for slippershells are banded, mottled sculpins and johnny darter. There seems to be a decent johnny darter population in the AOC, but sculpins were not observed during this project. There have been populations of slippershells found upstream from the AOC and it is very possible that this species is still using the AOC stretch. Possible points to look are points, 4, 5, 6, 7,8,11, and 12.

*Villosa iris* Rainbowshell END.

Living populations of this species have been found in a tributary to the Sheboygan River. The known host fish include smallmouth, largemouth bass and rock bass.

Simpsonaias ambigua Salamander mussel S.C.

Unlike most other mussels the salamander mussels host is the mudpuppy (*Necturus maculosus*). If locations are found with living mudpuppy populations mussel surveys should be done to see if salamander mussels are also present.

Appendix VII
Important areas for mussels within the AOC.
Site #1- This site is important to monitor and survey for mussels over time. Live mussels or future populations may exist if the water quality improves. Low dissolved oxygen may be a long term issue for mussel survivability and recruitment.
Site #2 - Is a good site to monitor the impacts of industry adjacent to the river.
Site #3- Has a decent mussel population and restoration work could be done on the terrestrial land adjacent to the river to reduce the overland flow of water. Any dredging and streambed work will be very damaging to the mussel population.
Site #4- Has a good mussel population and has multiple live elktoe mussels currently using the stretch. In-stream projects and dredging should be minimized and monitored and future bridge projects should be aware of this population.
Site #5- All road and bridge projects should be made aware of the mussel population that exists here.
Site #6 Great site to conduct long term mussel monitoring to assess the rivers integrity. This stretch should be in any long term monitoring plan for mussels.
Site #8- Live elktoe found here.
Site #9- Below dam there may be decent mussel populations downstream from here due to the barrier for fish. Lots of cobble and boulders and fast moving water make the immediate area poor quality habitat for mussels.
Site #10- Is a ponded area created by the dam. It may hold different species and provide habitat for a species like the paper pondshell.(*Utterbackia* imbecillis)
Site #11- This site could be a good site to find live sllipershells or ellipse mussels. A second wading survey was conducted to look for both but did not find any live or relict specimens. This would be a site to look at again for these two species if monitoring continues.
Site #11- Great site to include in any long term monitoring project for mussels. With the Onion River joining the Sheboygan just upstream from this spot, there is a potential for new species to be added to the list for the AOC.

# Appendix F – Lower Sheboygan River Restoration Area of Concern Mussel Inventories

# *Lower Sheboygan River Restoration Area of Concern Mussel Inventories*

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June 27, 2017

Wabash Pigtoe. *Fusconaia flava* (Jason M. Dare)

Technical Report#17008DEM

## Executive Summary

Mussels are very important components of aquatic ecosystems. They can be long-lived filter feeders (20+ years) and highly sensitive to changes in water quality, habitat degradation, and the presence of contaminants. The primary goals of this project were to determine the presence and distribution status of native unionid mussel species, create a species list, develop a quantitative monitoring point and locate areas were suitable habitat exists for mussels in the Lower Sheboygan River Restoration AOC. These surveys along with other species surveys will determine if the Lower Sheboygan River Restoration AOC is meeting determined delisting targets for the various beneficial use impairments (BUIs). Qualitative and Quantitative surveys were conducted in the Sheboygan River in the summer of 2016. Some of these sites visited in 2016 were surveyed in 2011. Of these sites surveyed qualitatively 3 of the five sites were also surveyed in 2011. Eleven native mussel species were found within the Lower Sheboygan River Restoration AOC during this survey. Three of these were found only as relict shells. The qualitative data collected adds to the 2011 preliminary qualitative data and provides an initial baseline for the long-term monitoring of the Lower Sheboygan River mussel communities. The 2016 quantitative data provides a baseline for future surveys to occur and can be used for possible comparison. Additional live populations may be present within the AOC that were not detected or surveyed in this study. Future inventories should focus to add data to fill in these distribution and presence gaps. The Eight species found alive were the White Heelsplitter, Floater, Creeper, Elktoe, Plain Pocketbook, Fat Mucket, Wabash Pigtoe, and Fluted Shell.

## 1. Introduction

Of all the faunas, freshwater mussels are the most vulnerable in the world. 73% of all mussel fauna are possibly extinct or imperiled (Master 1990). In Wisconsin, 55% of the native freshwater mussel (28 of 51) species are listed as endangered (12), threatened (7), special concern (6), or extirpated (3) (WDNR 2003). They also have considerable economic and cultural value, are used for ornamentation (e.g., buttons, pearls), food and tools (hoe, bowls, spoons; Machtinger 2007, Watters et al. 2009). Factors thought to be responsible for their decline include over-harvest, siltation, channelization, habitat alteration, pollution, and competition from exotic species. Mussels filter-feed on detritus, zooplankton, algae and bacteria, which they extract from the water by creating a current with cilia on their gills, (which are much larger than is needed for respiration) through the inhalant aperture. Juveniles feed on interstitial nutrients using cilia on their foot, gills, and mantle for several years before changing to a filter-feeding mode (Tankersley et al. 1997). Adults are typically partially buried, with the posterior edge of the shell exposed during much of the year, rendering them susceptible to predators, desiccation, temperature and other environmental extremes. Some species have life spans of 20-30 years or more, and may spend much of their life buried several centimeters within the stream sediment, relying on water to percolate between the substrate particles for food and oxygen. The creation of sperm and eggs is initiated by changes in water temperature and/or light levels. There seem to be temperature thresholds or light levels that prompt reproduction (Watters 2009). Sperm is transferred between sexes by the water current during a typically annual breeding season. Nearly all freshwater mussels are obligate vertebrate parasites as larvae, mostly on fish. The Salamander Mussel (*Simpsonaias ambigua*) however is one species believed to use exclusively a non-fish host, the Mudpuppy (*Necturus maculosus*; Howard 1915, 1951). For this reason, mussel conservation is closely tied to conservation of their aquatic host species (mostly fish), many of which are also in decline (Marshall and Lyons 2008). Mussels are also especially sensitive to contaminants (Watters et al. 2009), which have been a pervasive problem in many urban streams in the historically industrial Midwest. Because they accumulate toxins in their tissues over their sedentary lives, they can be useful bio indicators to monitor contaminant levels and assess aquatic community health (Phillips 1976, Tanabe et al. 1987, Gulf of Maine Council 2004). A number of strategies can be employed to address mussel conservation, including dam removal, pollution abatement, propagation, translocations, repatriation, habitat improvements, predator control, and invasive species management.

