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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Cover. Posterized photograph of Lake Michigan coastline and Algoma Lighthouse by Barbara Scudder Eikenberry with color and effects added by Michelle Greenwood.

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SALLY JEWELL, Secretary

U.S. Geological Survey
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Conversion Factors

International System of Units to Inch/Pound

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)
	Volume	
liter (L)	0.2642	gallon (gal)
	Mass	
gram (g)	0.03527	ounce, avoirdupois (oz)

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).

Datums

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).

Abbreviations

AOC	Area of Concern
BUI	Beneficial Use Impairment
HD	Hester-Dendy (artificial substrate sampler)
IBI	Index of Biotic Integrity
MMSD	Milwaukee Metropolitan Sewerage District
PCBs	polychlorinated biphenyl compounds
USGS	U.S. Geological Survey
WDNR	Wisconsin Department of Natural Resources
WSLH	Wisconsin State Laboratory of Hygiene

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

By Barbara C. Scudder Eikenberry, Amanda H. Bell, Hayley A. Templar, and Daniel J. Burns

Abstract

Recent data are lacking to assess whether impairments still exist at four of Wisconsin's largest Lake Michigan harbors that were designated as Areas of Concern (AOCs) in the late 1980s due to sediment contamination and multiple Beneficial Use Impairments (BUIs), such as those affecting benthos (macroinvertebrates) and plankton (zooplankton and phytoplankton) communities. During three seasonal sampling events ("seasons") in May through August 2012, the U.S. Geological Survey collected sediment benthos and water plankton at the four AOCs as well as six less-degraded non-AOCs along the western Lake Michigan shoreline to assess whether AOC communities were degraded in comparison to non-AOC communities. The four AOCs are the Lower Menominee River, the Lower Green Bay and Fox River, the Sheboygan River, and the Milwaukee Estuary. Due to their size and complexity, multiple locations or "subsites" were sampled within the Lower Green Bay and Fox River AOC (Lower Green Bay, the Fox River near Allouez, and the Fox River near De Pere) and within the Milwaukee Estuary AOC (the Milwaukee River, the Menomonee River, and the Milwaukee Harbor) and single locations were sampled at the other AOCs and non-AOCs. The six non-AOCs are the Escanaba River in Michigan, and the Oconto River, Ahnapee River, Kewaunee River, Manitowoc River, and Root River in Wisconsin. Benthos samples were collected by using Hester-Dendy artificial substrates deployed for 30 days and by using a dredge sampler; zooplankton were collected by net and phytoplankton by whole-water sampler. Except for the Lower Green Bay and Milwaukee Harbor locations, communities at each AOC were compared to all non-AOCs as a group and to paired non-AOCs using taxa relative abundances and metrics, including richness, diversity, and an Index of Biotic Integrity (IBI, for Hester-Dendy samples only). Benthos samples collected during one or more seasons were rated as degraded for at least one metric at all AOCs. In the Milwaukee Estuary, benthos richness was lower in the Milwaukee River subsite spring and summer samples and in the Menomonee River subsite spring sample relative to the paired non-AOCs. Benthos diversity and IBIs at the Menomonee River subsite and IBIs at the Milwaukee River subsite and Sheboygan River were significantly lower than at all non-AOCs as a group across all seasons and therefore were rated as degraded. In addition, IBIs at the Lower Menominee River were significantly lower than those at the paired non-AOCs during all seasons and were therefore rated degraded. Benthos at both Fox River subsites and the Milwaukee River subsite were significantly different from their paired non-AOCs during all three seasons, based on a comparison of the relative abundances of taxa using multivariate testing. Metrics for plankton at AOCs were not significantly lower than those at the paired or group non-AOCs during all seasons; however, zooplankton richness in spring at the Sheboygan River and in fall at the Menomonee River subsite was rated as degraded in comparison to paired non-AOCs. Also, zooplankton richness in fall at the Fox River near Allouez subsite and in spring at the Milwaukee River subsite was rated degraded overall because values were lower than at all non-AOCs as a group and lower than at the paired non-AOCs. Zooplankton diversity in fall at the Fox River near Allouez subsite and the Lower Menominee River was rated degraded in comparison to paired non-AOC comparison sites. Zooplankton communities at the Fox River near Allouez subsite were significantly different from the paired non-AOCs when multivariate comparisons were made without rotifers other than *A. priodonta*. Overall, benthos and zooplankton BUIs remained at the AOCs in 2012 but no AOCs with a phytoplankton BUI were rated degraded in comparison to non-AOCs. The use of a multiple ecological measures, structural and functional, and multiple statistical analyses, biological metrics and multivariate statistics, provided assessments that defined 2012 status of communities relative to less-impaired non-AOCs in the Great Lakes area.

Introduction

Within the Laurentian Great Lakes, certain sites were designated Areas of Concern (AOCs) under the United States and Canada Great Lakes Water Quality Agreement (International Joint Commission United States and Canada, 1987), because severe environmental degradation had affected the areas' ability to support aquatic life. Along the Great Lakes shoreline, 43 AOCs were designated: 26 in U.S. waters and 17 in Canadian waters, and 5 AOCs are shared by the nations. The State of Wisconsin has jurisdiction over five AOCs contained entirely within the U.S. borders on two of the Great Lakes—one AOC along the Lake Superior shoreline and four AOCs along the Lake Michigan shoreline (fig. 1). The four AOCs along the Lake Michigan shoreline are the Lower Menominee River, Lower Green Bay and Fox River, Sheboygan River, and Milwaukee Estuary (Wisconsin Department of Natural Resources, 1990, 2013a). Each AOC is officially "listed" due to one or more Beneficial Use Impairments (BUIs); for example, degradation of fish and wildlife populations, degradation of benthos (bottom dwelling or benthic invertebrates), or degradation of plankton populations (free floating invertebrates and algae, or zooplankton and phytoplankton, respectively). Hereafter, unless otherwise specified, use of the term "plankton" in this report implies both zooplankton and phytoplankton. An AOC cannot be "delisted" until remediation has resulted in meeting set goals and objectives, thus allowing the removal of all BUI designations for the AOC except in cases where a beneficial use cannot be fully restored (U.S. Policy Committee, 2001). A BUI may be removed without a full recovery only in cases where: 1) the BUI is due to natural rather than human causes, 2) the BUI is not limited to the AOC but is instead typical of lakewide, regionwide, or areawide conditions, or 3) the impairment is caused by stressors outside of the AOC (Grapentine, 2009).

The Great Lakes Water Quality Agreement (International Joint Commission United States and Canada, 1987) defines 14 BUIs, and the four Lake Michigan AOCs in Wisconsin have most of them (Wisconsin Department of Natural Resources, 2011, 2012b, 2013b; Wisconsin Department of Natural Resources and Michigan Department of Environmental Quality, 2011). All four of Wisconsin's Lake Michigan AOCs have the degraded benthos BUI designation, and the Lower Green Bay and Fox River, Sheboygan River, and Milwaukee Estuary AOCs have the degraded phytoplankton and zooplankton BUI, although few historical data are available for comparison of conditions at the time of listing to current conditions. Degradation of benthos is one of the most widespread BUIs in the United States, and it is most often related to sediment contamination; however, water chemistry, substrate type, inadequate food supply, and river flows may also be important (Reynoldson, 1987; U.S. Environmental Protection Agency, 1994; Wisconsin Department of Natural Resources, 2009, 2011). Degradation of plankton may be the result of poor water quality due to excessive nutrient enrichment or toxic chemicals, inadequate food supplies, high flows at river mouths, and other physical, chemical, and biological stresses (Gannon and Stemberger, 1978; Wisconsin Department of Natural Resources, 1994).

The macroinvertebrate community of the benthos in nonwadable rivers and their harbors on large lakes is comprised of worms, insect larvae, crustaceans, clams, snails and other organisms. Many of these organisms are filter feeders on phytoplankton and detritus but some eat other animals (Merritt and Cummins, 1996). Oligochaete worms may be abundant and diverse in the soft sediments. Midges, the most common group of larval insects in the benthos, may also be abundant and diverse. Together, oligochaetes and midges may dominate this environment, especially in polluted rivers, though not all of these taxa are considered tolerant (Thorp and Covich, 1991; Canfield and others, 1996). In general, the dominant zooplankton taxa in freshwater environments are rotifers, microcrustaceans such as cladocerans and copepods, and protozoans. Rotifers and microcrustaceans may compete for food resources but in general, many rotifers are outcompeted by microcrustaceans because of lower clearance rates and smaller size requirements for food particles (Wallace and Snell, 1991). However, due to short development times and high population growth rates in response to increased temperatures, rotifers can take advantage of new environmental conditions more than many microcrustaceans can and so they may become more abundant. 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 form the basis of aquatic food chains in large rivers and lakes, and communities are usually dominated by diatoms and the percentage of diatoms tends to decrease with pollution (Flotemersch and others, 2006). Changes in the phytoplankton community from a dominance by diatoms to a dominance by green or blue-green algae can have a cascading effect on secondary consumers and this in turn can have a cascading effect on organisms that feed on them (Wisconsin Department of Natural Resources, 1993).

Lake Michigan's water quality is greatly affected by development on its western shores in the State of Wisconsin. Each of Wisconsin's four Lake Michigan AOCs has historical and ongoing practices that contribute to degraded benthos and plankton. High sediment concentrations of arsenic, coal tar, and paint sludge from historical pollution are listed as the reasons for degraded benthos in the Lower Menominee River AOC (Wisconsin Department of Natural Resources and Michigan Department of Environmental Quality, 2011); the Lower Green Bay and Fox River AOC received industrial discharge along the banks of the Fox River for many years (Wisconsin Department of Natural Resources, 2012a). Toxic substances from point source discharges, primarily polychlorinated biphenyls (PCBs), and nutrients, primarily phosphorus, from nonpoint runoff draining agricultural and urban lands, are responsible for degraded benthos and plankton BUIs (Wisconsin Department of Natural Resources, 2012a). The sediments of the Sheboygan River AOC are contaminated predominantly by PCBs and polycyclic aromatic hydrocarbons

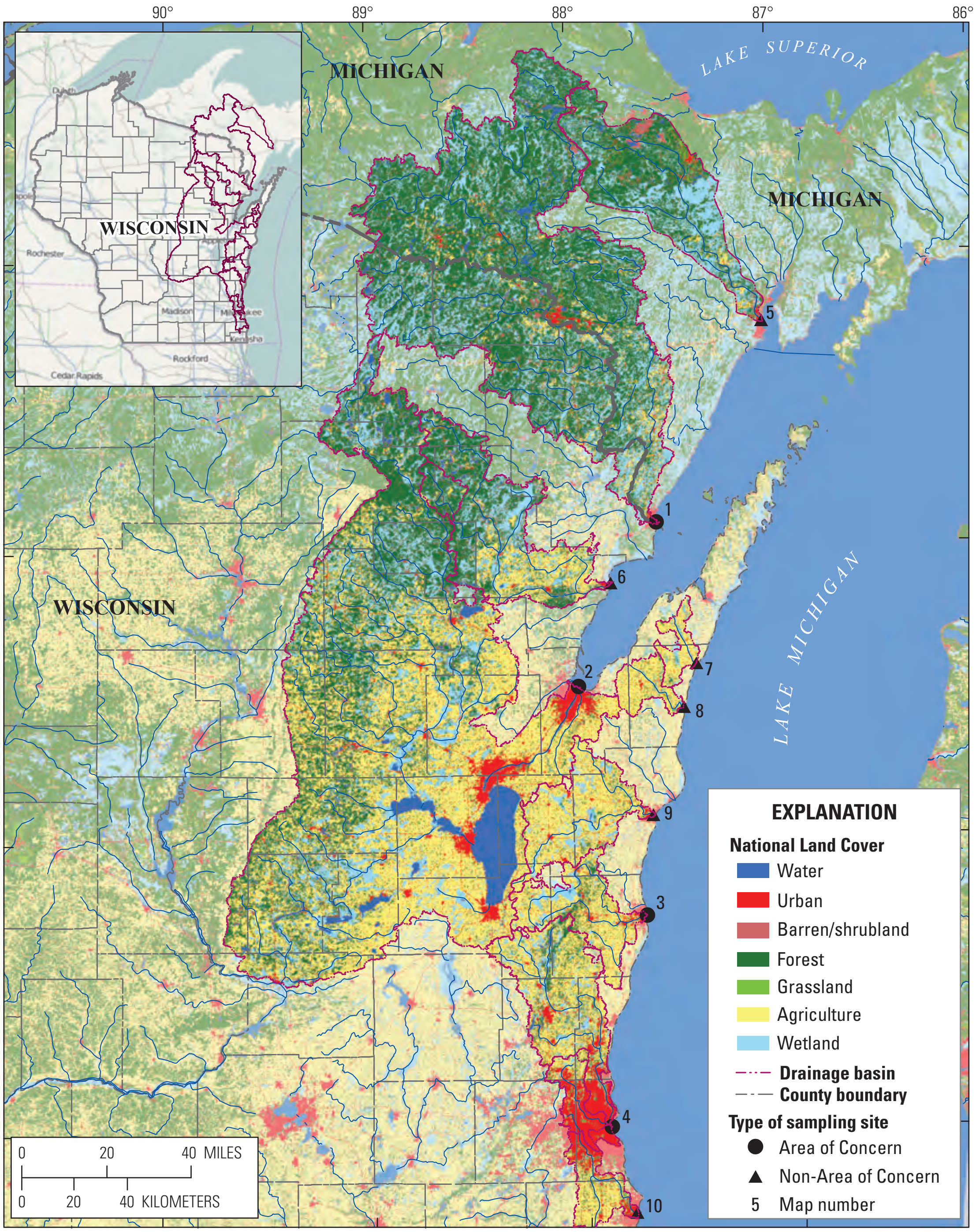


Figure 1. Sampling sites and subsites investigated for the evaluation of benthos and plankton communities at Wisconsin's four Lake Michigan Areas of Concern (AOCs) and six non-Area of Concern comparison sites in Wisconsin and Michigan.

