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

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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).

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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

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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

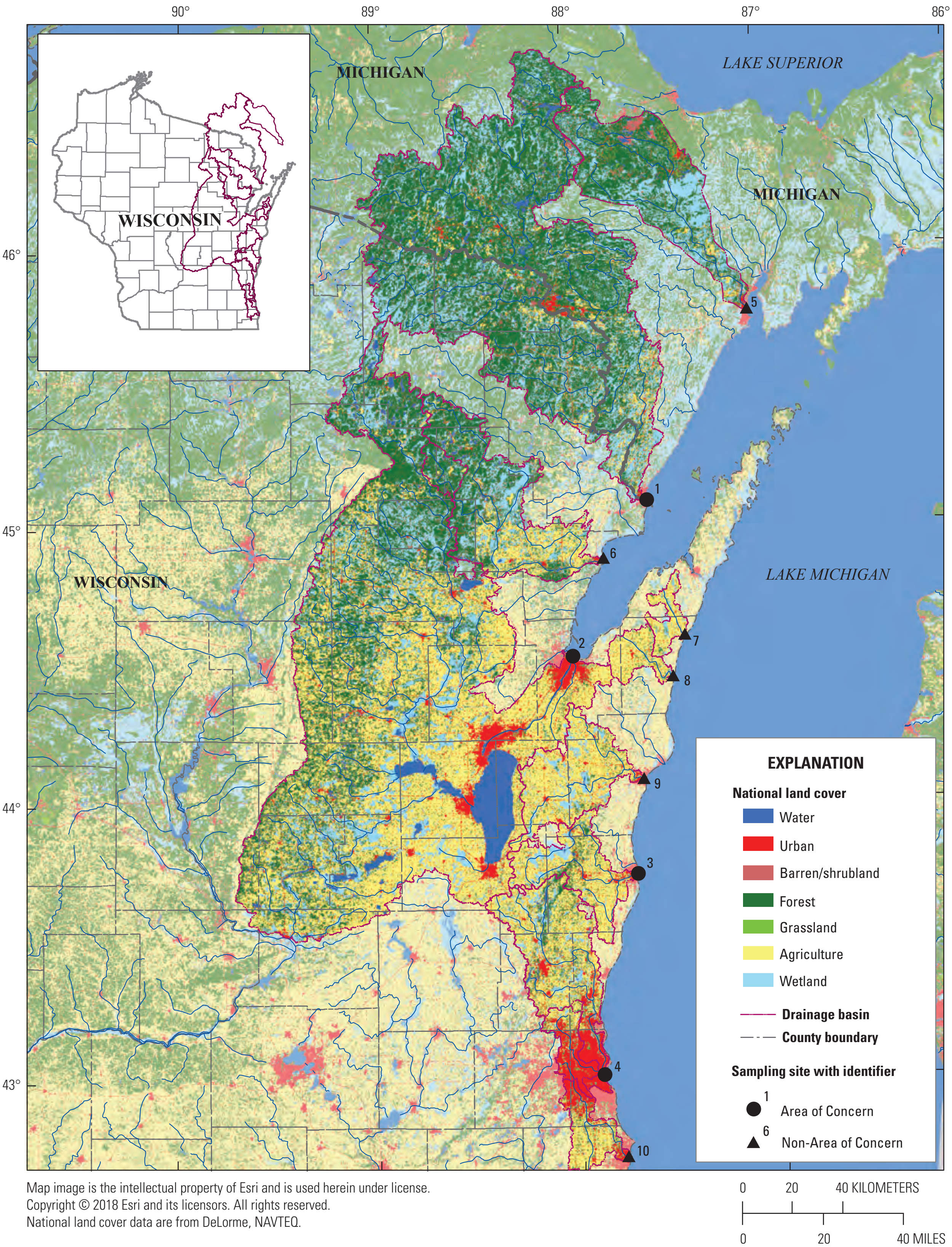


Figure 1. Sampling sites and subsites investigated for the evaluation of benthic and planktonic assemblages at Wisconsin's 4 Lake Michigan Areas of Concern and 6 non-Area of Concern comparison sites in Wisconsin and Michigan. Site and subsite numbers with names are provided in table 1.