## 2. Methods

A number of survey protocols have been used to develop species lists and assess mussels in the Midwest (Piette 2005). One qualitative protocol was used to obtain presence data during this survey. The protocol used was developed by the author in 2012 and has different periods of the survey that must be completed. These periods and how one proceeds are determined by detection times. We conducted reconnaissance trips in the summer of 2016 to locate suitable mussel habitat in the AOC reaches of the Sheboygan River trying to focus more points near where restoration work had been conducted. The survey area ranged from New Jersey Avenue to Esslinger Park. Five qualitative and two quantitative sampling areas were chosen within the river based on the presence of suitable mussel habitat and accessibility. (See Table 1)

Table 1 Survey Site Locations

| | |
|---|---|
| Site 1 *New Jersey Ave* | 43.747084 87,729842 |
| *Site 2 Wildwood Is.* | 43.744645 87.732562 |
| *Site 3 Julson Ct.* | 43.743102 87.736186 |
| *Site 4 Taylor Ave.* | 43.740809 87.741551 |
| *Site 5 Esslinger Park* | 43.740396 87.748142 |
| *Site 6 Quantitative 1* | Start 43741031 87744914<br>End 43740966 87745401 |
| *Site 7 Quantitative 2* | Start 43741032 87744248<br>End 43741034 87744447 |

Selection of sampling areas and all surveys were performed by Jason M. Dare, Principal Ecologist of Dare Ecosystem Management, LLC. Selection of sampling areas was discussed with Rich Staffen, Camille Bruhn and Victor Pappas of the WI DNR. We did not perform comprehensive surveys of all suitable habitats within the Sheboygan River AOC. Only representative areas were sampled due to time, budget and accessibility constraints.

Surveys consisted of timed qualitative, searches of all likely mussel habitats. At each station, timed shoreline searches were conducted to locate dead mussel shells drifted onto or near shore from past high waters, or in mammalian middens (mounds of shells left behind by predators after eating mussels). Each terrestrial assessment lasted a minimum of 10 minutes on each shoreline. If a high number of shells would be found or the investigator thought that the habitat may support additional species, the surveys would extend to 30 minutes. After the shoreline search was conducted and survey starting points were determined, a qualitative wading/snorkeling survey was completed. The start of the survey began at the base of a riffle or in a run habitat and proceeded upstream. For (Dare 2012), a standard one-hour search time was done in period one. If after a half hour the surveyor has not found a relict mussel or live mussel and feels the habitat is not suitable the survey can end. Otherwise after the first hour of surveying, a detection time is determined for the last new live species detected in period one. This determines how long the surveys search is for period two. So, during the first period, if the last new mussel species was found at minute 35, the second survey period (and possibly the whole survey) must last at least 35 more minutes. During this second period, if a new live species is detected, the investigator must go another half hour after every new live species is found. For example, if in this second period a new mussel is found at minute 15, the survey must go another half hour after the fifteen minutes. If no new live species are found during the time in this period, they can stop.

Examples for Dare 2012 protocol:

Example 1: First period (last detection time 35 minutes), Second period (new species found minute 1), no new species are found after minute 1. However, the survey must still go four more minutes to be completed. Total time 1 hr 35 min per surveyor.

Example 2: First period (last detection 44 minutes), Second period (no new species found in 44 min) Total time= 1 hr 44 min per surveyor

Example 3: First period (last detection 20 minutes), Second period (new species found at 15 minutes), surveyors go half hour past fifteen minutes (no new species found). Total time 1 hr 45 min per surveyor.

Example 4: First Period (last detection 40 minutes) Second period (new species found at 35 minutes), surveyors go another half hour (no new species) Total time 2 hr 05 min per surveyor.

Quantitative surveys consisted of a 25-meter-long transect, where 2 meters was searched on either side of the transect along its length. The sediment was disturbed and excavated often to locate mussels that could not be seen at the surface. With this survey, you have a unit of m2 from the distance and width.

After the mussel survey was completed at each sampling area, water depth, water temperature and the location was recorded using a GPS unit (Garmin 450). As the survey was conducted and while collecting the physical data, other notable habitat and biological observations were recorded when observed.

## 3. Results

### 3.1 Survey Results

Eleven native mussel species were found among the seven sampling station areas within the Sheboygan River restoration areas. (Table 2). Of the eleven-species found in 2016, three were not found alive: Spike, Cylindrical Papershell and Fragile Papershell. The Eight species found alive were the White Heelsplitter, Floater, Creeper, Elktoe, Plain Pocketbook, Fat Mucket, Wabash Pigtoe, and Fluted Shell. The major abundance of live mussels found was documented at stations (#4) and (#5). This could be partially due to the past issues with contaminated sediment and more suitable substrate. However, one major factor was the difference in the effort required to locate mussels in these stations (#4, #5) verse downstream stations that were in deeper, more turbid water.