from industrial facilities and leaking landfills located on the shore of the river (Wisconsin Department of Natural Resources, 1995, 2012b). Contaminated sediment is listed as a source for degraded benthos, and contaminated sediment as well as point and nonpoint runoff pollution are listed as the sources for degraded plankton. The Milwaukee Estuary AOC is located at the confluence of three urban rivers: the Milwaukee, Menomonee, and Kinnickinnic Rivers. Toxic substances, such as PCBs and polycyclic aromatic hydrocarbons, together with sewage overflows, thermal discharges, nonpoint runoff, and physical habitat alterations that include dams, drop structures, concrete lined channels, poorly sized culverts, and shoreline alterations are concerns in the Milwaukee Estuary AOC (Wisconsin Department of Natural Resources, 2011; Wisconsin Department of Natural Resources and Milwaukee River Basin Land and Water Partners Team, 2001).

Based on guidelines for benthos and plankton BUIs as stated in the U.S. Policy Committee report (U.S. Policy Committee, 2001), if the structure of the communities at an AOC are not found to differ significantly from one or more unimpacted control sites with comparable physical and chemical characteristics, then the target criteria are considered to have been met to remove that particular BUI. The International Joint Commission listing guideline states that an impairment will be listed when the community structure "significantly diverges from unimpacted control sites of comparable physical and chemical characteristics (Ohio Environmental Protection Agency, 2008)."

In 2012, 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 conducted a study of the benthos and plankton at 10 sites in rivers and harbors along Wisconsin's Lake Michigan shoreline. Four sampling sites were AOCs and six additional sites were relatively less-impacted comparison sites with similar physical and chemical characteristics (referred to hereafter as non-AOCs). Results were then compared to historical studies at the AOCs to provide context and evaluate potential progress in site remediation benefits. Sediment remediation was ongoing in 2012 at all four AOCs: the Lower Menominee River, Fox River, Sheboygan River, and Milwaukee Estuary.

Purpose and Scope

The purpose of this study is to inform the decision process for removal of the BUI for degradation of benthos and the BUI for degradation of plankton population by determining whether or not the benthos or plankton community differed significantly from one or more less-impaired sites with comparable physical and chemical characteristics. The report presents an assessment of the status of aquatic communities at the four AOC and six non-AOC comparison sites in 2012. The results of the study are then compared to those of historical studies of the AOCs to provide context and evaluate potential progress in site remediation benefits in the four AOCs.

Methods

Four AOC sites and six non-AOC comparison sites were selected for this study (fig. 1, table 1). "Sites" refer to the geographic areas that were sampled; for example, the Lower Green Bay and Fox River AOC. The term "subsite" in this report is used when more than one area was sampled within an AOC site. Detailed site and location information is provided in Scudder Eikenberry, Bell, and others (2014). Although there are no rivermouths or harbors along the western Lake Michigan shoreline that are truly unimpacted, the six non-AOCs selected for comparison were not within any AOC and were representative of the region; therefore, they were assumed to have biological communities similar to those that would be present in the AOCs if the AOCs did not have the specific contamination that was identified during designation and listing.

The selection of good comparison sites can be a challenge for making quantitative assessments of the degree to which aquatic communities at sites were impaired, and this is especially true of Great Lakes rivermouths and harbors. Aquatic communities in rivermouths and harbors are often quite different from either the upstream riverine communities or the downstream lake communities (Larson and others, 2013). With the possible exception of remote northern sites, there are no unimpaired rivermouths or harbors in the Great Lakes for making comparisons to "unimpacted control" or reference sites as stated in the target criteria for AOCs (U.S. Policy Committee, 2001). Quantitative historical data also may be unavailable due to decades of pollution at a site. Comparison of sites within an AOC with those in unimpacted or less-impacted areas is an approach that has been used to delist BUIs in other Great Lakes states, such as Michigan and Ohio (Michigan Department of Environmental Quality, 2008; Ohio Environmental Protection Agency, 2008).

Sample Collection and Processing

Benthos and plankton assemblages were sampled at each of the four AOC sites and six non-AOC comparison sites during three visits approximately six weeks apart in late May and early June, mid-July, and late August. Hereafter for simplicity, the three sampling events are referred to as the "spring," "summer," and "fall "seasonal samples. All sites were nonwadable and samples were collected from a boat. During each sampling period, *in situ* water-quality samples were collected at each site for pH, specific conductance, and water temperature using a Hydrolab Quanta™ Sonde. All data and detailed method descriptions can be found in Scudder Eikenberry and others (2014). Many areas of the Midwest and Wisconsin were experiencing a stretch of high heat and drought during the summer and fall sampling periods that resulted in lower than average stream discharges to some sampling locations, most notably the Lower Menominee River (*MENI*), Menomonee River subsite (*MENO*), and Root River

Table 1. U.S. Geological Survey sampling sites and subsites during 2012 at four Lake Michigan Areas of Concern in Wisconsin and six non-Area of Concern comparison sites in Wisconsin and Michigan.

[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. km², square kilometer; Mich., Michigan; NA, not applicable]

Site or subsite name	Abbreviated name	Site or subsite number	Latitude (decimal degrees)	Longitude (decimal degrees)	Drainage area (km²)	Comparison site or subsite number
Areas of Concern						
Lower Menominee River	*MENI*	1	45.09810	–87.60772	10,490	5, 6
Lower Green Bay-Fox River		2				
Lower Green Bay[1]	*GREE*	2a	44.57751	–87.98600	16,584	NA
Fox River near Allouez	*FOXR*	2b	44.49499	–88.02424	16,178	7, 8
Fox River near De Pere[2]	*FOXD*	2c	44.46251	–88.05776	16,011	7, 8
Sheboygan River	*SHEB*	3	43.74887	–87.70352	1,043	8, 9
Milwaukee Estuary		4				
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-Areas of Concern						
Escanaba River, Mich.	*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, 2c
Kewaunee River	*KEWA*	8	44.46073	–87.50205	354	2b, 2c, 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]Benthos samples were not collected in Green Bay.

[2]Plankton samples were not collected at the Fox River near De Pere.

(*ROOT*) where annual mean discharge in 2012 was about two-thirds or less than the historical mean annual discharge at nearby stream gages (Scudder Eikenberry and others, 2014).

Benthos samples were collected from bottom sediment at each site using two methods: dredge samples and Hester-Dendy (HD) artificial substrate samplers (U.S. Environmental Protection Agency, 1994; Weigel and Dimick, 2011). Three to five Ponar® dredge samples were composited into one sample per site (U.S. Environmental Protection Agency, 2010a). A small amount of material (less than 50 grams) from each composited dredge sample was placed into two plastic bags: one for analysis of sediment size fractions for estimating substrate sizes and types and another for analysis of volatile-on-ignition for estimating the amount of organic matter. Size fraction samples were analyzed by the University of Wisconsin Soils Laboratory in Madison, Wisconsin, using the hydrometer method (Bouyoucos, 1962), and volatile-on-ignition samples were analyzed by the USGS using a combustion method (U.S. Geological Survey, 1989; Wentworth, 1922). Fine sediments were removed from the composite sample by sieving; large debris and empty shells were examined for any attached invertebrates before being discarded. The retained debris and organisms were stained with rose bengal dye and preserved with 10 percent buffered formalin for later sorting and identification of individual macroinvertebrates. With the exception of Green Bay, four individual HDs were deployed for 6 weeks at each site during each season (Weigel and Dimick, 2011). Once retrieved, three of the four HDs were randomly chosen to represent the site. Only two of the four HDs were available for analysis from the summer samples at the Oconto River (*OCON*) and *ROOT* non-AOCs. All organisms were scraped off, composited into one sample per season for the site, stained with rose bengal dye, and preserved with 10 percent buffered formalin. All benthos samples were identified and counted by the Lake Superior Research Institute at the University of Wisconsin-Superior (U.S. Environmental Protection Agency, 2010b).

Zooplankton samples were collected using plankton nets towed from a depth of 5 meters (m), or just above the bottom if less than 5 m to the surface using a 63-micrometer (µm) mesh net (U.S. Environmental Protection Agency, 2010f). 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. Subsamples were collected from the whole water sample for chlorophyll *a*, total and volatile suspended solids, identification and enumeration of “soft” algae phytoplankton (blue-greens, cryptomonads, desmids, dinoflagellates, euglenoids, and greens), and identification and enumeration of diatom phytoplankton (U.S. Environmental Protection Agency, 2010d).

The samples for zooplankton and phytoplankton identification were preserved with glutaraldehyde to a 1 percent final solution. Identification and counting were done at the Wisconsin State Laboratory of Hygiene (WSLH) for soft algae and the WDNR for zooplankton and diatoms (Karner, 2005; U.S. Environmental Protection Agency, 2010c, e). Rotifers other than the large taxon *Asplanchna priodonta* were not identified and counted in the summer zooplankton samples from Lower Green Bay (*GREE*) and Ahnapee River (*AHNA*) nor for any seasons at the Fox River near Allouez (*FOXR*) due to interference from large amounts of algae in samples. Chlorophyll *a* and suspended solids (total suspended solids and volatile suspended solids) were determined at the WSLH (American Public Health Association, American Water Works Association, and Water Environment Federation, 2006; Kennedy-Parker, 2011).

Data Analysis

The benthos and plankton community data were compared among the sites using various metric analyses as well as multivariate nonmetric analyses of the relative abundance of each taxon, which is the abundance of each taxon compared to the total abundance of all taxa in a sample. Briefly, the metrics used for comparisons were taxa richness (the total number of taxa), the Shannon Diversity Index (Shannon, 1948), and, for HD sampler data only, a multi-metric macroinvertebrate Index of Biotic Integrity (IBI) designed for use with HD sampler data for large, nonwadable rivers of Wisconsin was calculated (Weigel and Dimick, 2011).

The two largest AOCs, the Lower Green Bay and Fox River AOC and the Milwaukee Estuary AOC, are larger and far more complex systems than any other harbors or rivermouths along the western Lake Michigan shoreline. For the Lower Green Bay and Fox River AOC, samples were collected at three locations or subsites (table 1). Benthos and plankton samples were collected from the Fox River near Allouez (*FOXR*), and additional benthos samples were collected each season (spring, summer, and fall) from the Fox River near De Pere (*FOXD*); only plankton samples were collected in Lower Green Bay (*GREE*). The Milwaukee Estuary AOC was sampled at three locations or subsites: the Menomonee River (*MENO*), Milwaukee River (*MILR*), and the Milwaukee Harbor (*MILH*). Due to their size and complexity, it was assumed that *GREE* and *MILH* would likely have different community structures than the non-AOC comparison sites. Therefore, except for *GREE* and *MILH*, each AOC site and any associated subsites were matched to two non-AOC comparison sites (“paired non-AOCs”) as closely as possible, based on available environmental data and discussions between USGS and WDNR personnel who are familiar with the individual systems.

A multitiered analysis was used to compare metrics from AOCs and non-AOCs for evaluating community degradation (fig. 2). With the exception of the metric values for *GREE* and *MILH*, metric values were compared on three levels:

- Level 1, between an AOC and all six non-AOCs as a group for each seasonal sample
- Level 2, between an AOC and its paired non-AOCs for each seasonal sample, and
- Level 3, between an AOC and its paired non-AOCs across all seasonal samples using median metric scores.