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)]

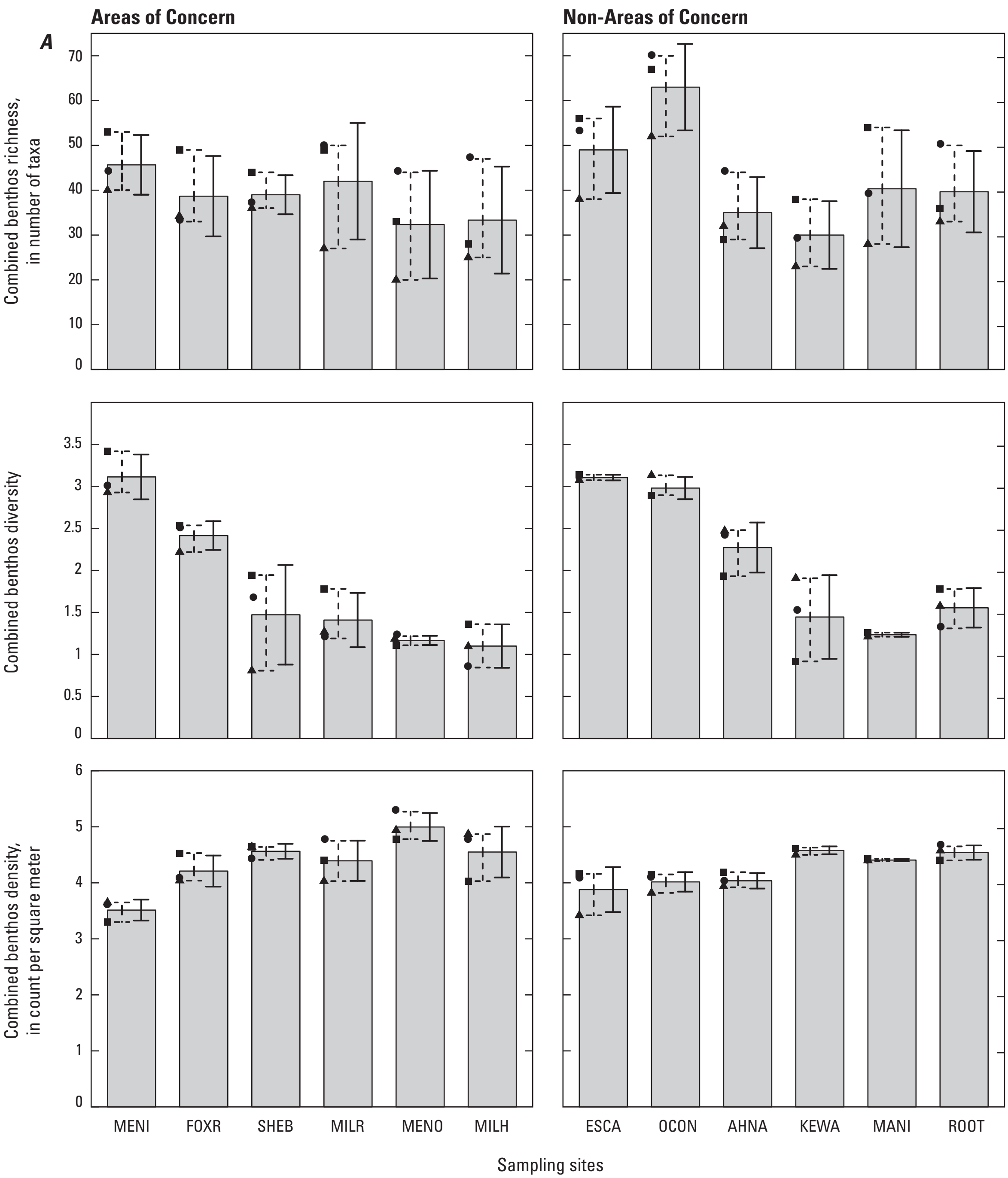
Statistic	Year	Dredge		Hester-Dendy			Combined benthos[1]					
		All taxa		All taxa			All taxa			EPT taxa		
		Richness	Diversity	Richness	Diversity	IBI[2]	Richness	Diversity	Density[3]	Richness	Density[3]	EPT percentage[4]
ROOT non-Area of Concern comparison site												
Mean	2012	10.7	0.9	24.0	1.9	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.



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]

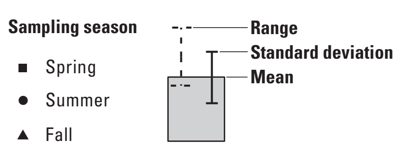


Figure 2. Metric values for benthos from 4 Lake Michigan Areas of Concern and 6 non-Area of Concern comparison sites. *A*, Richness, diversity, and total density of combined benthos (dredge and Hester-Dendy samples combined); and *B*, Ephemeroptera-Plecoptera-Trichoptera (EPT) density and EPT richness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