Table 2: Survey Results

| Species | Common Name | State Status | Site 1 | Site 2 | Site 3 | Site 4 | Site 5 | Site 6 | Site 7 |
|---|---|---|---|---|---|---|---|---|---|
| *Lasmigona complanata* | White Heelsplitter | | x | x | 2 | 20 | 12 | 9 | 11 |
| *Strophitus undulatus* | Creeper | | | | | 12 | 10 | 8 | 7 |
| *Elliptio dilatata* | Spike | | x | x | | x | x | | |
| *Pyganodon grandis* | Giant Floater | | | | x | 7 | x | | |
| *Alasmidonta marginata* | Elktoe | S.C. | | | | 1 | 2 | | |
| *Lampsilis cardium* | Plain Pocketbook | | x | | | 21 | 22 | 10 | 19 |
| *Lampsilis siliquoidea* | Fat Mucket | | x | x | 4 | 10 | 22 | 6 | 15 |
| *Fusconaia flava* | Wabash Pigtoe | | | | | 1 | x | | |
| *Lasmigona costata* | Flutedshell | | | | | 2 | | | 1 |
| *Anodontoides ferussacianus* | Cylindrical Papershell | | | x | | | | | |
| *Venustaconcha ellipsiformis* | Ellipse | THR. | | | | | | | |
| *Leptodea fragilis* | Fragile Papershell | | | | | x | x | | |
| | | | | | | | | | |
| | Total live mussels found per site | | 0 | 0 | 6 | 74 | 68 | 33 | 53 |

#s= Live individuals; X = relict shells only; THR = Wisconsin Threatened Species; SC= Special Concern Species

### 3.1.1 Site 1: New Jersey Ave.

This survey point was chosen as a back up to conducting one at Kiwanis Park due to the extreme difficulty to observe anything in the waters at the park. As will be mentioned again below, Kiwanis Park was the most difficult area to survey and after a half hour of tactile surveys and not locating a relict mussel, the survey was ended and moved upstream. New Jersey Avenue's visibility was better, but not drastically improved. The site has deep holes that cannot be surveyed by snorkel and the depth to substrate was hard to survey. The turbidly and the velocity of the river made surveying this stretch difficult. However, it was easier to conduct the survey than at Kiwanis Park. No live mussels were found and four relict species were collected during the survey. There is good substrate for mussels and populations of poor water quality tolerant species like White Heelsplitters, Floaters, and Creepers could exist in this substrate among other species. Surveys were not conducted at this point in 2011 to compare.

### 3.1.2 Site 2 Wildwood Island

In 2016 survey locations were conducted downstream form Julson court to better establish a baseline for future surveys of the Wildwood Island restoration area. The overall habitat should be better for mussels in the future, but the stretch still provided many of the difficulties that all the downstream units from Taylor avenue had. Depth to substrate, turbidity, clarity, and velocity of the river made searching for mussels difficult. There were areas near the island where searching was easier and should provide good substrate for mussels, but no live mussels were observed. Much of this substrate will be very suitable for White Heelsplitters, Floaters, and Creepers. Relict mussels observed were the White Heelsplitter, Spike, Fat Mucket, And Cylindrical Papershell. This point was not surveyed in 2011 and so this data is the baseline for future studies.

### 3.1.3 Site 3 Julson Court

This site had below average habitat, but live mussels existed. The substrate was covered with a thin layer of silt where gravel did exist. Most of the substrate was dominated by silt. Even so, gravel and silt intermixed with a small percentage of sand provided some substrate for mussels. The rocks that were found in the unit often looked like mussels because of the thin layer of clay silt on them. This lead to a lot of searching only to conclude it was a rock. Some areas along the substrate were too hard and compacted for mussels to get established but most of the section is physically available for mussels. As with the rest of the downstream survey, this stretch was difficult to survey. However, the north end of the island located here has the best visibility within the stretch. One dilemma is once you locate what looks like a mussel you reach for it and a plume of clay is created when you remove it from the sediment and often it will be a rock. Due to depth to substrate, turbidity and velocity of the river locating mussels is difficult. The site is adjacent to an industrial park and effluent is leaving the parking area via underground pipes. Algal blooms were also very prominent throughout this survey point. Consequently, this point would be a good point to monitor if the local water quality is improving, and if more uncommon unionids begin to re-colonize this stretch in the future. The total number of mussels observed in 2016 was down from 2011.Two White Heelsplitters were observed in 2016 compared to Seventeen in 2011. Four Fat Mucket were observed in 2016 compared to 1 in 2011. However, no Creeper or Floaters were observed in 2016 alive as they were in 2011. The point was a little harder to survey in 2016 because the depth of the river had increased in 2016. Future surveys should be conducted by scuba if other points will be surveyed as well via this technique.

### 3.1.4 Site 4 Taylor Ave

This site has good habitat within the lower AOC restoration areas for mussels. This section total number of live mussels found went up in 2016 compared to 2011. All species total numbers went up on this site except for Creeper and Floater. The White Heelsplitter, Fat Mucket and Plain Pocketbook observations increased in 2016. Three new species were found alive in this stretch in 2016 the Elktoe (special concern species), Fluted shell, and the Wabash Pigtoe. One new species to the river was found as a relict in 2016 the Fragile Papershell. The Spike was once again not found alive during 2016 surveys. The substrate was dominated by cobble however a good even consistency of silt sand and gravel existed in between the rocks and made for good substrate for mussels. The site is a good area to use to educate people about mussels. The site has a similar turbidity as most of the lower AOC section with a cloudy water column from the clay sediments. Although this site and upstream are slightly less turbid than the downstream survey points. Fish were observed but not in high numbers. The land adjacent to the river has been planted to a recreated prairie which should help to collect more overland runoff.

### 3.1.5 Site 5 Esslinger park

This section provides a great mix of silt and gravel in much of the stretch. The presence of fish is good and darters were seen often during the survey. The total number of live mussel was up in 2016 compared to 2011. This was mostly due to an increase in Plain Pocketbook and Fat Mucket individuals located in 2016. All other species, White Heelsplitter, Creeper, Floater, Elktoe, were observed less in 2016 than 2011. One new species was found in the river, the Fragile Papershell in 2016 as a relict. The upstream portion of this section is better for mussels than the downstream. However, in 2011 alive Elktoe were found in the downstream portion of this stretch and more effort was placed there this year to see if that substrate and habitat produced more of them and other species of interest. It would be better to start more upstream in future surveys to assess the mussels for this section, as this downstream portion turned out to not be as good of substrate for mussels. This area that can be excluded is closer to the bridge for future reference. A higher total number of mussels probably would have been collected if this 2016 survey would have started further upstream. The riparian area next to the river was left un-mowed and provided more erosion control, siltation reduction, and nutrient retention along the river. This was a restoration project that was easy to recognize the reduction of erosion.