Standard statistical tests could not be used for making comparisons within a single season because of small sample sizes. Therefore, for only single-season comparisons, one standard deviation or 10 percent of the mean was chosen by the WDNR as an acceptable, but still defensible, cutoff for comparison of single-season samples. The reasoning was that if the variation at non-AOCs was high, then the standard deviation also would be high; however, if there was little variability in metric values at non-AOCs, then the value(s) for an AOC should be within that range if the community was not degraded (Andrew Fayram, WDNR, Madison, Wis., oral communication, 2012). Hereafter, when the term “standard deviation” is used in seasonal metric comparisons, it refers to either the standard deviation of the non-AOC values or 10 percent of the mean, whichever is greater. For Level 1, if a single seasonal metric value for an AOC site or subsite was more than one standard deviation below the range of all six non-AOC metric values, then the seasonal sample was rated to have a community more degraded than all non-AOCs as a group. For Level 2, if the seasonal metric value of an AOC site or subsite was below either of the seasonal metric values for the paired non-AOCs by one standard deviation of the mean of the paired seasonal metric values, then the seasonal sample was rated to have a community more degraded than the paired non-AOCs. For Level 3, if the median metric value of all seasons at an AOC site or subsite was more than one standard deviation below the median metric value for all seasons at the paired non-AOCs, then the AOC seasonal samples were rated to have communities more degraded than the paired non-AOCs. Also, if two or more seasonal samples were rated to be degraded, or if samples at two or more levels were rated to be degraded, then the AOC community overall was rated to be degraded.

For additional statistical robustness, Mann-Whitney and paired t-tests were added to Level 1 and 3 to compare metrics across the three seasons. Except for single season metric comparisons where a higher significance level was used (see above), if the median metric value was significantly ($p<0.05$) below the median metric value of all non-AOCs as a group (Level 1) or below the median metric value of the two non-AOC comparison sites (Level 3), then the community in that AOC was rated more degraded than the communities of the selected non-AOCs. Unless otherwise stated, use of the term “significant” refers to values of $p<0.05$. Lack of a

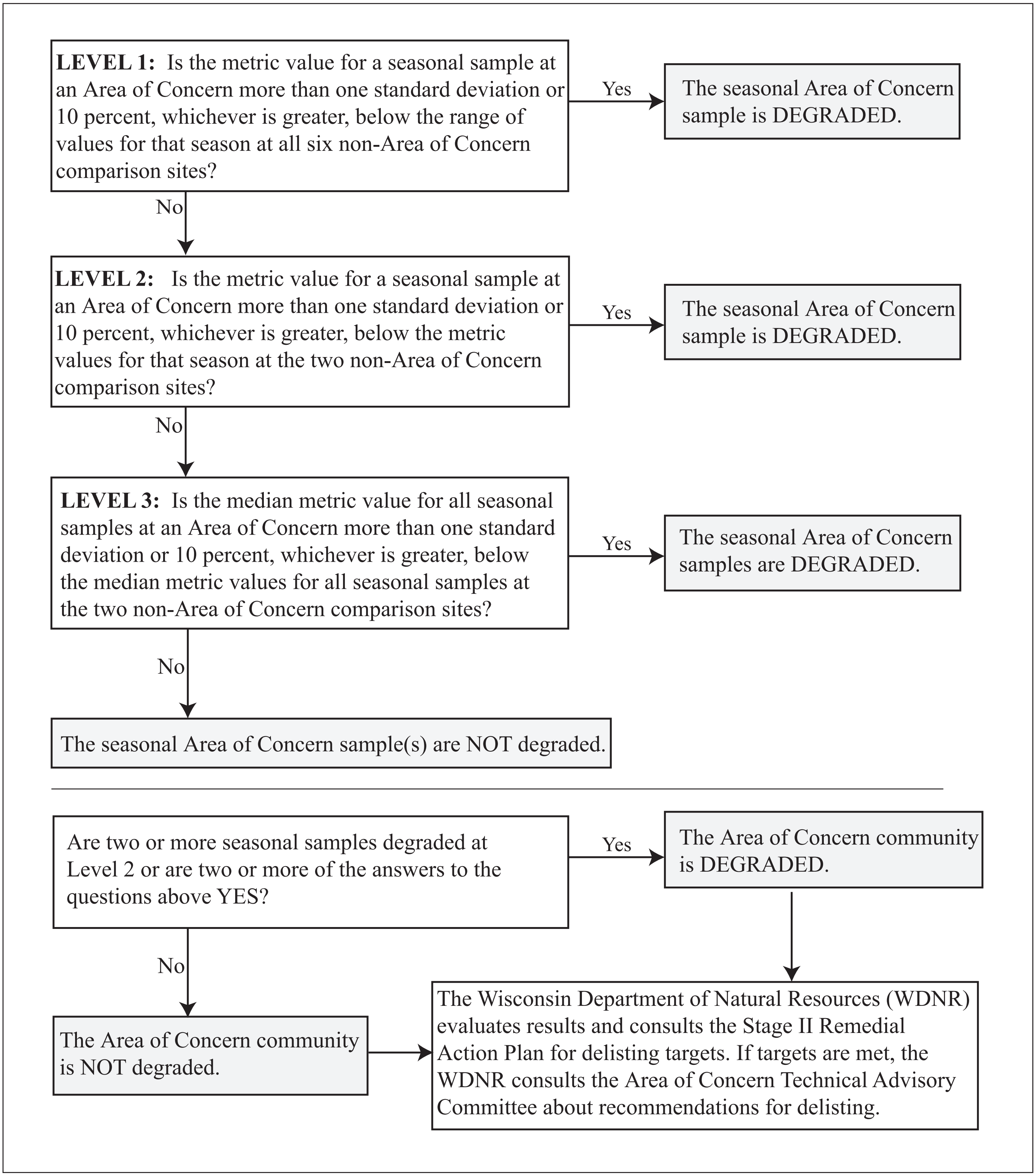


Figure 2. Diagram of the decision tree for comparing benthos and plankton community metrics at four Lake Michigan Areas of Concern in Wisconsin and six non-Area of Concern comparison sites in Wisconsin and Michigan.

significant difference does not infer that the AOC community is not degraded, but rather that it was not degraded in comparison to the selected non-AOCs in 2012.

Five PRIMER software (Clarke and Gorley, 2006) routines were used for multivariate analyses of the relative abundance of taxa. The PRIMER routines used were:

1. DIVERSE—to calculate diversity in $\log_e$, and
2. SIMilarity PERcentage (SIMPER)—to assess differences in the relative abundance of taxa among each AOC and its paired nonAOCs, among primary and replicate samples collected each season at the Sheboygan River AOC and Manitowoc River non-AOC, and among subsites within the Lower Green Bay and Fox River AOC (benthos only) and the Milwaukee Estuary AOC,
3. Multi-Dimensional Scaling (MDS)—nonmetric method based on relative abundances of taxa—to derive benthos and plankton community site scores and create ordination plots of sites and (or) samples, and
4. ANalysis Of SIMilarity (ANOSIM)—to compare communities among sites and samples using similarity matrices in a procedure analogous to an analysis of variance.
5. Bio-Env+STepwise (BEST)—to discern the top five taxa for benthos, zooplankton, and phytoplankton that accounted for the most variability between AOCs as a group and non-AOCs as a group.

For multivariate analyses in PRIMER routines, the relative abundance of each taxon was determined for each sample, and a Bray Curtis similarity matrix was calculated for the sample using a fourth root transformation of relative abundance data. These Bray Curtis similarity matrices formed the basis of MDS, SIMPER, and ANOSIM comparisons. A one-way ANOSIM, based on site-specific scores generated with MDS, was used to determine the extent to which relative abundances of taxa in benthos and plankton communities varied across sites by sampling event and across sampling seasons. A one-way ANOSIM also was used to compare HD data from the current study with one historical data set (Weigel and Dimick, 2011) that also used HD samplers. Site scores based on similarities between communities were used to determine whether the community composition of a sample or site was statistically different from other sites, with a 90 percent confidence limit.

Taxa in a data set are ambiguous when some individuals cannot be classified to the species level due to immaturity or damage, and then abundances are reported for multiple, related taxonomic ranks. This can lead to erroneously high richness and diversity values. Ambiguous “parents” are taxa that are not identified to the species, while ambiguous “children” are the species that were identified and fall under the same classification of the “parent.” Ambiguous taxa were resolved prior to calculating metrics and prior to multivariate analyses by distributing counts for the parent to the children present within each site, taking into account 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 data was applied to the benthos, zooplankton, and diatom taxa; there were no ambiguous soft algal taxa. For zooplankton, immature copepod taxa were distributed to the children when present within a sample; in samples without children, Calanoid and Cyclopoid copepodites were kept as distinct taxa when analyzing data.

Rare taxa, or taxa that contributed less than 1 percent of the total abundance in a sample, were removed prior to multivariate analyses so that the total numbers of taxa would not exceed three times the total number of samples. Rare taxa are sometimes excluded in studies to reduce noise in the data analyses (Cao and others, 2001; Marchant, 2002). For the combined benthos samples (dredge and HD), a total of 203 benthic invertebrate taxa were identified across all sites and dates and, after removing rare taxa, 87 benthic taxa remained. Removal of rare taxa reduced the taxa list from 164 to 85 for HDs and from 106 to 65 for dredge samples. A total of 95 zooplankton taxa were identified, including immature copepods (nauplii and copepodites); removal of rare taxa reduced this total number to 73. Removing rare taxa from soft algae reduced the taxa list from 48 to 43; a total of 277 diatom taxa were identified across all sites and dates, and 193 taxa remained after rare taxa were eliminated.

Richness was computed by totaling the number of taxa with ambiguous taxa resolved and rare taxa included; diversity was calculated using the Shannon Diversity Index ($\log_e$) on raw abundances of taxa without data standardization or transformation, but with ambiguous taxa resolved and rare taxa included in $\log_e$. Richness and diversity were calculated separately for the two benthos sampling types—dredge and HDs—as well as for the combined benthos samples. Richness and diversity were also calculated separately for soft algae and diatom phytoplankton, as well as for combined phytoplankton (soft algae and diatoms together). An IBI was calculated for only the HD samples. IBI calculations followed the methods described by Weigel and Dimick (2011), with possible scores ranging from 0 (worst) to 100 (best). IBI calculations for benthos were designed by Weigel and Dimick (2011) for HD sampler data only; this metric ranges from 0 (worst) to 100 (best) (Weigel and Dimick, 2011).

Benthos HD data collected in 2012 were compared quantitatively to historical benthos HD data from Weigel and Dimick (2011). In addition, the 2012 plankton data for the Milwaukee Estuary AOC were compared to plankton data from the Milwaukee Metropolitan Sewerage District (MMSD; Eric Waldmer, MMSD, electronic files accessed April 22, 2013). 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 do not conform to the standards required for quantitative comparison. Taxonomic resolution and changes in taxonomic classifications over time—especially for the phytoplankton community—posed the largest problems with using the historical data.

Even when site locations match fairly closely, field collection methods can vary greatly between studies, and quality assurance and quality control procedures are not always reported. For the comparison to the study by Weigel and Dimick (2011), the metrics used were taxa richness; the IBI, and selected metrics that comprise the IBI: the number of insect taxa and the number of insect taxa that are Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies) , and the percentages of total taxa comprised of insects, chironomids, and oligochaetes, and the combined percentage of individuals in the Orders Ephemeroptera, Plecoptera, and Trichoptera that is commonly known as the EPT index.

For quality assurance and quality control, the SIMPER routine was run on the replicate samples from the Sheboygan River (*SHEB*) AOC and Manitowoc River (*MANI*) non-AOC comparison site collected during each sampling event to evaluate the similarity between taxa and their relative abundances in the samples. Similarities greater than 60 percent were considered acceptable for quality assurance purposes (Kelly, 2001). Except for diatoms, replicates within each seasonal sample at a site had similarities of 60 percent or greater. In general, diatom density was relatively low for all samples. For example, *MANI* summer samples had a similarity of 0, likely because less than 10 diatom taxa were identified in each of the two samples, and none of those taxa were identified in both samples. This demonstrates the difficulty of characterizing taxa at sites with low abundances and patchy distributions. By combining soft algae and diatom taxa and abundances in comparisons with AOCs, the effect of the diatom taxa on the overall phytoplankton comparisons was decreased.

Condition of the Benthos and Plankton Communities

Characterization of what taxa or abundances should comprise an unimpaired benthos or plankton community is a challenge for Great Lakes rivermouths and harbors. A study of benthos at 50 nearshore “reference” sites in lakes Superior, Huron, Erie, and Ontario by Bailey and others (1995) found that the top four taxa were chironomids, oligochaetes, bivalves, and sponges; however, that study found considerable variation in the benthos across sites and suggested that there was not a single, defined “healthy ecosystem.” Relatively diverse fauna with at least modest abundances of some taxa in a healthy, downstream community in a temperate rivermouth or harbor would be expected (Larson and others, 2013).

In the present study, differences in benthos and plankton communities at AOCs were evaluated by comparing computed biological metrics as well as relative abundances of taxa comprising the aquatic communities at each site. Metrics for each site are shown graphically in figs. 3–5, and values are provided in tables 2 and 3 for the benthos and plankton, respectively. Invasive species and potentially harmful algal taxa were also characterized.