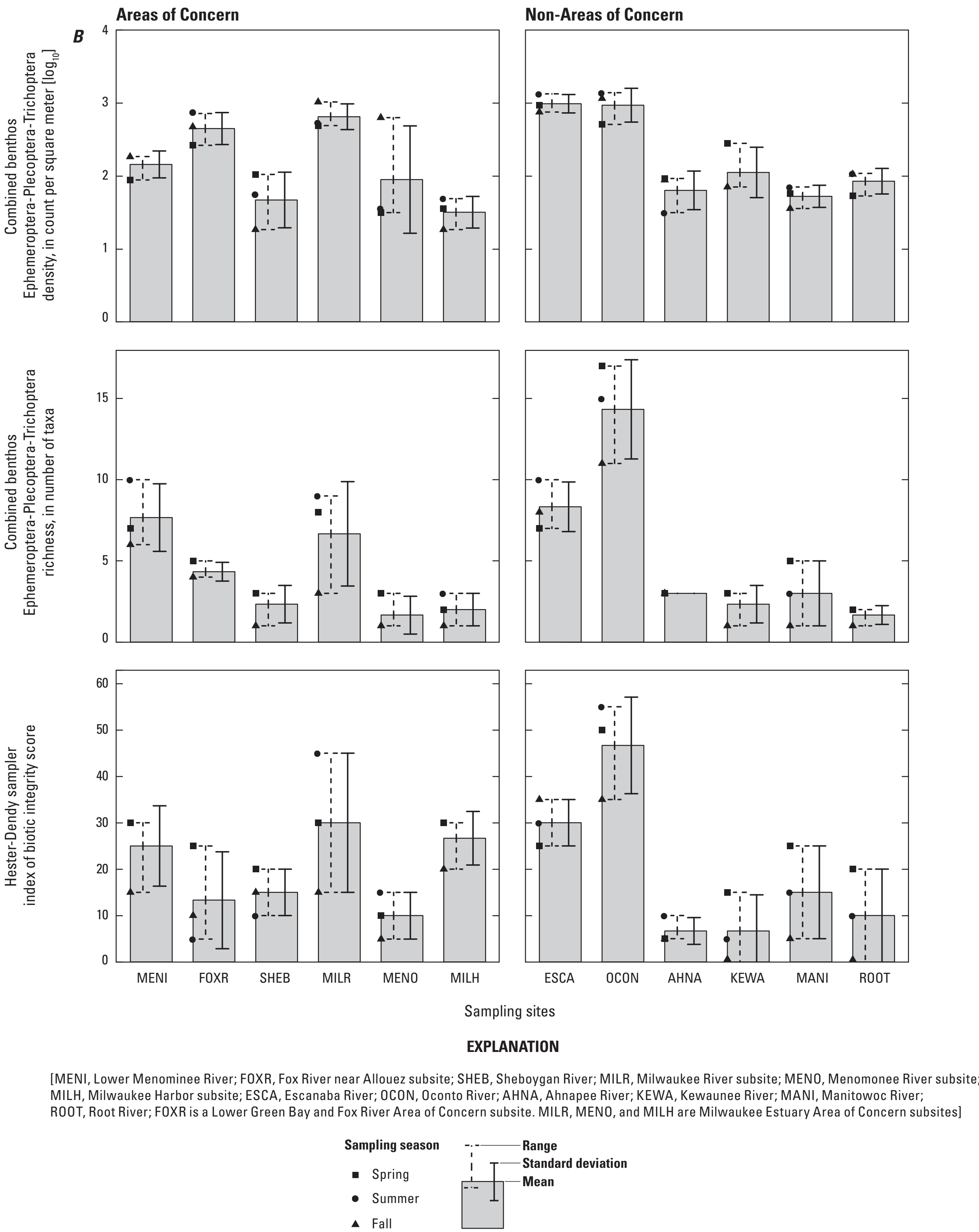


Figure 2. Metric values for benthos from 4 Lake Michigan Areas of Concern and 6 non-Area of Concern comparison sites. *A*, Richness, diversity, and total density of combined benthos (dredge and Hester-Dendy samples combined); and *B*, Ephemeroptera-Plecoptera-Trichoptera (EPT) density and EPT richness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