Site 5 Esslinger Park (Jason M. Dare)

### 3.1.6 Site 6 Taylor Ave Quantitative 1

This quantitative point was conducted upstream of the Taylor Avenue bridge. It was started 60 meters from the bridge. The survey was started about 10 meters to the south of the most northern bridge footing that is in the water. For another visual cue, this survey was started where a Box Elder tree on the south bank near a Wood Duck box exists. It ended upstream lined up with an Ash tree on the south bank. The Plain Pocketbook was the most abundant followed by White Heelsplitter, Creeper and Fat Mucket.

### 3.1.7 Site 7 Taylor Ave Quantitative 2

This quantitative point was conducted downstream of the Taylor Avenue bridge. It was started 25 meters from the bridge heading upstream. To center the survey for future replication, the surveyor should aim for the northeast corner of the metal observation area located on the bridge. The survey ended right as you get under the bridge. Plain Pocketbook was once again the most abundant followed by Fat Mucket, White Heelsplitter, Creeper and Fluted Shell. One dead Zebra mussel was located near the bridge. It is possible with all the equipment in the river that downstream populations may have been moved upstream with the construction equipment, metal, and tubes in the water. No live or other relict shells were collected. It's possible that a new pioneer population just got started and this should be monitored.

### 3.1.8 Kiwanis Park. (Not an official survey)

An attempt was made to locate mussels again at Kiwanis park in 2016. In 2011 the water was lower and that made the surveys more practical and productive. In 2016 the water depth was almost five feet in places and the turbidity was tremendous. When snorkeling one could only do tactile searches while surveying. Kiwanis Park should be surveyed in the future, but it should be done via scuba.

## 4. Discussion

### 4.1 Overall Health

The mussel community within the lower AOC restoration areas is a moderately diverse and has varying abundance depending on the site. In the upstream portions of the lower AOC restoration areas Esslinger Park and Taylor Ave have good populations of most of the living species. Upstream portions of the river and AOC in general have locations of better water quality than the lower portion of the industrialized river. There are probably hotspots found throughout the river as you move upstream from Esslinger Park. However, Esslinger and Taylor held their own in 2011 and good numbers were found in 2016. The restoration work has provided structurally suitable substrate downstream. If the contaminants are gone, it should in theory provide a better substrate for mussel recruitment.

It is recommended that more surveys be conducted ten years after the restoration and remediation work had been conducted. Mussel populations may take time to recolonize new sediments as fish move them around and they begin to potentially grow. If sediments are contaminated with heavy metals, pcbs, and ammonia, it may be hard for mussel juveniles to become adults. In ten years we should have a better picture if this is occurring within the restoration areas of the lower AOC. More pollution tolerant species like the White Heelsplitter, Floater, Creeper, and Fat Mucket should be able to inhabit degraded stretches of the river if these sediments are not to toxic for their grown and life history. Even nearby present species such as Wabash Pigtoe and Fluted shells can inhabit moderately degraded water quality.

In comparing the lower AOC mussel community to other Southeastern Wisconsin Rivers the lower AOC has been highly impacted by urban degradation. Species such as the Spike and Ellipse were not found alive (Fragile Papershell was not as well but it is probably present alive).The overall mussel health of the lower AOC is not tremendous. It may take time for these areas to rebound. But with average populations upstream, fish should be able to move these species around and recolonization should begin to happen for many of the present live species found at the Taylor and Esslinger survey points.

## 4.2 Present Species of Local Conservation Interest

Elktoe (*Alasmidonta marginata*) (Jason M. Dare)

Four mussel species from the species list were chosen as Species of Local Conservation Interest (SLCI) for the Sheboygan River. This exercise is intended to assist in guiding the development of conservation plans; identifying species which can be the focus of projects; and/or used to evaluate project success through monitoring of their population responses. SLCIs are species that are at least one of the following: a) listed as either state or federally Endangered, Threatened, or Special Concern; b) listed as Species of Greatest Conservation Need in the State Wildlife Action Plan; c) considered to be locally rare or declining; or d) are of social value to stakeholders and considered to be desirable to the community (Casper Dare 2013). Reported habitat preferences for mussels is an area of active research; and many attributes used to describe habitat may ultimately turn out to be of minor importance with a smaller set of critical parameters such as substrate type and stability, dissolved oxygen, temperature regime, and turbidity being the major influences on mussel occurrence. For this reason, we urge caution in applying habitat criteria too rigorously.

### Ellipse (*Venustaconcha ellipsiformis*)

The Ellipse is currently listed as Threatened in Iowa, Minnesota and Wisconsin (NHI 2012, Iowa DNR 2013, Minnesota Department of Natural Resources 2013). This species prefers shallow, flowing, good current, clean, small to medium streams with stable substrate in gravel or mixed sand gravel. It is often found alive within southeastern Wisconsin streams. In larger southeastern Wisconsin rivers where it seems to be declining, as living specimens have not been found during recent inventories on several rivers

(J. M. Dare, personal observations). More surveys need to be conducted to better evaluate its conservation status and population trends. Because it inhabits small streams and headwaters, this mussel is particularly vulnerable to siltation and pollution from runoff. In the Sheboygan River, the Ellipse may benefit from habitat protection and water quality improvements. Conservation should include managing and protecting host darter species populations and habitat. Making sure gravel bars, sand/gravel sediments, and sand gravel deposit areas are not disturbed during any in-stream construction activities is highly important. Where known mussel beds must be disturbed, translocations and habitat restoration or replacement is strongly recommended. The first goal will be to locate living populations of the species in the AOC. Maintaining areas with cobble that provide habitat for darters would be beneficial for Ellipse as well. It may be a candidate for restoration in the Sheboygan River in areas of suitable habitat. This species was found in Kiwanis park as a relict in 2011 and in upstream portions of the AOC in 2011. It was not found alive or as a relict in 2016.