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

Although Lower Green Bay (*GREE*) and Milwaukee Harbor (*MILH*) were not included in direct comparisons with paired non-AOCs, results of this study provide an ecological assessment of the subsites for *MILH* benthos and plankton and *GREE* plankton that can be used for BUI evaluations and comparison to historical studies at the AOCs.

In plankton samples from *GREE*, zooplankton richness was highest in spring at 28 and lowest in summer at 13; diversity was also highest in spring at 2.49 and lowest in summer at 1.55 (table 3). Summer richness and diversity values reflected the lack of data for rotifers in the summer samples because of interference from high algal counts; rotifers contributed at least half the richness in the spring and fall zooplankton samples at this site. The rotifer *Conochilus unicornis* was the dominant species in the spring zooplankton community, and the microcrustaceans *Chydorus sphaericus* and immature Cyclopoid copepods were dominant in fall. Phytoplankton richness (soft algae and diatoms combined) was lowest in spring at 17 and highest in fall at 75; diversity ranged from 1.90 in spring to 3.14 in summer, and 3.12 in the fall, with blue-green algae dominant in spring, summer, and fall samples.

In benthos samples from *MILH*, richness in dredge samples was lowest in spring at 10, and highest at 18 in the fall; benthos richness in HD samples ranged from 40 in spring to 11 in fall (table 2). Diversity was highest in fall for dredge samples (1.25), but highest in spring for HD samples (3.09). For combined benthos samples, the median richness and diversity values were 28 and 1.11, respectively. The IBIs for HD samples were lowest in summer at 10 and highest at 25 in the fall. In the spring and summer samples, oligochaetes were dominant in the dredge and HD samples; immature worms of the tubificids were dominant in dredge samples and *Nais* spp. were dominant in the HD samples. In the fall samples, zebra mussels were dominant in both sample types. Midges were also important members of the benthos community in the *MILH* samples. Oligochaetes and midges are considered to be relatively tolerant in general and some taxa can be abundant in degraded waters (Brinkhurst and Gelder, 1991; Kennedy, 1966; Lenat, 1993; Rodriguez and Reynoldson, 2011). A study of other AOCs in New York, Indiana, and Michigan by Canfield and others (1996) determined that the absence of midges was indicative of a grossly contaminated sample.

For plankton at *MILH*, zooplankton richness was lower in spring (18) than fall (27) samples (table 3). Rotifers and zebra mussel veligers were the dominant species in the zooplankton community, with rotifers primary in spring and fall but secondary in summer samples. Phytoplankton richness ranged from 11 in summer to 30 in fall and diversity ranged from 1.79 in summer to 2.90 in fall. Diatoms were dominant in the spring, cryptophytes and diatoms were dominant in summer, and green and blue-green algae were dominant in fall as diatoms dropped to third in abundance.

Table 2. Richness, diversity, and Index of Biotic Integrity values for benthos samples collected in 2012 by the U.S. Geological Survey at four Lake Michigan Areas of Concern in Wisconsin and six non-Area of Concern comparison sites in Wisconsin and Michigan.

[Site abbreviations: *MENI*–Lower Menominee River; Lower Green Bay and Fox River subsites: *GREE*–Lower Green Bay, *FOXR*–Fox River near Allouez, and *FOXD*–Fox River near De Pere; *SHEB*–Sheboygan River; Milwaukee Estuary subsites: *MILR*–Milwaukee River; *MENO*–Menomonee River; and *MILH*–Milwaukee Harbor; *ESCA*–Escanaba River, Mich.; *OCON*–Oconto River; *AHNA*–Ahnapee River; *KEWA*–Kewaunee River; *MANI*–Manitowoc River; and *ROOT*–Root River]

Site	Season	Dredge		Hester-Dendy			Combined benthos	
		Richness[1]	Diversity[2]	Richness	Diversity	IBI[3]	Richness	Diversity
Areas of Concern[4]								
MENI	Spring	14	2.20	30	2.21	15	40	2.73
	Summer	17	2.34	36	2.64	20	51	3.10
	Fall	9	1.80	17	0.99	20	25	1.37
FOXR	Spring	12	1.54	45	2.97	10	49	2.15
	Summer	8	0.71	14	0.37	20	18	0.94
	Fall	12	1.10	12	0.70	20	21	1.53
FOXD	Spring	25	2.14	23	2.25	15	41	2.63
	Summer	16	1.80	13	1.09	20	26	1.86
	Fall	11	1.74	16	1.51	10	26	2.30
SHEB	Spring	20	0.95	30	2.68	10	48	1.72
	Summer	12	0.42	29	2.36	5	38	0.61
	Fall	7	0.28	18	1.05	10	20	1.01
MILR	Spring	14	0.63	25	2.13	10	36	1.07
	Summer	8	0.32	18	1.26	10	23	0.67
	Fall	15	0.98	13	1.88	0	24	1.41
MENO	Spring	13	1.08	26	2.52	10	36	1.16
	Summer	12	0.76	27	1.69	0	32	0.99
	Fall	16	1.01	10	1.30	5	26	1.32
MILH	Spring	10	0.84	40	3.09	20	45	1.11
	Summer	11	0.65	20	1.64	10	28	0.72
	Fall	18	1.25	11	0.49	25	21	1.15
Non-Areas of Concern								
AHNA	Spring	5	1.30	29	2.58	10	32	1.96
	Summer	2	0.21	32	2.36	15	33	1.75
	Fall	4	0.84	13	1.51	10	18	1.92
ESCA	Spring	19	2.48	27	2.55	25	43	3.03
	Summer	11	2.07	22	2.35	25	28	2.73
	Fall	20	1.61	21	2.13	30	33	2.09
KEWA	Spring	10	0.91	20	2.09	10	29	2.26
	Summer	10	0.51	13	1.29	0	21	1.45
	Fall	7	0.49	5	0.59	15	12	1.14
MANI	Spring	8	0.46	41	1.92	15	47	0.65
	Summer	7	0.64	37	2.42	10	41	0.96
	Fall	7	0.64	23	2.05	0	27	1.44
OCON	Spring	21	1.30	40	2.19	25	51	1.80
	Summer	16	2.26	24	2.35	30	36	2.68
	Fall	23	2.36	45	3.14	50	60	2.70
ROOT	Spring	15	0.76	35	2.32	20	43	2.16
	Summer	8	0.81	28	2.64	15	32	1.40
	Fall	9	1.03	9	0.76	5	18	1.16

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

[2]Shannon Diversity, calculated as $\log_e$.

[3]Index of Biotic Integrity designed for use with Hester-Dendy artificial substrates in nonwadable rivers, calculated as in Weigel and Dimick (2011). The Index of Biotic Integrity ranges from 0 (worst) to 100 (best).

[4]Benthos samples were not collected in Green Bay.

Table 3. Richness and diversity values for plankton samples collected in 2012 by the U.S. Geological Survey at four Lake Michigan Areas of Concern and six non-Area of Concern comparison sites in Wisconsin and Michigan.

[Site abbreviations: *MENI*–Lower Menominee River; Lower Green Bay and Fox River subsites: *GREE*–Lower Green Bay, *FOXR*–Fox River near Allouez, and *FOXD*–Fox River near De Pere; *SHEB*–Sheboygan River; Milwaukee Estuary subsites: *MILR*–Milwaukee River; *MENO*–Menomonee River; and *MILH*–Milwaukee Harbor; *ESCA*–Escanaba River, Mich.; *OCON*–Oconto River; *AHNA*–Ahnapee River; *KEWA*–Kewaunee River; *MANI*–Manitowoc River; and *ROOT*–Root River]

Site	Season	Zooplankton[1]		Soft algae		Diatoms		Combined phytoplankton[4]	
		Richness[2]	Diversity[3]	Richness	Diversity	Richness	Diversity	Richness	Diversity
Areas of Concern[5]									
MENI	Spring	27	2.51	13	1.86	28	2.67	41	2.96
	Summer	35	2.92	8	1.09	8	1.28	16	1.88
	Fall	25	1.81	15	1.22	14	2.53	29	2.57
GREE	Spring	28	2.49	12	1.60	5	0.81	17	1.90
	Summer	13	1.55	22	1.91	36	2.99	58	3.14
	Fall	23	2.14	18	1.67	57	3.18	75	3.12
FOXR	Spring	10	1.50	12	1.42	55	3.01	67	2.91
	Summer	8	1.22	14	1.46	65	3.11	79	2.98
	Fall	5	0.98	13	0.68	50	2.77	63	2.42
SHEB	Spring	18	1.87	14	1.71	29	1.61	43	2.35
	Summer	19	2.25	13	1.50	37	2.66	50	2.78
	Fall	24	2.08	8	1.21	70	3.16	78	2.89
MILR	Spring	13	1.97	15	1.87	38	3.22	53	3.24
	Summer	21	1.90	8	0.73	35	3.11	43	2.61
	Fall	27	2.32	19	1.96	56	3.26	75	3.30
MENO	Spring	24	2.18	8	1.53	47	3.26	55	3.09
	Summer	14	2.25	12	1.90	33	3.19	45	3.24
	Fall	20	2.28	19	2.11	10	2.22	29	2.86
MILH	Spring	18	1.27	8	1.59	20	2.69	28	2.83
	Summer	18	1.81	8	1.11	3	1.08	11	1.79
	Fall	27	1.48	15	2.04	15	2.37	30	2.90
Non-Areas of Concern									
AHNA	Spring	19	1.36	14	0.83	24	2.52	38	2.37
	Summer	7	1.26	15	0.81	24	2.83	39	2.51
	Fall	20	2.15	18	0.94	19	2.12	37	2.22
ESCA	Spring	24	2.35	9	1.27	32	3.00	41	2.83
	Summer	21	1.62	13	1.95	12	2.25	25	2.79
	Fall	27	2.64	11	1.31	12	2.30	23	2.50
KEWA	Spring	27	1.71	13	2.15	2	0.64	15	2.09
	Summer	14	1.66	17	1.71	45	2.64	62	2.87
	Fall	22	1.93	17	1.86	55	2.71	72	2.98
MANI	Spring	23	2.40	6	1.08	30	2.25	36	2.36
	Summer	14	1.25	16	0.90	9	2.01	25	2.15
	Fall	25	1.00	16	1.38	23	2.79	39	2.78
OCON	Spring	18	2.21	6	0.82	10	2.27	16	2.34
	Summer	25	2.04	16	2.08	28	2.84	44	3.16
	Fall	23	2.40	8	1.28	32	2.82	40	2.75
ROOT	Spring	20	1.49	15	1.99	71	3.67	86	3.52
	Summer	15	0.54	14	1.44	24	2.19	38	2.51
	Fall	24	1.34	14	1.64	15	2.46	29	2.74

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

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

[3]Shannon Diversity, calculated as $\log_e$.

[4]Phytoplankton richness and diversity comparisons in this table were calculated for combined soft algae and diatoms.

[5]Plankton samples were not collected at the *FOXD*.

Comparison of Subsites for the Lower Green Bay and Fox River and the Milwaukee Estuary Areas of Concern

Sampling was done at separate subsites in the Lower Green Bay and Fox River AOC (Fox River near De Pere [*FOXD*] and Fox River near Allouez [*FOXR*]) and the Milwaukee Estuary AOC (Milwaukee River [*MILR*], Menomonee River [*MENO*], and Milwaukee Harbor [*MILH*]) due to concerns about potential differences in environmental conditions at those sites. Similarity analyses of relative abundance with no ambiguous or rare taxa confirmed that community compositions at subsites within each AOC were sufficiently dissimilar to warrant separate analysis in order to properly characterize the AOCs.

For the combined benthos samples, SIMPER results showed that similarities in community composition between the two Fox River subsites (*FOXR* and *FOXD*) in the Lower Green Bay and Fox River AOC were 50.0 percent for spring, 61.1 percent for summer, and 57.6 percent for fall; because two of the three similarities were below the 60 percent threshold, benthos data for these AOC subsites were kept separate in metric comparisons and multivariate analyses. For the three Milwaukee Estuary AOC subsites (*MILR*, *MENO*, and *MILH*), similarities were 60.0 percent for spring, 55.1 percent for summer, and 49.0 percent for fall, which indicated that these subsites were also dissimilar; therefore, these sites were also kept separate in the analyses. The relative abundance of immature oligochaetes (Tubificinae) was important for defining these dissimilarities for the two Fox River and three Milwaukee Estuary AOC subsites; however, immature oligochaetes dropped to second in number to zebra mussels (*Dreissena polymorpha*) in the summer sample at the Fox River subsites.