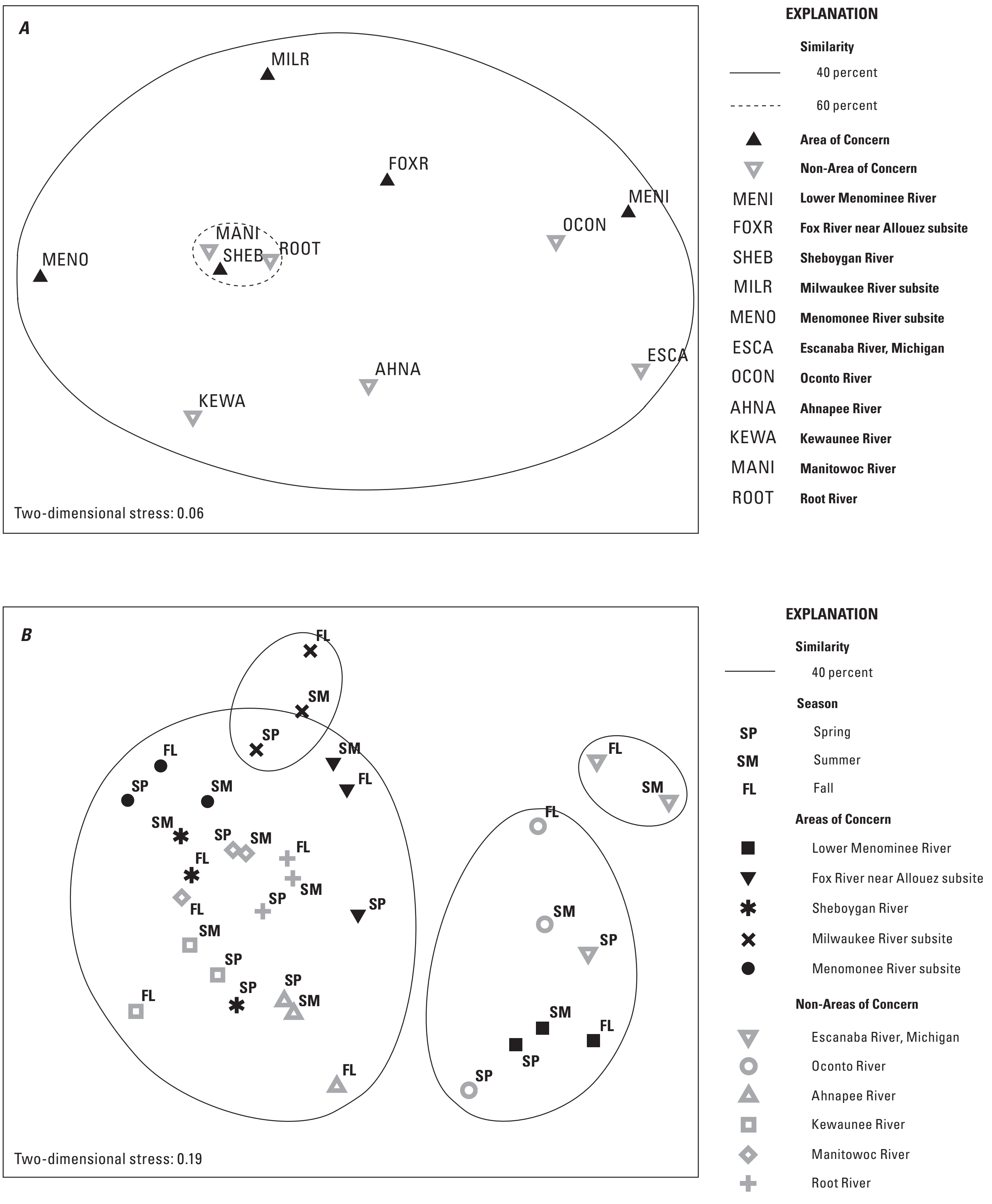


Figure 3. Multidimensional scaling ordination plots for combined benthos (dredge and Hester-Dendy samples combined) at 4 Lake Michigan Areas of Concern and 6 non-Area of Concern comparison sites, based on relative abundance with no rare or ambiguous taxa. *A*, Seasons combined; and *B*, seasons separate. 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).