### Spike (*Elliptio dilatata)*

Mathiak (1979) considered the Spike abundant in Wisconsin statewide. It is often found alive within southeastern Wisconsin streams, and was once a very common species, but is now often found in low numbers (J. M. Dare, personal observations). In recent surveys of three large rivers in southeastern Wisconsin, no live Spike were observed (J. M. Dare, unpublished data). In many small streams with water quality issues, no live Spike can be found (J. M. Dare, personal observations) In Minnesota, the Spike has been listed as a Species of Concern since 1996 since it has been found alive in only a small number of Minnesota drainages (Minnesota Department of Natural Resources 2013). The state of Illinois lists the Spike as Threatened (Mankowski 2012). In the Illinois River, the once most abundant Spike is now considered rare or absent (Warren 1995). It is uncommon in the Fox River basin in Illinois and Wisconsin (Schanzle et al. 2004). Stansbury (1965) considered Spike highly intolerant of pollution. It occurs in medium streams to large rivers, primarily in shoal habitat of unimpounded streams and rivers, but can occasionally be found in tailwaters of dams in water 4-8 m deep, and can even be found in lakes under some conditions (Williams et al. 2008). No live individuals were found during this study in 2016. It however was found as a relict at every qualitative point in 2016 except Julson Court. It was found as a relict in 2011 at Julson court as with most every point surveyed in the 2011 Sheboygan River AOC. Although abundant as relict shells, more searching for live Spike is recommended in the Sheboygan River. Because of factors such as declining water quality, it may have been extirpated from the Sheboygan River AOC; or simply have been missed on surveys due to a low detection probability. It is not unusual to find many relict shells of this species, but few to no live individuals (J. M. Dare, personal observations). Since historically it was an abundant and common species statewide, not finding it alive in the 2011 or 2016 survey is a concern. Additional surveys and research are recommended to better evaluate its conservation status and population trends.

### Fragile Papershell (*Leptodea fragilis*)

This species is common in Wisconsin and is not in need of conservation efforts statewide (WI DNR). However, locally the species was a new addition to the species list for the river in 2016. This species is not in need of management or restoration attention but it needs future research and inventory focus. More inventory work should be conducted upstream, specifically past Esslinger Park to determine its distribution in the river and locate live individuals. It is predicted that this species is a more recent inhabitant of these Lake Michigan basin rivers. The host for this species is the Freshwater Drum (Aplodinotus grunniens) (Cummings and Watters 2004). The Fragile Papershell has been found in streams of all sizes in mud, sand or gravel (Cummings Mayer 1992).

### Elktoe (*Alasmidonta marginata*)

The Elktoe is currently listed as Special Concern in Wisconsin (NHI 2012) and Threatened in Minnesota (Minnesota Department of Natural Resources 2013). It is found in various-sized streams with flowing water, silt, mud, sand, gravel, or rock substrates that are stable. The known host fishes include widespread species such as Redhorse, Suckers and Rock Bass. Leaving natural shoreline with vegetation, roots, logs, and natural structures that create stable sediments should help this species. It is also found in mud or silt, as long as the sediments are stable. Restored shorelines that mimic natural shorelines and maintain stable sediments can provide habitat for this species even if conditions are silty. Maintaining or enhancing stable run areas is important for this species. Elktoe were found alive at Esslinger Park and upstream from there at other points in 2011. In 2016 live individuals were observed at the Esslinger Park and Taylor Ave points. While usually not extremely abundant, this species is often found in southeastern Wisconsin streams. This species may be detected alive in more points as future inventories and work is completed in the watershed.

## 4.3 Recruitment

Not much recruitment was observed during the surveys. At Taylor and Esslinger Park, sub adult Fat Mucket and sub adult White Heelsplitter were found. At Esslinger park, sub-adult Plain Pocketbook was also found. The new observation for the river in 2016 is the Fragile Papershell relicts were not old shells or sub adults. But being newer relicts, there is obviously new recruitment going on in the lower part of the river. This species was not found in 2011 or reported in other surveys prior to 2016.

## 4.4 Abundance

By far the most abundant sites within the lower AOC restoration areas for mussels is at Esslinger Park and Taylor Ave. The difficulty to locate mussels downstream is somewhat based on the conditions being harder for a surveyor to see mussels due to turbidity, depth to substrate and velocity of the river. These areas downstream should be given time to recover. Ten years from the time of the restoration work, surveys for mussel populations should be conducted again. As fish begin to use this habitat and populations of mussels can potentially become established, a survey would be useful to see how this taxa group is responding to the restoration work. This survey should be conducted by a surveyor utilizing scuba. This will allow for an easier way to assess the substrate. Snorkel can be used if drought conditions happen to occur in year ten.

## 4.4 Important Areas

Taylor Ave and Esslinger park are the most important areas in the restoration areas of the Sheboygan River. We know stable populations exist there; and if populations are reduced downstream, these areas can serve as a source as fish may take them downstream to recolonize newly created habitats and substrate. The key will be for those downstream sediments to be suitable for mussel growth. If they still contain contaminants or high amounts of ammonia, they may not be suitable.

## 4.5 Population in new habitat

It is hard to definitively say how much of an impact the removal of sediments in the river has produced for mussels at this time. Structurally, the sediments are suitable in these restoration areas. In the Wildwood Island's survey point, there are silt deposits that are building up that should support common species overtime as fish reintroduce these individuals to the areas. As long as the sediments are conducive to juvenile and sub-adult growth, populations can carry out their life history needs. Another assessment should be done in 2021 with scuba to asses if these areas are suitable for mussels.