Similarities in zooplankton community composition between the *MILR* and *MENO* subsites in the Milwaukee Estuary AOC were 48.8 percent for spring, 60.0 percent for summer, and 54.9 percent for fall. Because not all three similarities were 60 percent or more, the *MILR* and *MENO* communities were considered to be distinct and were kept separate in metric and multivariate comparisons with non-AOCs. The relative abundance of zebra mussels and several rotifer taxa (especially *Pompholyx sulcata*, *Gastropus stylifer*, *Brachionus calyciflorus*, and *Synchaeta oblonga*) was important in defining spring dissimilarities between these subsites. Another rotifer, *Keratella crassa*, became fourth and then first in importance in summer and fall dissimilarities, respectively, and *Brachionus calyciflorus* was second in importance. As mentioned earlier, plankton were collected from the *FOXR* but not from *FOXD* in the Lower Green Bay and Fox River AOC, so similarities could not be compared between these two subsites for zooplankton or phytoplankton.

For combined phytoplankton, similarities in community composition among *MILR* and *MENO* in the Milwaukee Estuary AOC were 44.6 percent for spring, 24.1 percent for summer, and 43.4 percent for fall. Because all similarities were less than 60 percent, phytoplankton communities in *MILR* and *MENO* were considered to be distinct from each other and therefore were kept separate in metric comparisons and multivariate analyses. The colonial green alga of the genus *Scenedesmus* was determined to be important in defining these dissimilarities; peak abundance occurred in spring, and decreased in summer when the colonial blue-green alga of the genus *Merismopedia* became primary, with both taxa contributing fairly equally in the fall sample.

Benthos Community Comparisons Between Areas of Concern and Non-Areas of Concern

Benthos taxa richness was generally lower for dredge samples than for HDs at all sites, possibly reflecting the greater suitability of the hard, artificial substrate of HDs in comparison to the soft, natural substrates collected in dredge samples (table 2). Dredge samples represent the habitat that is most affected by contaminants. For combined benthos, mean richness across seasonal samples at AOCs ranged from 27.7 ± 7.2 (mean ± standard deviation) for the Milwaukee River (*MILR*) to 38.7 ± 13.1 for the Lower Menominee River (*MENI*); mean taxa richness at non-AOCs ranged from 20.7 ± 8.5 for the Kewaunee River (*KEWA*) to 49.0 ± 12.1 for the Oconto River (*OCON*) (fig. 3*A*). Combined benthos richness for all seasons at each AOC was not significantly different from richness at non-AOCs as a group at Level 1, based on Mann-Whitney tests ($p<0.05$) (table 4), and it was not different within seasons. However, at Level 2, seasonal richness values at two subsites in the Milwaukee Estuary AOC—spring and summer at *MILR* and spring at the Menomonee River (*MENO*)—were below a seasonal value for the paired non-AOCs by more than one standard deviation of the mean of seasonal values for the paired sites (table 5). At Level 3, Mann-Whitney tests showed that combined benthos richness at each AOC was not significantly lower than their paired non-AOCs across all seasons, so no sites were rated as degraded. However, because two seasonal samples were rated as degraded at Level 2 for richness, the benthos community for *MILR* was rated as degraded overall. Sand that was relatively low in organic matter was dominant in the substrate at *MILR* and may not have been suitable for many invertebrates. Although sand was dominant in most samples from *MENO*, silt made up a greater proportion of the substrate at *MENO* than at *MILR*.

Diversity in benthos samples also tended to be lower for dredge samples than for HDs overall. For combined benthos, mean diversity for AOCs ranged from 1.1 ± 0.4 at *MILR* to 2.4 ± 0.9 at *MENI*; mean diversity at non-AOCs ranged from 1.0 ± 0.4 at the Manitowoc River (*MANI*) to 2.4 ± 0.5 at *OCON* (fig. 3*B*). Mann-Whitney tests showed that the diversity for combined benthos across all seasons at the *MENO* subsite in the Milwaukee Estuary AOC was significantly lower than diversity at non-AOCs as a group and therefore was rated

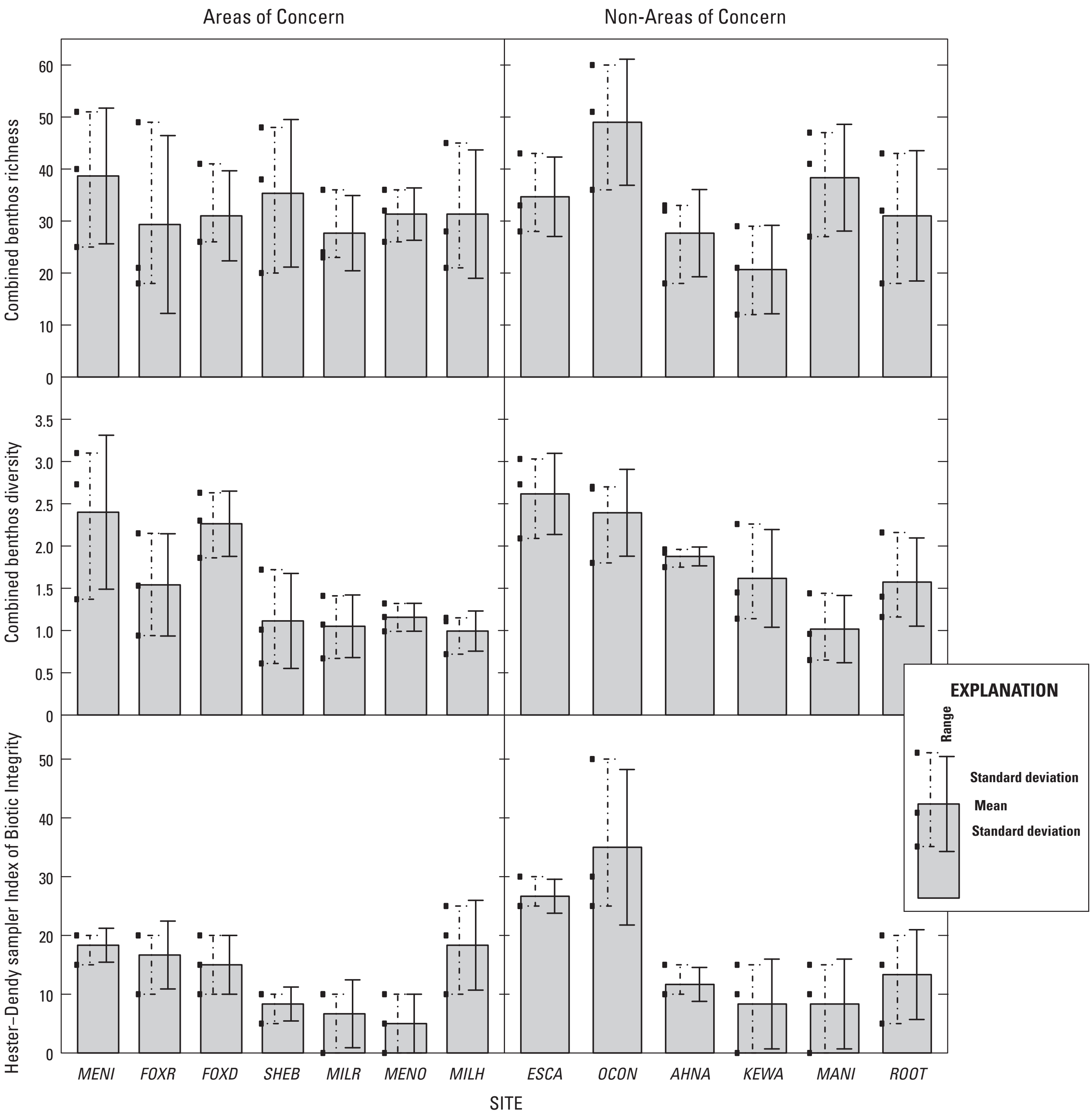


Figure 3. Boxplots of *A*, richness, *B*, diversity metric values for combined benthos (dredge and Hester-Dendy data combined), and *C*, Index of Biotic Integrity (Hester-Dendy samples) from four Lake Michigan Areas of Concern and six non-Area of Concern comparison sites. Site abbreviations: *MENI*–Lower Menominee River; Lower Green Bay and Fox River subsites: *FOXR*–Fox River near Allouez and *FOXD*–Fox River near De Pere; *SHEB*–Sheboygan River; Milwaukee Estuary subsites: *MILR*–Milwaukee River, *MENO*–Menomonee River, and *MILH*–Milwaukee Harbor; *ESCA*–Escanaba River, Mich.; *OCON*–Oconto River; *AHNA*–Ahnapee River; *KEWA*–Kewaunee River; *MANI*–Manitowoc River; and *ROOT*–Root River.

Table 4. Significance values (p) for Mann-Whitney tests and paired t-tests for metric comparisons between each of the four Lake Michigan Areas of Concern and the six non-Area of Concern comparison sites as a group or the specific non-Areas of Concern pair across the spring, summer, and fall seasons in 2012.

[Site abbreviations: *MENI*–Lower Menominee River; Lower Green Bay and Fox River subsites: *FOXR*–Fox River near Allouez and *FOXD*–Fox River near De Pere; *SHEB*–Sheboygan River; Milwaukee Estuary subsites: *MILR*–Milwaukee River and *MENO*–Menomonee River; *ESCA*–Escanaba River, Mich.; *OCON*–Oconto River; *AHNA*–Ahnapee River; *KEWA*–Kewaunee River; *MANI*–Manitowoc River; and *ROOT*–Root River. **Bold** values indicate that Area of Concern metric values were significantly lower at $p<0.05$. Benthos samples were not collected in Green Bay; AOC, Area of Concern; nc, plankton samples were not collected at the Fox River near De Pere; IBI, Index of Biotic Integrity computed as in Weigel and Dimick (2011); richness computed as the number of unique taxa in sample; Shannon diversity calculated as $\log_e$]

Area of Concern site or subsite	Comparison sites	Combined benthos		Hester-Dendy benthos	Zooplankton		Combined phytoplankton	
		Richness	Diversity	IBI[3]	Richness	Diversity	Richness	Diversity
MENI	NonAOCs	1.000	0.507	0.507	0.111	0.268	1.000	1.000
	ESCA; *OCON*	1.000	0.667	**0.046**	0.400	0.737	1.000	1.000
FOXR	Non-AOCs	0.667	0.667	0.507	0.053	0.121	0.111	0.507
	AHNA; *KEWA*	1.000	1.000	0.102	0.111	0.281	0.111	0.444
FOXD	Non-AOCs	0.507	0.222	0.667	nc	nc	nc	nc
	AHNA; *KEWA*	0.825	0.222	0.400	nc	nc	nc	nc
SHEB	Non-AOCs	1.000	0.400	**0.046**	1.000	0.311	0.111	0.507
	KEWA; *MANI*	0.800	0.667	1.000	1.000	0.102	0.667	0.800
MILR	Non-AOCs	0.444	0.111	**0.046**	1.000	0.109	0.111	0.102
	MANI; *ROOT*	0.667	0.400	0.268	1.000	0.400	0.444	0.444
MENO	Non-AOCs	1.000	**0.038**	**0.038**	1.000	([1])	0.667	([1])
	MANI; *ROOT*	0.667	0.184	0.444	0.667	([1])	1.000	0.400

[1]*MENO* values significantly higher, not lower, than comparison sites ($p<0.05$).

Table 5. Summarized results of metric comparisons for benthos and plankton samples collected in 2012 by the U.S. Geological Survey at four Lake Michigan Areas of Concern and six non-Area of Concern comparison sites.

[Site abbreviations: *MENI*–Lower Menominee River; Lower Green Bay and Fox River subsite: *FOXR*–Fox River near Allouez; *SHEB*–Sheboygan River; Milwaukee Estuary subsites: *MILR*–Milwaukee River and *MENO*–Menomonee River; and *AHNA*–Ahnapee River. Sites in ***bold italics*** were significantly lower in richness, diversity, or the IBI than non-AOCs for all seasons (Mann Whitney test or paired t test, $p<0.05$); sites not in bold italics were evaluated for single seasons based on comparison of standard deviations and ranges because of the small sample numbers; AOC, Area of Concern; IBI, Index of Biotic Integrity]

Community	Metric	Degraded at Level 1 (AOC:nonAOC group)	Degraded at Level 2 (AOC:nonAOC pair, per season)	Degraded at Level 3 (AOC:nonAOC pair, all seasons)	Degraded overall
Combined benthos	Richness	None	*MILR* Spring, Summer *MENO* Spring	None	*MILR*
	Diversity	***MENO***	*MENI* Fall *FOXR* Summer *SHEB* Summer	None	None
Hester-Dendy benthos	IBI	***SHEB*** ***MILR*** ***MENO***	*MENI* Spring, Summer *MILR* Spring *MENO* Spring, Summer	***MENI***	*MENI* *MENO*
Zooplankton[1]	Richness	*FOXR* Fall *MILR* Spring	*FOXR* Fall *SHEB* Spring *MILR* Spring *MENO* Fall	None	*FOXR* *MILR*
	Diversity	None	*FOXR* Fall *MENI* Fall	None	None
Combined phytoplankton	Richness	None	None	None	None
	Diversity	None	*MENI* Summer	None	None

[1]High algal counts precluded identification of rotifers other than *Asplanchna priodonta* in zooplankton for *AHNA* summer samples and all *FOXR* samples. Therefore, zooplankton comparisons *FOXR* excluded all other rotifers at non-AOCs.

as degraded at Level 1. Diversity in fall at *MENI*, summer at the Fox River near Allouez (*FOXR*), and summer at the Sheboygan River (*SHEB*) AOC were rated as degraded at Level 2 in comparison to their paired non-AOCs. No AOCs were rated as degraded at Level 3, based on results for Levels comparisons, Mann-Whitney tests, or paired t-tests, and so none were rated as degraded overall with regard to diversity when compared to the non-AOC sites.