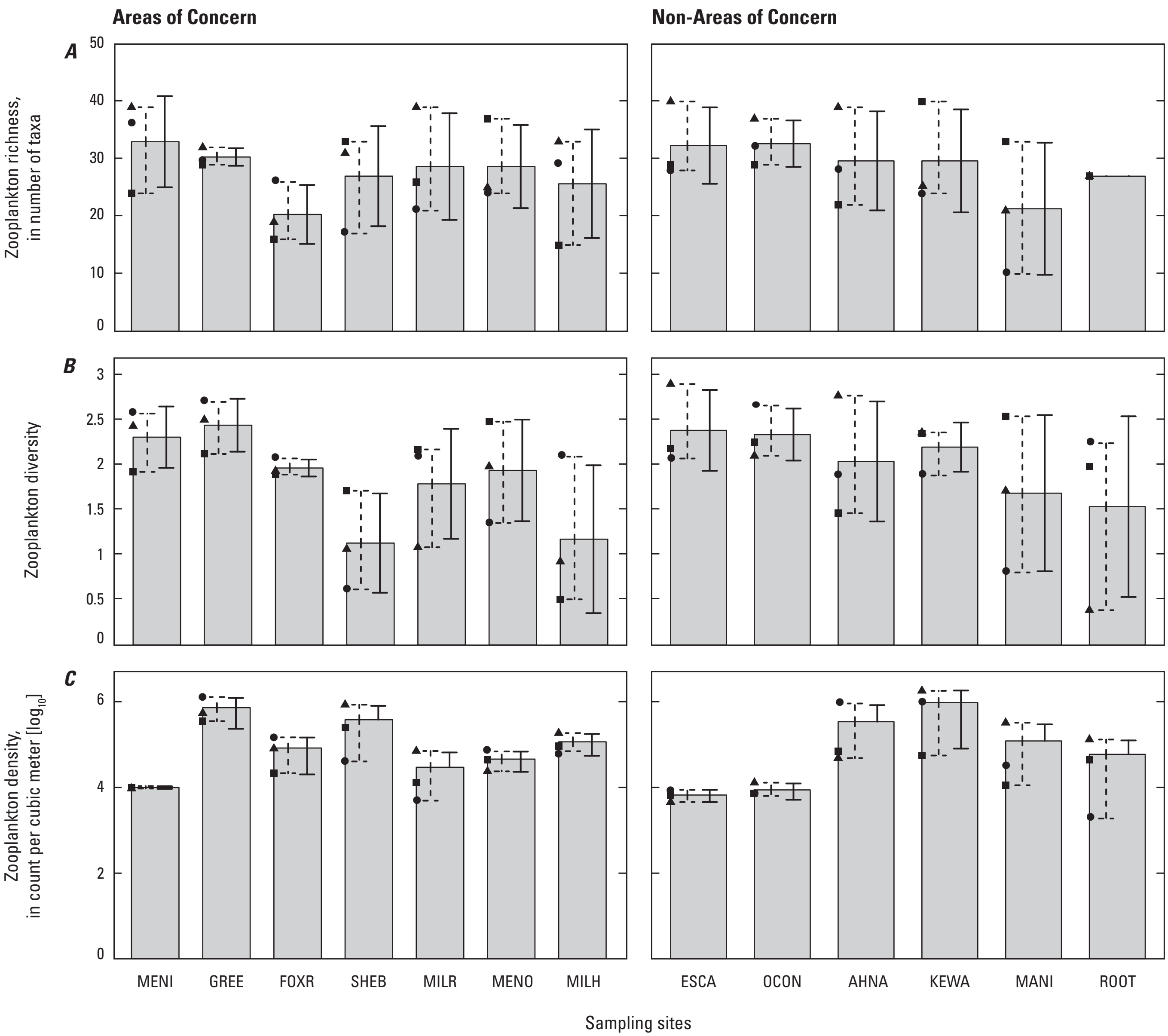
Metrics for combined phytoplankton at MENI did not differ from either the mean of all non-AOCs or the mean of the two non-AOC comparison sites (fig. 6, table 9). Richness was higher in 2014 than in 2012 (table 7), and this was because the diatom richness was higher in 2014 ($p<0.01$). Diversity and total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



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]

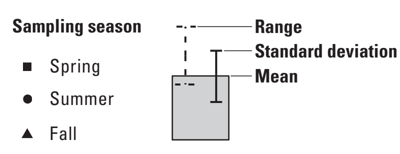


Figure 4. Metrics for zooplankton at 4 Lake Michigan Areas of Concern and 6 non-Area of Concern comparison sites. *A*, Zooplankton richness; *B*, zooplankton diversity; and *C*, zooplankton density.