## 4.6 Threats

### Contaminants and Water Quality

Mussels are very sensitive to contaminants (Havlik and Marking 1987, Farris and Van Hassel 2007). Although the effects of pesticides are often species-specific, in general, sub-lethal levels of PCBs, DDT, Malathion, Rotenone, and other compounds inhibit respiratory efficiency and accumulate in the tissues. Mussels are particularly sensitive to heavy metals (Keller and Zam 1991); and again, responses may be species-specific. Adult mussels may be able to survive short-term exposure through behavioral responses (Keller 1993); but chronic exposure at lower levels may have significant impacts. For example, low levels of metals may interfere with the ability of glochidia to attach to the host (Huebner and Pynnönen 1992). Glochidia are also very sensitive to ammonia from wastewater treatment plants (Goudraeu et al. 1993). At sub-lethal exposures, adult mussels exhibit decreased respiratory efficiency (Anderson et al. 1978). Ellis (1931) discovered that mussels found below sewage outfalls had dead glochidia in the marsupia contaminated with bacteria and fungi. There is circumstantial evidence that salinity is lethal to some glochidia as well, which may be a problem in the AOC from runoff contaminated with salt used on roads in winter (Liqouri and Insler 1985, Anders and Wiese 1993). Urban runoff is one of the main threats to mussels overall, and can be addressed through existing water quality improvement programs.

The degree to which past pollution is affecting mussels is unknown for the river. However, current and/or past pollution events have likely had major impacts on mussels in the Sheboygan River. What we now observe is a post-impact community of survivors. Currently, reproduction and recruitment in some mussels appears to be a problem as evidenced by some species being represented mainly or only by dead shells (i.e. Spike, Ellipse, Fragile Papershell not included). Reproduction and recruitment in these species should be assessed further, through special efforts to find juveniles and sub-adults, to determine if successful reproduction is occurring. More intensive quantitative surveys are also needed to assess population demographics in the AOC. Past and current pollution can affect the endocrine system of mussels (Ciocan et al. 2010). These disruptors may influence the reproduction of fish and amphibian hosts and mussels alike. Very little is known about how toxins affect growth, reproduction and behavior of mussels at sub-lethal doses including the complex mix of endocrine disruptors and pharmaceutical drugs often found in physiologically significant concentrations in urban waterways (Ternes and Joss 2008). Just as some of the new toxins of today may influence mussel populations, contaminants from the past like metals, PCBs, and polycyclic aromatic hydrocarbons could impact current and future mussel populations. Many of these pollutants concentrate in the sediments of aquatic systems, being minimally soluble in water. Exposure to contaminated sediments can be detrimental to juvenile mussels that carry out their life living and feeding in these sediments.

There were very few sub-adult mussels observed during this study. The sub-adult observed were mostly more tolerant species. Unlike adults, juveniles cannot tolerate low dissolved oxygen or high ammonia levels (Goudraeu et al. 1993), so recruitment can be compromised by these water quality problems. Ammonia is very toxic to mussels and it is typically found more often in sediments rather than in the water column (Goudraeu et al. 1993). It is generally believed that ammonia has increased in aquatic systems over the past century. Studies evaluating ammonia and dissolved oxygen concentrations in the AOC would help to address mussel conservation issues. In general, oxygen levels are increased by cooler temperatures and mixing with air, such as in rapids. They are decreased by bacterial contamination, such as sewer overflows. Shading banks with overhanging trees and shrubs, implementing water quality improvements that reduce runoff and sewer overflows and maintenance of rapids would all benefit mussels.

Non-point source pollution from overland flow is a common problem in major cities along rivers. Activities that would benefit mussels include creating more vegetated buffers along streams, protecting and restoring wetlands to filter water before it enters streams, and creating rain gardens for retention and infiltration of water. Educating the public and local officials about non-point source pollution is also important to success. Due to the soil types common in the watershed, the river can be turbid from suspended fine silts and clays after rain events. Fine sediments can stay suspended in the water column for a significant time period; and have the potential to negatively impact mussel populations by clogging gill membranes and burying interstitial spaces in coarse gravel needed for proper filtration. Habitat alteration was observed in multiple spots as fine silts had filled in spaces where gravel and small rocks existed. While some species can tolerate this process, other species habitats are damaged or lost from this disturbance. Minimizing the amount of erosion of upstream and riverine corridor soil is therefore an important conservation action that would have substantial benefits.

### Predation

Elevated predation levels from inflated mammalian predator populations such as Raccoon (Procyon lotor) and Common Muskrat (Ondatra zibethicus) can be highly detrimental to native mussel populations, significantly suppressing or even eliminating them (Neves and Odom 1989). Such human-subsidized predators can become super-abundant in urban areas, where they take advantage of abundant food and shelter (e.g. garbage and gardens, buildings), and their natural predators are largely absent. While Common Muskrat do not appear to be abundant in the Greenway, Raccoon are. Trapping can be effective in controlling these mammals, but is problematic in urban settings where there may be social value conflicts and safety issues. Research into the extent of Raccoon predation on mussels in the AOC, along with testing socially acceptable means of suppressing such predation through trapping or deterrent programs may be productive.

### Non-native Invasive Species

Many non-native invasive species could impact mussels in the AOC (Strayer 1999). Zebra Mussel, Quagga Mussel, and Asian Clam could all possibly invade. There is little evidence to support the idea that Asian Clams are directly detrimental to native mussels (Strayer 1999), but they may compete for food and ingest the gametes of native mussels. Zebra and Quagga mussels belong to the family Dreissenidae (“false” mussels), and are highly detrimental to native mussels (Strayer 1999). Zebra Mussels can form a pavement on gravel substrates such that native mussels are dislodged and cannot rebury themselves. They congregate on native mussel shells interfering with food and oxygen uptake. Their extremely strong byssal threads may fasten the two shells of native mussels together so they cannot open. Clusters of Zebra mussels attached to the ends of native mussels may create drag pulling the native mussels out of the substrate where they are swept ashore to die. Up to 10,000 Zebra Mussels have been found on a single native mussel; and once they arrive, they can spread extremely rapidly. In the Mississippi River, colonization rates of Zebras on natives increased from 27% to 99.7% within a year (Tucker 1994). At sub-lethal levels of infestation, native mussels experience lowered glycogen levels and increased stress, resulting in decreased fitness (Haag et al. 1993). The first individual Zebra mussel was found at Taylor Ave in 2016. This area should be monitored to decrease this population.