Mean IBIs at AOCs ranged from 5.0 ± 5 at the *MENO* subsite to 18 ± 2.9 at *MENI*; mean IBIs at non-AOCs ranged from 8.3 ± 7.6 at both *KEWA* and *MANI* to 35 ± 13 at *OCON* (fig. 3*C*). Mann-Whitney or paired t-tests showed that the IBIs at *SHEB*, *MILR*, and *MENO* were significantly lower than non-AOCs as a group across all seasons and so were rated as degraded at Level 1 (tables 4 and 5). IBIs in spring and summer at *MENI*, spring at *MILR*, and spring and summer at *MENO* were rated as degraded at Level 2. Mann-Whitney tests showed that the IBIs across all seasons at *MENI* were also significantly lower than the paired non-AOCs for Level 3. Because two seasonal samples were rated as degraded at Level 2, the benthos communities for *MENI* and the *MENO* subsite were rated as degraded overall with regard to benthos IBIs. Similar to its paired non-AOCs—*ESCA* and *OCON*, the substrate at *MENI* was primarily hard sand (84–91 percent), making dredge grabs difficult to obtain; volatile-on-ignition analyses indicated low amounts of organic matter in the samples. Multiple independent studies during the 1970s and 1980s characterized the benthos of the Lower Menominee River AOC 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).

Nonmetric multidimensional scaling (MDS) is a multivariate method that represents objects, such as samples, in two or three dimensions where dissimilar objects plot far apart and similar objects plot close together. Stress values <0.2 are optimal with regard to how well relationships between objects are represented in an MDS plot. A small R (0.14) and moderate stress (0.21) in the two-dimensional MDS plot of benthos samples indicate that the relative relationships between samples are well represented in fig. 4. Based on ANOSIM tests with relative abundances of combined benthos taxa when seasons were treated separately, the differences between each AOC and all non-AOCs as a group were not significant. However, benthos at *FOXR* and *FOXD* were significantly different from their paired non-AOCs (Ahnapee River [*AHNA*] and *KEWA*). Additional SIMPER testing showed that *FOXD* was 60 percent dissimilar, with zebra mussels, the caddisfly *Cyrnellus fraternus*, and the clam *Sphaerium* contributing most to distinguishing between the sites; *FOXR* was 64 percent dissimilar, and differences between the AOC and its non-AOC comparison sites were due mostly to differences in relative abundances of zebra mussels, the midge *Procladius*, and the oligochaete *Limnodrilus cervix*. The benthos community at *MILR* was significantly different when compared to the paired non-AOCs *MANI* and *ROOT*. The community at *MILR* was 48 percent dissimilar from the paired non-AOCs, with differences between the sites due mostly to differences in relative abundances of the oligochaete *Aulodrilus pluriseta*, the isopod *Caecidotea intermedia*, and the midge *Procladius*. ANOSIM tests indicated that relative abundances of combined benthos taxa at *MENI*, *SHEB*, and *MENO* were not significantly different than the paired non-AOCs across seasons.

For the combined benthos data, the taxon that accounted for most of the variability between AOCs as a group and non-AOCs as a group was immature tubificid oligochaete worms, Tubificinae (r_s=0.56). The five taxa that accounted for most of the variability between the two groups were immature tubificid oligochaetes as well as adult oligochaetes *Limnodrilus hoffmeisteri* and *Nais pardalis*, and chironomid midges *Tanytarsus* spp. and *Ablabesmyia* spp. (r_s=0.78). The tubificid oligochaete *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 conditions (Bode and others, 2002).

Plankton Community Comparisons between Areas of Concern and Non-Areas of Concern

Plankton communities at AOCs were compared using richness and diversity metrics. For zooplankton, rotifers made up at least half of the taxa in samples on average (Scudder Eikenberry and others, 2014), and this had a large effect on richness and diversity. With the exception of the large rotifer *Asplanchna priodonta*, data for the Fox River near Allouez (*FOXR*) and one of its paired non-AOCs, the Ahnapee River (*AHNA*), lacked rotifer data for the summer sample because large algal blooms obscured rotifer counts. For this reason, zooplankton comparisons for *FOXR* were made by excluding rotifers other than *A. priodonta* from metric comparisons. Mean zooplankton richness at AOCs ranged from 7.7 ± 2.5 at *FOXR* (without rotifers) to 29.0 ± 5.3 at the Lower Menominee River (*MENI*) (with rotifers); mean richness at non-AOCs ranged from 8.7 ± 2.1 at *AHNA* (without rotifers) to 24.0 ± 3.0 at the Escanaba River (*ESCA*) (with rotifers) (fig. 5*A*). At Level 1, the *FOXR* fall and the Milwaukee River (*MILR*) spring samples for zooplankton richness were rated as degraded for richness (tables 4 and 5). At Level 1, zooplankton richness at *FOXR* was lower than non-AOCs as a group, but was not quite significantly lower (p=0.053). At Level 2, *FOXR* and *MILR* were also rated as degraded for richness, so the zooplankton communities at these AOCs were rated as degraded overall in comparison to their paired non-AOCs. The Sheboygan River (*SHEB*) AOC spring and Menomonee River (*MENO*) fall samples were rated as degraded at Level 2; no sites were rated as degraded at Level 3 with regard to zooplankton richness.

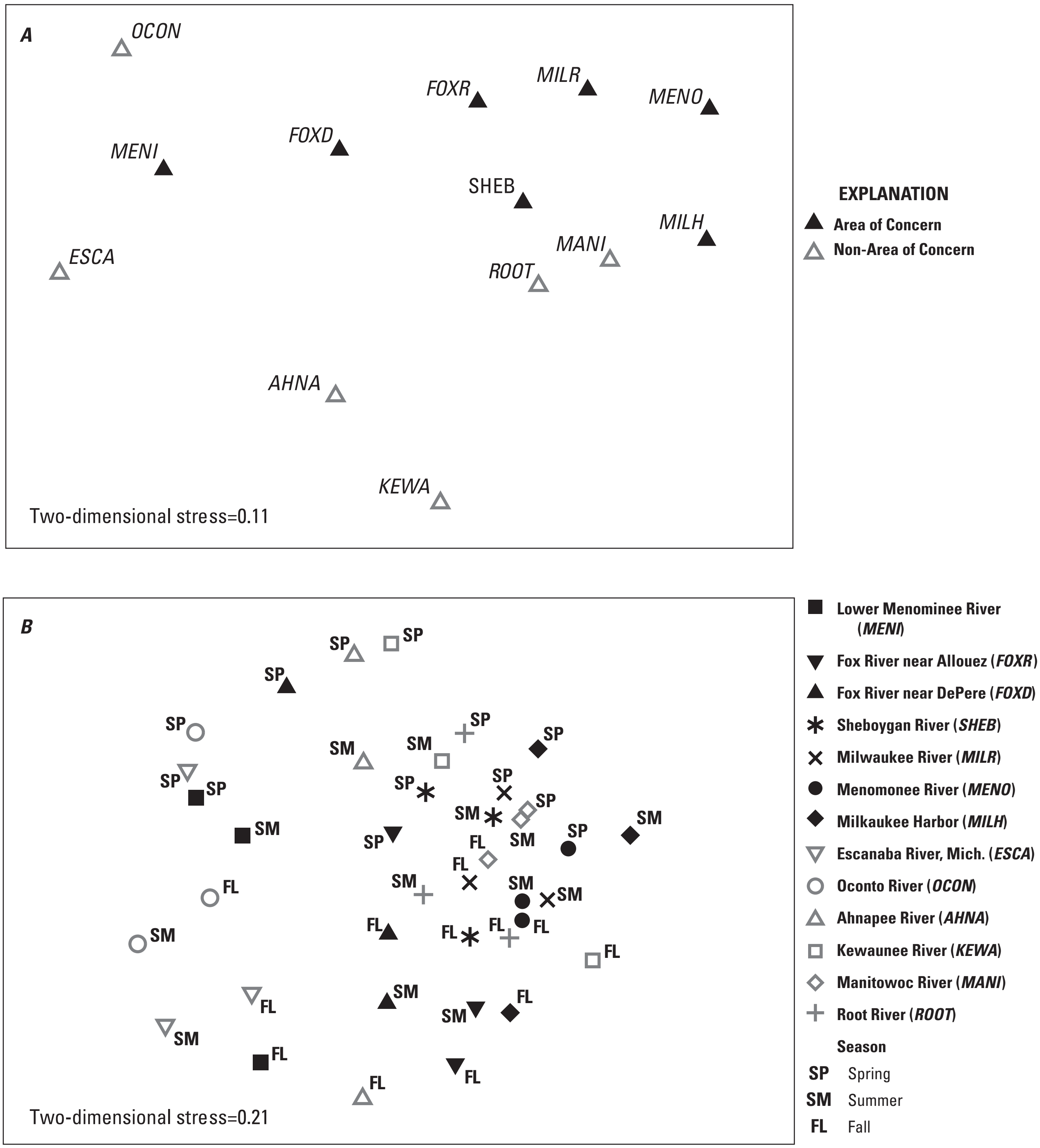


Figure 4. Multi-Dimensional Scaling ordination plots for the combined benthos (dredge and Hester-Dendy samples) at four Lake Michigan Areas of Concern, including subsites for the Milwaukee Estuary Area of Concern, and non-Area of Concern comparison sites, based on relative abundance with no rare or ambiguous taxa, *A*, with seasons combined, and *B*, with seasons separate. Distances between sites are representative of their similarity or dissimilarity to each other.

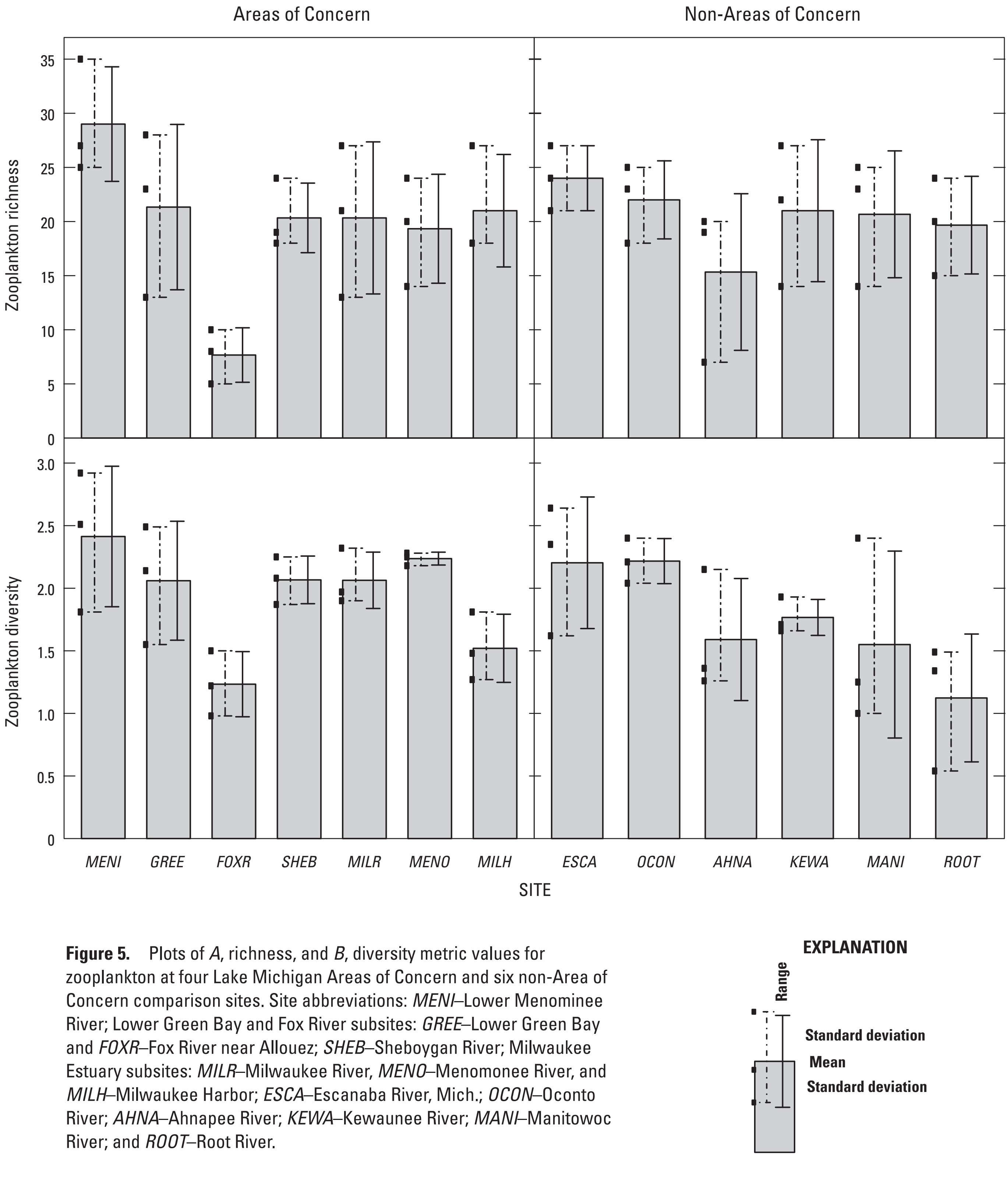


Figure 5. Plots of *A*, richness, and *B*, diversity metric values for zooplankton at four Lake Michigan Areas of Concern and six non-Area of Concern comparison sites. Site abbreviations: *MENI*–Lower Menominee River; Lower Green Bay and Fox River subsites: *GREE*–Lower Green Bay and *FOXR*–Fox River near Allouez; *SHEB*–Sheboygan River; Milwaukee Estuary subsites: *MILR*–Milwaukee River, *MENO*–Menomonee River, and *MILH*–Milwaukee Harbor; *ESCA*–Escanaba River, Mich.; *OCON*–Oconto River; *AHNA*–Ahnapee River; *KEWA*–Kewaunee River; *MANI*–Manitowoc River; and *ROOT*–Root River.