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*

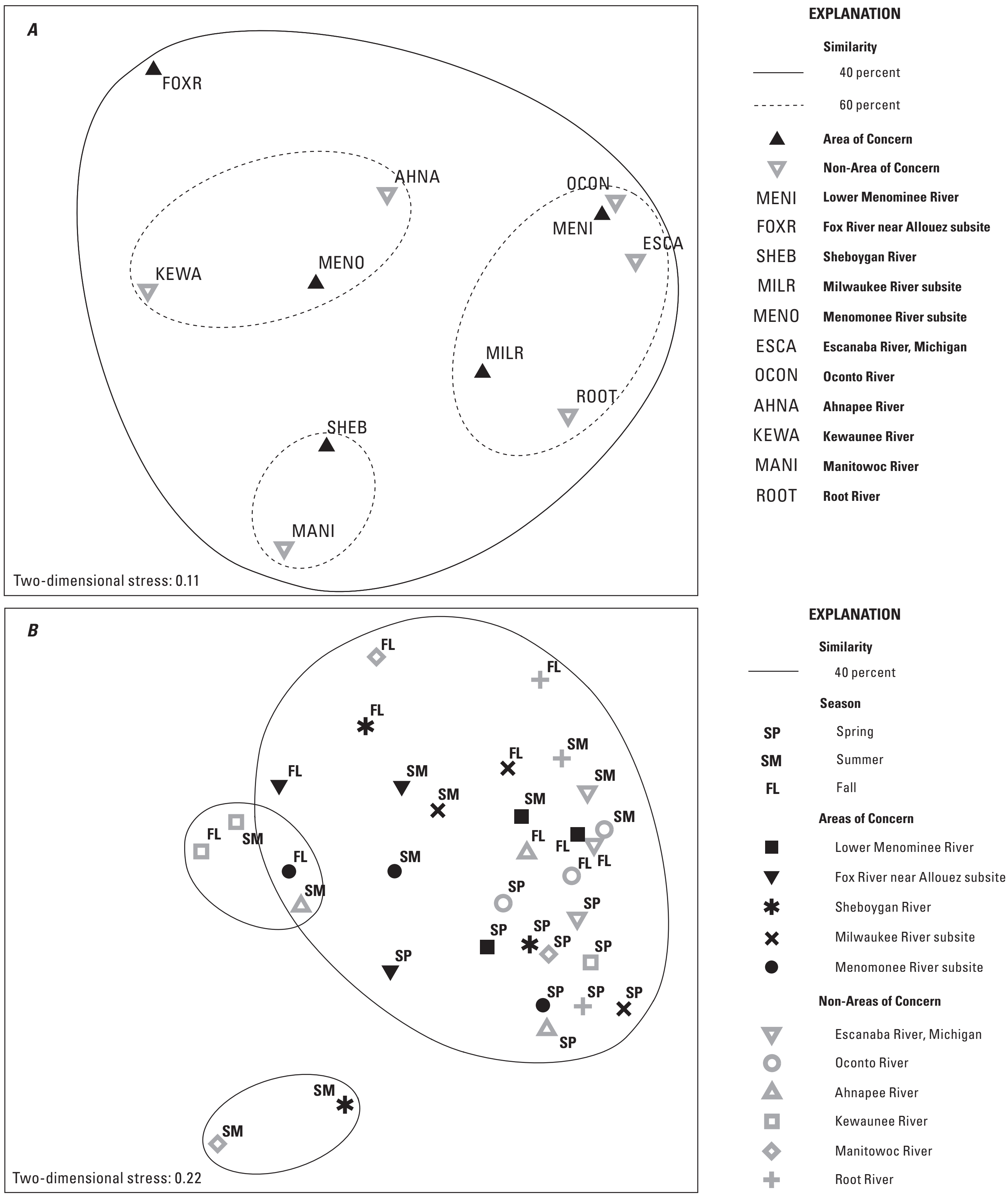


Figure 5. Multidimensional scaling ordination plots for zooplankton at 4 Lake Michigan Areas of Concern and 6 non-Area of Concern comparison sites, based on relative abundance (fourth-root transformed) with no rare or ambiguous taxa. *A*, Seasons combined; and *B*, seasons separate. [The Fox River near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