Non-native Common Carp are also present in the AOC and are damaging mussel habitat by uprooting vegetation, destabilizing substrates, and disturbing sediments. Control and management of this and other non-native fishes is problematic; often involving chemical (i.e., Rotenone) applications, which may also damage many mussels and their native host fishes. Effective management of Common Carp while avoiding damage to native mussels and host fishes is needed.

### Parasites and Diseases

The influx of new parasites and diseases brought on by our increased ability to move water and organisms from all over the world to new locations may also influence mussel populations negatively. This has been especially problematic in the Great Lakes. While control of human behavior is always difficult, education may be effective in reducing the spread of invasive species, parasites and disease. Control of ballast water release in the Great Lakes shipping industry would also be extremely beneficial in limiting future threats.

### Climate Change

Climate change is another pervasive issue that will impact aquatic communities. In Wisconsin, the effects of climate change are expected to be greater flashiness (more extreme weather events), warmer temperatures, and lower water flows in summer. These processes are already underway. These climate impacts are expected to increase in the coming decades (Wisconsin's Changing Climate: Impacts and Adaptation 2011); and efforts to limit the pace and extent of climate change would have many benefits for mussels and other organisms.

## 4.7 Historical Data

There were three periods of surveys conducted for mussels in the Sheboygan River. 1996, 1999, and 2003 were the repective years. The separate years species list are below.

Downstream of the village of Kohler-1996

Fatmucket Lampsilis siliquoidea

Cylindrical papershell Anodontoides ferussacianus

White heelsplitter Lasmigona complanata complanata

Creeper Strophitus undulatus undulatus

Sheboygan River Mussel Community Assessment – 1999.

Mussel Species Upstream of Sheboygan Marsh and Dam Manitowoc Co. Upstream of Kiel Marsh and Dam Sheboygan Co. Downstream of Millhome Dam and upstream of Franklin Dam Downstream of Franklin Dam and upstream of Johnsonville Impoundment Downstream of Johnsonville Dam and upstream of Sheboygan Falls

Fluted-shell Lasmigonta costata

Fatmucket Lampsilis siliquoidea

Giant floater Anodonta grandis grandis

Wabash pigtoe Fusconaia flava

Cylindrical papershell Anodontoides ferussacianus

White heelsplitter Lasmigona complanata complanata

Creeper Strophitus undulatus undulatus

Plain pocketbook Lampsilis cardium

Slippershell mussel Alasmidonta viridis

Ellipse Venusaconcha ellipsiformis

Various surveys upstream of Sheboygan Falls- 2003

Deertoe Truncilla truncate (most likely misidentified)

Fatmucket Lampsilis siliquoidea

Giant floater Anodonta grandis grandis

Wabash pigtoe Fusconaia flava

Cylindrical papershell Anodontoides ferussacianus

White heelsplitter Lasmigona complanata complanata

Creeper Strophitus undulatus undulatus

Slippershell mussel Alasmidonta viridis

Creek Heelsplitter Lasmigona compressa

There are no known surveys for the lower Sheboygan River except for what was conducted in 2011 by the WI DNR and Dare Ecosystem Management, LLC. The upstream surveys that were conducted in 1996,1999, and 2003 produced a species list that is similar to many rivers of its size in southeast Wisconsin. In the upstream reaches there are species like the Cylindrical Papershell, Slippershell, and Creek Heelsplitter. The downstream list resembles the same species found during the lower AOC survey of 2011.

## 5. Acknowledgments

We express thanks to The WI DNR for funding the project. The baseline inventory of our natural areas and aquatic areas are essential to know how to set goals, plan and manage our local biodiversity. The baseline information provided will help with prioritizing future aquatic decisions in the lower Sheboygan River AOC.

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# Appendix G – Letters of Support for BUI Removal

November 11, 2020

Brennan Dow
Sheboygan River AOC Coordinator
Wisconsin Department of Natural Resources
2300 N. Dr. Martin Luther King Jr. Drive
Milwaukee, WI 53212

Dear Mr. Dow:

The City of Sheboygan is pleased to join the Wisconsin Department of Natural Resources (WDNR) in initiating the process to remove the Degradation of Benthos Beneficial Use Impairment (BUI) from the Sheboygan River Area of Concern (AOC).

The Sheboygan River AOC community partnered with many local, state and federal agencies, non-governmental organizations, business groups, community leaders, and volunteers to clean up toxic sediments in the AOC. From 2011 through 2013, four dredging projects effectively removed over 400,000 cubic yards of contaminated sediment from the river. These included two Superfund projects, a Great Lakes Legacy Act dredging project, and a navigational dredging project designed by the Army Corps of Engineers. These projects resulted in a cleaner, deeper river.

The goals for removing contamination have been met and subsequent assessments provided information indicating that the benthos target is achieved, signifying an overall healthy population. We appreciate the efforts of the many partners who helped to carry out the sediment cleanups and evaluate the status of the benthos community. We concur that the Degradation of Benthos impairment has been adequately addressed and we look forward to celebrating the removal of this BUI. The City of Sheboygan is excited about the removal of another BUI impairment getting the Sheboygan River one step closer to being a valuable natural resource for the future.