Zooplankton diversity at AOCs ranged from 1.23 ± 0.26 at *FOXR* (without rotifers) to 2.41 ± 5.56 at *MENI* (with rotifers); diversity at non-AOCs ranged from 1.12 ± 0.51 at the Root River (*ROOT*) with rotifers to 2.22 ± 0.18 at the Oconto River (*OCON*) with rotifers (fig. 5*B*). No zooplankton samples were rated as degraded at Level 1 for diversity. The fall samples for the *FOXR* and *MENI* were rated as degraded for zooplankton diversity at Level 2 (table 5). No AOCs were rated as degraded at Level 3 or overall for zooplankton diversity in comparison to the non-AOCs.

One-way ANOSIM tests on relative abundances of zooplankton taxa were done with and without *FOXR* data because no rotifers, except *Asplanchna priodonta*, were counted at this site due to interference from high algal counts in the samples. In both cases, there was no significant difference ($p>0.10$) between zooplankton communities at all AOC and non-AOCs as a group (fig. 6*A* and *B*). However, zooplankton communities at *FOXR* were significantly different from the paired non-AOCs (*AHNA* and *KEWA*) when comparisons were made without rotifers other than *A. priodonta*. Additional SIMPER tests showed that *FOXR* was 57 percent dissimilar due in large part to differences in relative abundances of the rotifer *A. priodonta*, the copepod *Diacyclops bicuspidatus thomasi*, and cladoceran *Chydorus sphaericus*.

The zooplankton taxon that accounted for the most variability between AOCs as a group and non-AOCs as a group was the cladoceran *Bosmina longirostris* (r_s=0.51) when only microcrustaceans were considered and the rotifer *Brachionus calyciflorus* when both microcrustaceans and rotifers were considered (r_s=0.51). The five taxa that accounted for most of the variability between the groups were the cladoceran *Bosmina longirostris*, and rotifers *Brachionus calyciflorus*, *Ascomorpha saltans*, *Synchaeta oblonga*, and *Gastropus stylifer* (r_s=0.77).

Phytoplankton communities were compared between AOCs and non-AOCs using richness and diversity metrics calculated for soft algae and diatoms separately and combined. For combined phytoplankton, mean richness ranged from 26.3 ± 11.5 at *MENI* to 59.7 ± 5.0 at *FOXR*; mean richness at non-AOCs ranged from 26.3 ± 8.4 at *ESCA* to 45.0 ± 26.3 at Kewaunee River (*KEWA*) and 45.0 ± 21.2 at *ROOT* (fig. 7*A*). No AOC sites were rated as degraded for combined phytoplankton richness at any levels (table 5) in comparison to the sampled non-AOCs. Mann-Whitney tests showed no significant differences in chlorophyll-*a* concentration or total suspended solids and volatile suspended solids between AOCs and non-AOCs ($p<0.05$), indicating that phytoplankton biomass was not significantly different between AOCs and non-AOCs.

Mean diversity for combined phytoplankton at AOCs ranged from 2.42 ± 0.5 at *MENI* to 3.00 ± 0.20 at *MENO*, and at non-AOCs it ranged from 2.30 ± 0.16 at *AHNA* to 2.88 ± 0.48 at *ROOT* (fig. 7*B*). Combined phytoplankton diversity for the *MENI* summer sample was rated as degraded at Level 2 (tables 4 and 5). Combined phytoplankton communities were rated as not degraded at Level 3 or overall at any AOC sites in comparison to the sampled non-AOCs, possibly due to contributions of tolerant taxa to richness and diversity metrics for these sites.

No significant difference between AOCs and non-AOCs were found for combined phytoplankton communities in a one way ANOSIM using transformed relative abundance with no ambiguous or rare taxa if seasons were combined (fig. 8*A*) or kept separate (p=0.40 and 0.07, respectively; fig. 8*B*). However, the combined phytoplankton community at *FOXR* was significantly different from its paired non-AOCs (*AHNA* and *KEWA*); SIMPER testing showed that the Fox River was 72 percent dissimilar, due mostly to the diatoms *Aulacoseira granulata*, *Stephanodiscus hantzschii*, and *Stephanodiscus niagarae*.

The phytoplankton taxon that accounted for the most variability between AOCs as a group and non-AOCs as a group was *Cocconeis placentula* (r_s=0.49). The five taxa that accounted for most of the variability between the groups were *Cocconeis placentula*, *Amphora pediculus*, *Aulacoseira granulata*, *Rhoicosphenia abbreviata*, and *Staurosira construens* (r_s=0.71). All five taxa are diatoms that are alkalophilous (occurring mostly at pH>7) and fresh brackish (chloride <500 milligrams per liter, salinity <0.9 percent) (Van Dam and others, 1994), tolerant of moderately polluted waters (ß-mesosaprobous) but relatively sensitive with regard to overall pollution class/tolerance (Bahls, 1993; Lange-Bertalot, 1979).

Potentially harmful blue-green algae were identified in soft algae samples from three of four AOCs, including *MENI* in fall, *FOXR* in all seasons, and all three Milwaukee Estuary subsites (*MILR*, *MILH*, and *MENO*) in fall. In addition, these taxa were found at two non-AOCs: *KEWA* in summer and fall, and *ROOT* in summer. Taxa of the blue-green algae were identified as *Anabaena* sp., *Aphanizomenon flos aquae* and *Aphanizomenon. issatschenkoi*, *Cylindrospermopsis raciborskii*, *Microcystis aeruginosa*, and *Microcystis* sp. *Aphanizomenon issatschenkoi* was identified at six sites—more than any other blue-green algae. Blue-green algae were dominant in all phytoplankton samples collected from Lower Green Bay (*GREE*) and *FOXR*, in part due to high densities of the harmful algal taxa *Anabaena* in spring and *Microcystis* in summer and fall. Zebra mussels were first found in Green Bay in 1991 and are now (2012) very abundant. Their high densities and ability to filter large volumes of water in the Bay correlated with a change in dominance from green algae to blue-green algae, large increases in abundance of blue-green algae *Anabaena* and *Microcystis*, and an increase in phytoplankton biovolume and chlorophyll (De Stasio, Jr. and others, 2014). *Microcystis* is known to thrive in high nutrient conditions, such as those found at in 2012 samples at *GREE* and *FOXR*. These results may provide additional input to the BUI for excessive nutrients and undesirable algae in the Lower Green Bay and Fox River AOC and the Milwaukee Estuary AOC.

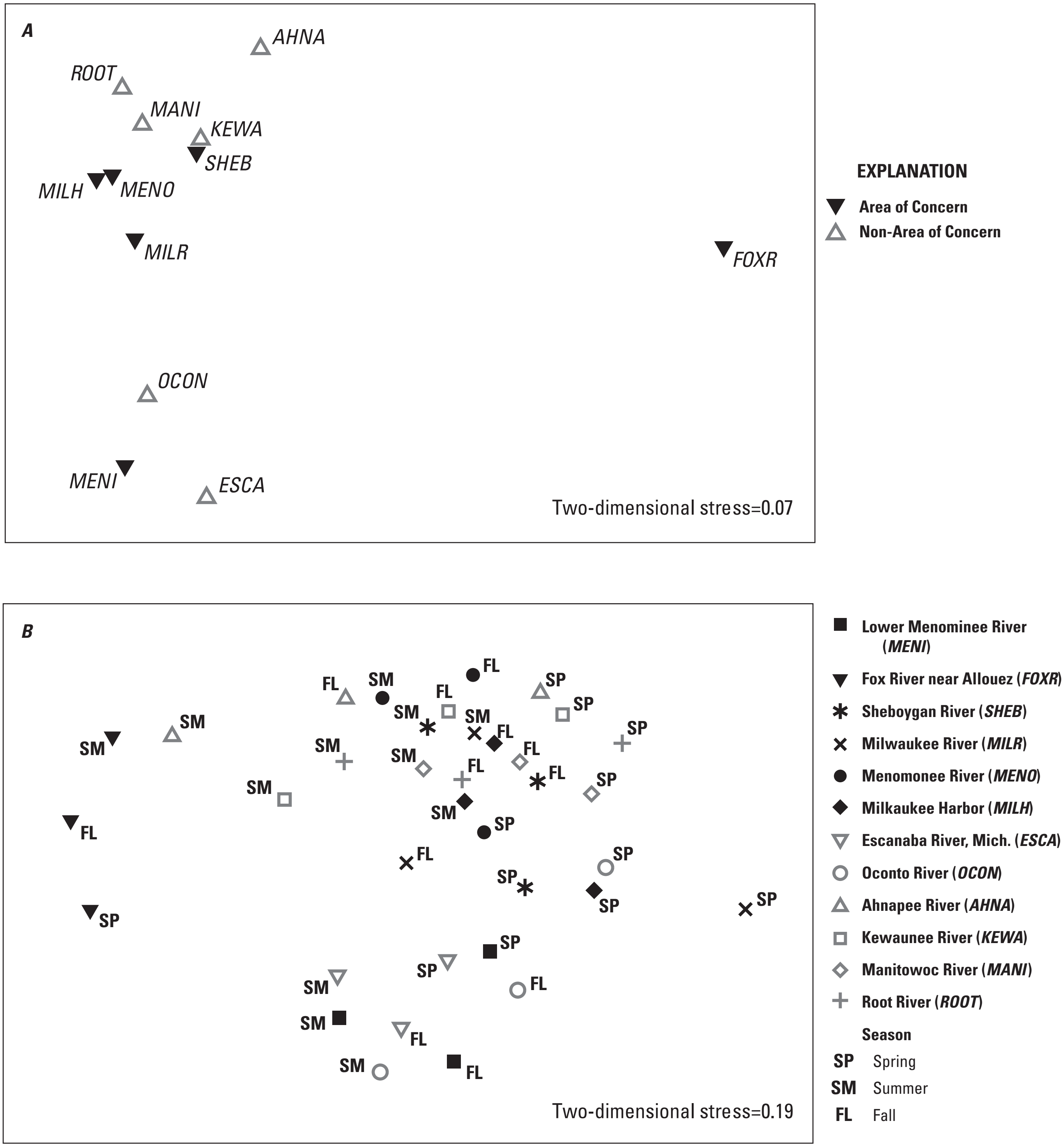


Figure 6. Multi-Dimensional Scaling ordination plots for zooplankton at four Lake Michigan Areas of Concern, including subsites for the Milwaukee Estuary Area of Concern, and six non-Area of Concern comparison sites, based on relative abundance (calculated by using fourth root transformation) with no rare or ambiguous taxa: *A*, with seasons combined, and *B*, with seasons separate.