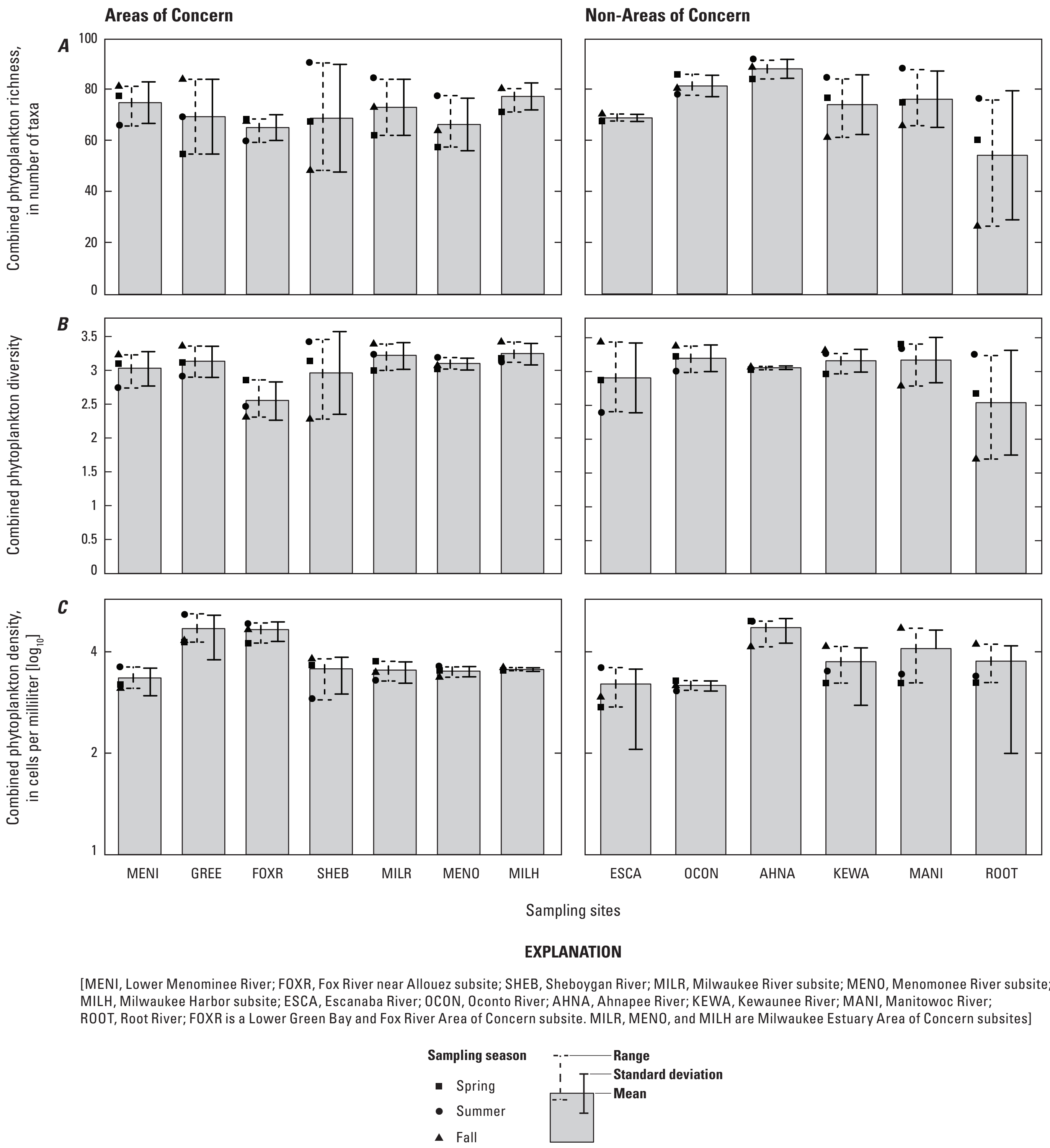


Figure 6. Metrics for combined (soft algae and diatoms) at 4 Lake Michigan Areas of Concern and 6 non-Area of Concern comparison sites. *A*, Combined phytoplankton richness; *B*, combined phytoplankton diversity; and *C*, combined phytoplankton density.

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.

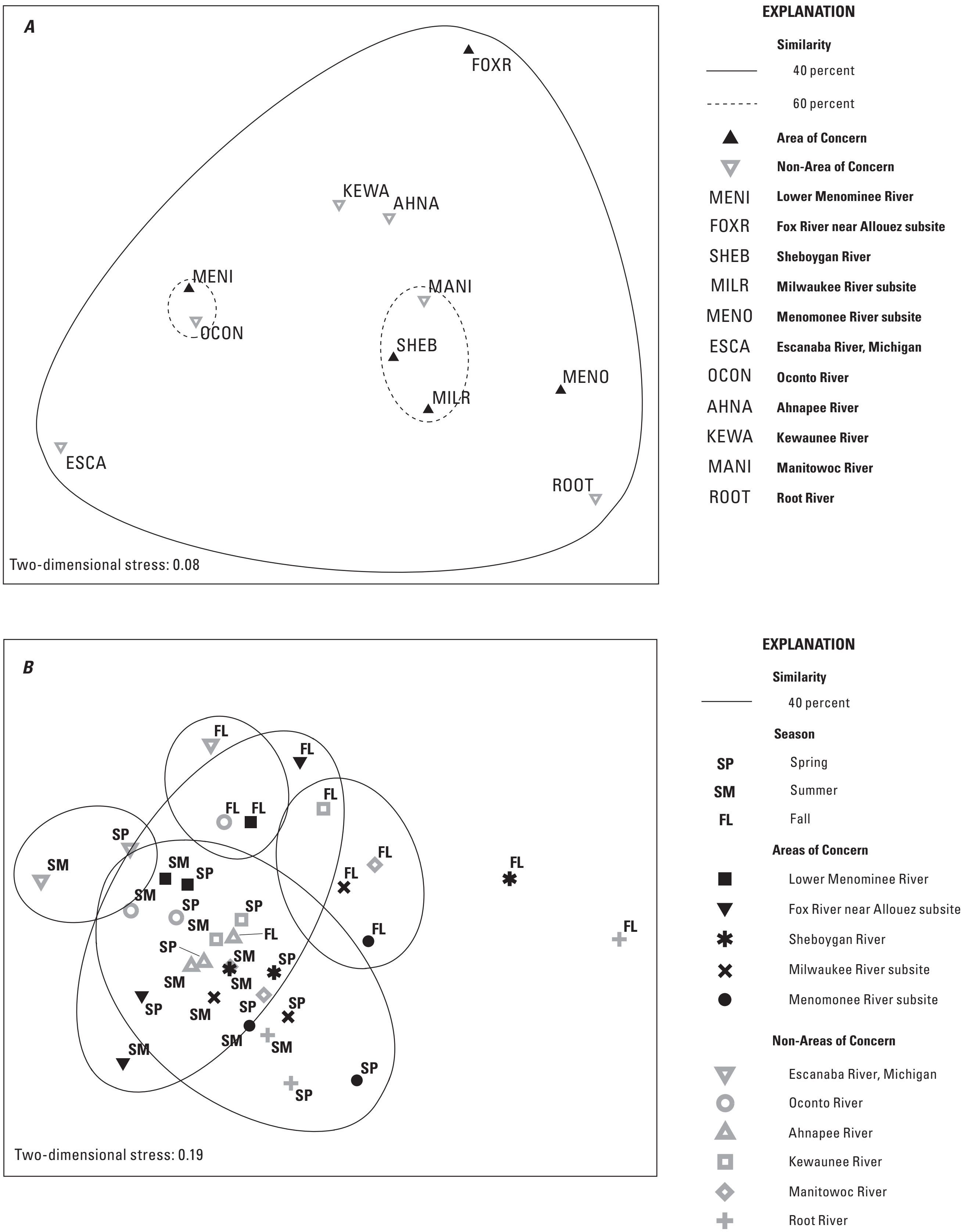


Figure 7. Multidimensional scaling ordination plots for combined phytoplankton (soft algae and diatoms) at 4 Lake Michigan Areas of Concern and 6 non-Area of Concern comparison sites, based on relative abundance (fourth-root transformed) with no rare or ambiguous taxa. *A*, Seasons combined; and *B*, 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