Sincerely,

Chad Pelishek

Chad D. Pelishek
Director of Planning and Development

DEPARTMENT OF PLANNING AND DEVELOPMENT

828 Center Avenue, Suite 208
Sheboygan, WI 53081

920-459-3377 (Phone)
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SHEBOYGAN COUNTY
WISCONSIN

# SHEBOYGAN COUNTY

**Vernon Koch**
*Chairman of the Board*

**Adam N. Payne**
*County Administrator*

*11/12/2020*

Brennan Dow, Sheboygan River AOC Coordinator
Wisconsin Department of Natural Resources
2300 N. Dr. Martin Luther King Jr. Drive
Milwaukee, WI 53212

Dear Mr. Dow,

Sheboygan County is pleased to join the Wisconsin Department of Natural Resources (WDNR) in initiating the process to remove the Degradation of Benthos Beneficial Use Impairment (BUI) from the Sheboygan River Area of Concern (AOC).

The Sheboygan River AOC community partnered with many local, state and federal agencies, non-governmental organizations, business groups, community leaders, and volunteers to clean up toxic sediments in the AOC. From 2011 through 2013, four dredging projects effectively removed over 400,000 cubic yards of contaminated sediment from the river. These included two Superfund projects, a Great Lakes Legacy Act dredging project, and a navigational dredging project designed by the Army Corps of Engineers. These projects resulted in a cleaner, deeper river.

The goals for removing contamination have been met and subsequent assessments provided information indicating that the benthos target is achieved, signifying an overall healthy population. We appreciate the efforts of the many partners who helped to carry out the sediment cleanups and evaluate the status of the benthos community. We concur that the Degradation of Benthos impairment has been adequately addressed and we look forward to celebrating the removal of this BUI.

Sincerely,

Vernon Koch
County Board Chair

Adam Payne
County Administrator

Aaron Brault
Planning & Conservation Director

**Administration Building**
Telephone (920) 459-3103
508 New York Avenue - Room 311
Sheboygan, WI 53081-4126
www.sheboygancounty.com

maywood

November 13, 2020

Brennan Dow, Sheboygan River AOC Coordinator
Wisconsin Department of Natural Resources
2300 N. Dr. Martin Luther King Jr. Drive
Milwaukee, WI 53212

Dear Mr. Dow,

The Maywood Environmental Park is pleased to join the Wisconsin Department of Natural Resources (WDNR) in initiating the process to remove the Degradation of Benthos Beneficial Use Impairment (BUI) from the Sheboygan River Area of Concern (AOC).

The Sheboygan River AOC community partnered with many local, state and federal agencies, non-governmental organizations, business groups, community leaders, and volunteers to clean up toxic sediments in the AOC. From 2011 through 2013, four dredging projects effectively removed over 400,000 cubic yards of contaminated sediment from the river. These included two Superfund projects, a Great Lakes Legacy Act dredging project, and a navigational dredging project designed by the Army Corps of Engineers. These projects resulted in a cleaner, deeper river. Maywood worked with UW-Extension and the DNR to conduct volunteer monitoring programs for birds, bats, frogs/toads, mussels, and nest boxes from 2013 – 2018.

The goals for removing contamination have been met and subsequent assessments provided information indicating that the benthos target is achieved, signifying an overall healthy population. We appreciate the efforts of the many partners who helped to carry out the sediment cleanups and evaluate the status of the benthos community. We concur that the Degradation of Benthos impairment has been adequately addressed and we look forward to celebrating the removal of this BUI.

Sincerely,

David R Kuckuk

David R. Kuckuk
Environmental Park Director

# Appendix H – GovDelivery Announcement for Public Comment Period

# Sheboygan River Area Of Concern

## Public Invited To Comment On Proposal To Remove Impairment In Sheboygan River Area Of Concern

*View of Wildwood Island, one of the important habitat restoration projects completed in the Sheboygan River AOC. / Photo Credit: Debbie Beyer*

MADISON, Wis. — The Wisconsin Department of Natural Resources is seeking public comments on the recommendation to remove the Degradation of Benthos Beneficial Use Impairment from the Sheboygan River Area of Concern.

After the Sheboygan River was listed as an Area of Concern (AOC) in 1987, the Remedial Action Plan identified “degradation of benthos” as one of nine environmental problems, called beneficial use impairments or BUIs, in the AOC program.

Communities of organisms that live on or in the bottom sediment of a waterbody are collectively referred to as benthic invertebrates or benthos. These essential creatures are at the base of aquatic food webs, which provide food for a wide array of fish, birds and other aquatic life.

The lower 14 miles of the Sheboygan River downstream from the Sheboygan Falls Dam, including the entire harbor and nearshore waters of Lake Michigan, were identified as an AOC primarily due to contamination from polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs).

These toxins were discharged directly into the river from municipal and industrial sources and settled to the river bottom, leading to many contamination-related impairments within the AOC. Because benthic organisms are in direct contact with the sediment and water, they are harmed by toxins, poor water and sediment quality, low dissolved oxygen, high ammonia and poor substrate conditions.

To address the harm to benthic organisms, several sediment remediation projects were completed to remove the sources of toxic pollutants in the AOC. Monitoring was then conducted to confirm if pollution cleanup and benthic community recovery goals have been met.

The monitoring results showed that removal targets are being met and multiple lines of evidence support a recommendation to remove this impairment from the AOC. The results of these studies, along with support from a team of technical experts, agency partners and stakeholders support this recommendation.

The removal recommendation document is available for public review and comment now until **Nov. 6, 2020**, using this link.

Questions and comments can be sent to Brennan Dow, a Sheboygan River and Milwaukee Estuary Area of Concern coordinator, at brennan.dow@wisconsin.gov or 414-263-8651.

To date, two of the nine impairments have been removed in the Eutrophication or Undesirable Algae and Restrictions on Dredging Activities AOCs. Once all impairments have met their targets and are removed, the AOC can be formally delisted.

The Sheboygan River AOC was designated as one of 43 sites on the Great Lakes with significant environmental damage by the United States and Canada under the

Great Lakes Water Quality Agreement. Federal Great Lakes Restoration Initiative funding, first authorized in 2010, is helping AOCs clean up pollution and restore waterways.