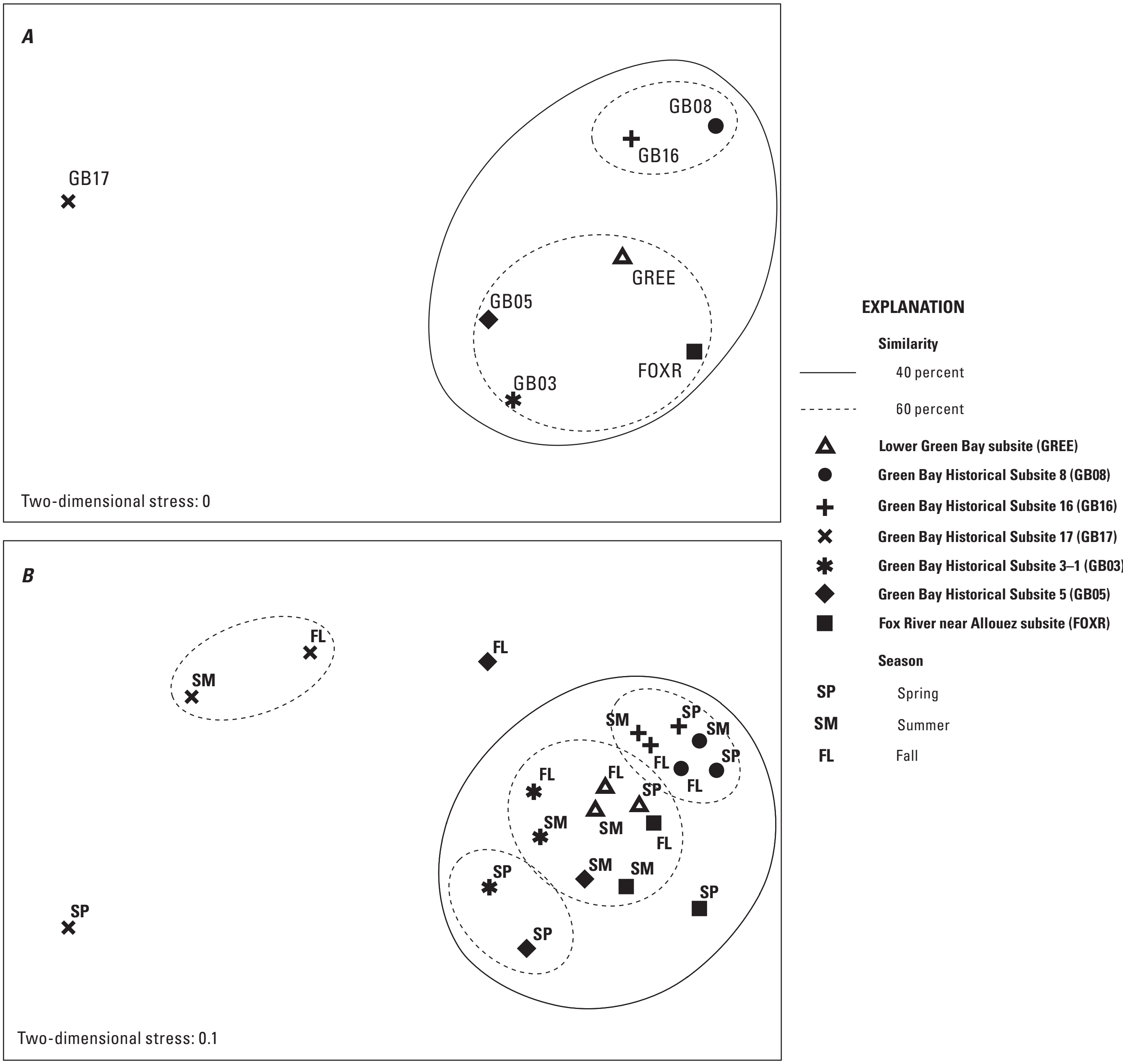


Figure 8. Multidimensional scaling ordination plots for the benthos collected by dredge at the Green Bay and Lower Fox River Area of Concern, based on relative abundance (fourth-root transformed) with no rare or ambiguous taxa. *A*, Seasons combined; and *B*, seasons separate.

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 cervi*x (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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