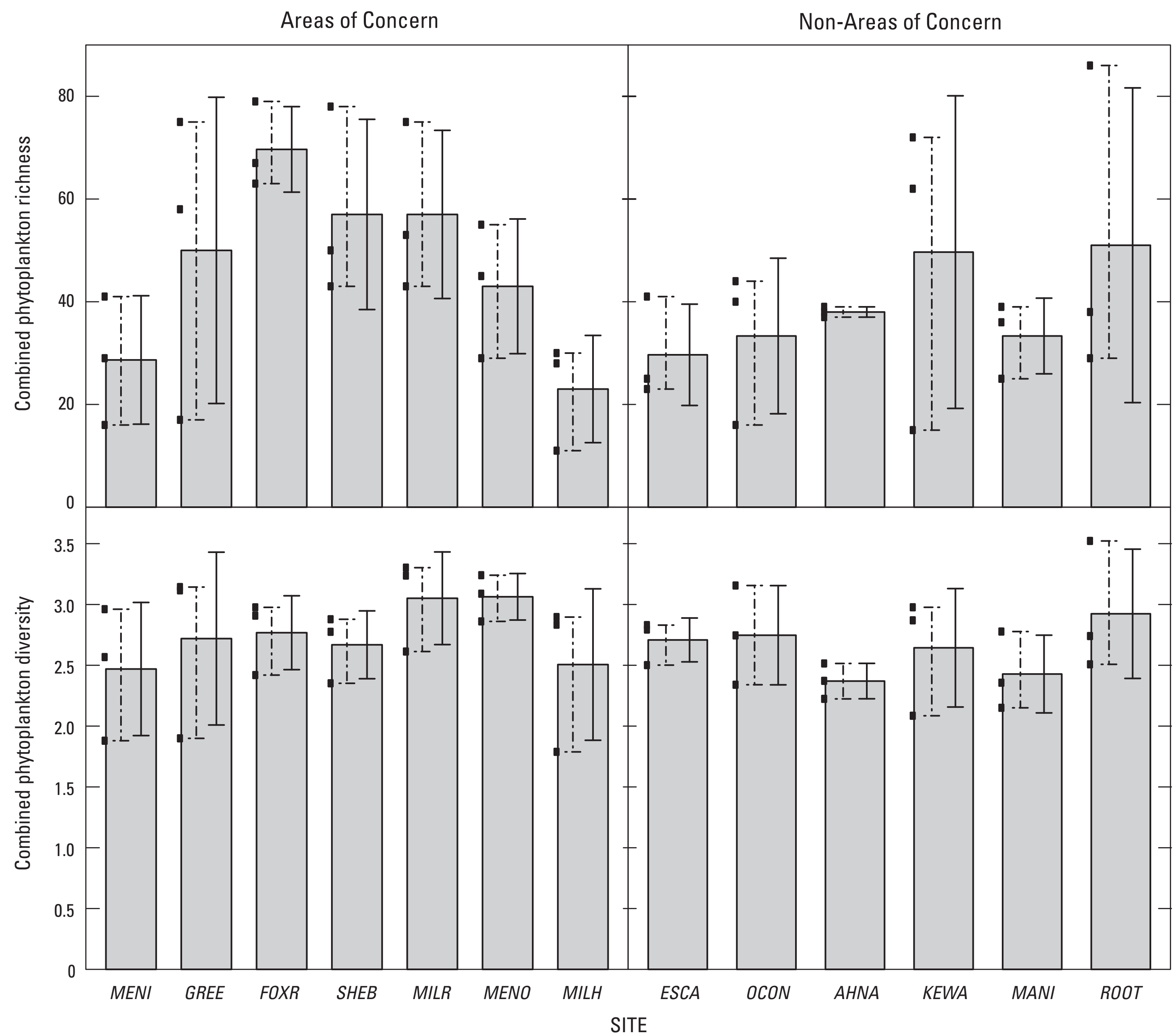
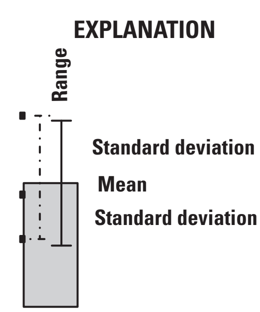


Figure 7. Plots of *A*, richness and *B*, diversity metric values for combined phytoplankton (soft algae and diatoms) at four Lake Michigan Areas of Concern and six non-Area of Concern comparison sites. Site abbreviations: *MENI*–Lower Menominee River; Lower Green Bay and Fox River subsites: *GREE*–Lower Green Bay and *FOXR*–Fox River near Allouez; *SHEB*–Sheboygan River; Milwaukee Estuary subsites: *MILR*–Milwaukee River, *MENO*–Menomonee River, and *MILH*–Milwaukee Harbor; *ESCA*–Escanaba River, Mich.; *OCON*–Oconto River; *AHNA*–Ahnapee River; *KEWA*–Kewaunee River; *MANI*–Manitowoc River; and *ROOT*–Root River.



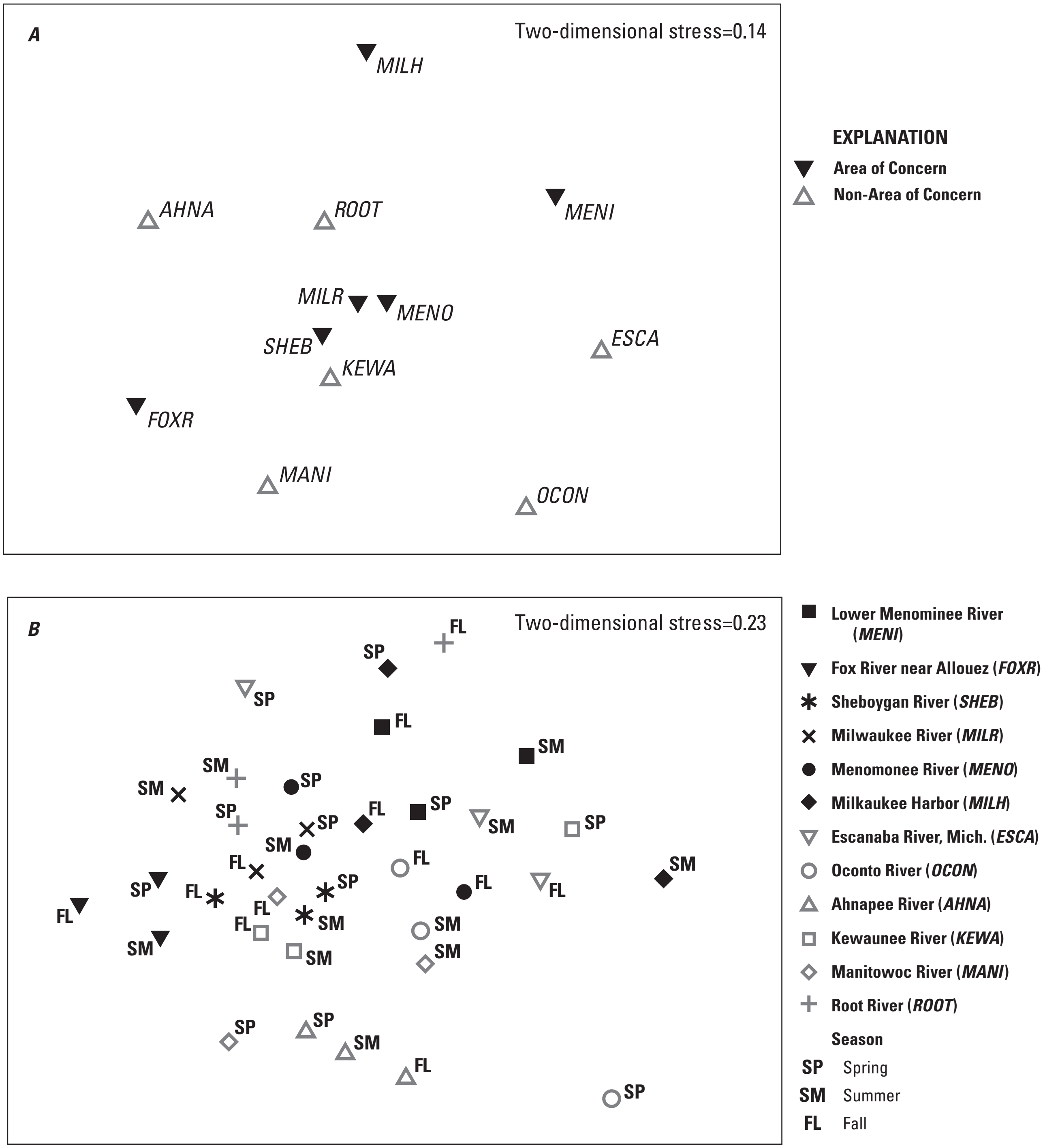


Figure 8. Multi-Dimensional Scaling ordination plots for the combined phytoplankton (soft algae and diatoms) at four Lake Michigan Areas of Concern, including subsites for the Milwaukee Estuary Area of concern, and six non-Area of Concern comparison sites, based on relative abundance (calculated by using fourth root transformation) with no rare or ambiguous taxa: *A*, with seasons combined, and *B*, with seasons separate.

Invasive Species at Areas of Concern and Non-Areas of Concern

Invasive benthos species were found in some samples. Two live, large specimens of the invasive Asian clam *Corbicula fluminea* were identified in dredge samples in July and August at the Menomonee River (*MENO*) subsite in the Milwaukee Estuary AOC. *Corbicula* were not identified at any other sample in 2012. *Corbicula* is an aquatic invasive species that has been found at other locations in the Great Lakes and in rivers of the continental United States (Carlisle and others, 2013; Foster, 2013). Invasive zebra mussels were found in benthos and plankton samples from all sites and were considered abundant at some AOCs and non-AOCs. The highest abundances for adult zebra mussels were found in both Fox River subsites (*FOXR* and *FOXD*) summer HD and Sheboygan River (*SHEB*) fall HD samples. Overall, zebra mussels were more commonly found in HD samples than in dredge samples, possibly due to the preference of zebra mussels for hard substrate over the soft natural substrates. Lastly, the invasive benthic amphipod *Echinogammarus ischnus* has established populations in the Fox River (De Stasio, 2013), and populations have been documented elsewhere in the Great Lakes (Nalepa and others, 2001; Witt and others, 1997). In our study, *Echinogammarus* sp. was identified in benthos samples—mostly in HD samples—from both Fox River subsites as well as from the Lower Menominee River (*MENI*) and *SHEB*, the Milwaukee Harbor (*MILH*) subsite, and the Escanaba River (*ESCA*), Ahnapee River (*AHNA*), and Manitowoc River (*MANI*) non-AOCs. However, because the identifications were not made past genus, we cannot confirm the presence of *E. ischnus* in any of our samples.

Benthos Community Comparisons to Other Studies

When values for eight invertebrate metrics from HD sampler data collected in the current study were compared quantitatively with values for data collected near the same AOC locations in 2003 through 2005 by Weigel and Dimick (2011), little difference was found between the two studies for these metrics as a group. Three sites, the Fox River near De Pere (*FOXD*), and the Lower Menominee (*MENI*) and Sheboygan River (*SHEB*) AOCs, had a single metric value that was significantly different between the studies. At *FOXD*, chironomids were dominant, making up 62 percent of the total taxa in 2005, and this was more than twice the highest value in 2012 (31.7 percent in spring, p=0.06). For *MENI*, the large river IBI scored 45 (fair) in 2005, and that was significantly higher (p=0.01) than the 2012 IBIs, which scored 15 (very poor) for spring and 20 (poor) in summer and fall. At *SHEB*, the percentage of Ephemeroptera-Plecoptera-Trichoptera taxa was 2.56, which was significantly (p<0.05) higher in 2003 compared to all seasons in 2012, which scored <1. There were no significant differences in metric values between 2005 and 2012 at the Milwaukee River (*MILR*). Although the large river IBI is designed for use in riverine sections with flows of at least 0.09 meters per second, this flow threshold may not have been met at times in our study, especially in the Milwaukee Estuary AOC; therefore values may not be comparable to other locations and studies.

Previous studies of the Lower Green Bay and Fox River AOC found the benthos to be low in diversity and predominantly tolerant tubificid oligochaete worms and chironomid midge larvae (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, State Board of Health, and Green Bay Metropolitan Sewerage Commission, 1939). A study by Canfield and others (1996) found that oligochaete worms and chironomid midge larvae made up over 90 percent of the benthos at three Great Lakes AOCs. The change from rocky to soft, silty bottom substrates along with toxins and low oxygen levels 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). Burrowing mayfly larvae (*Hexagenia* sp.), which are referred to as "fish flies" or "Green Bay flies" when adults, were abundant in the 1930s although the larvae were found in low densities in dredge samples of Lower Green Bay in 1938 and 1939 (Wisconsin State Committee on Water Pollution, State Board of Health, and Green Bay Metropolitan Sewerage Commission, 1939). The larvae also were collected at 16 of 51 stations in surveys of Green Bay by Balch and others (1956), but rarely in later years (Ball and Patterson, 1985; Wisconsin Department of Natural Resources, 2013b).

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 (Wisconsin Department of Natural Resources, 1991). River studies have been done of benthos in the Sheboygan River AOC (Wisconsin Department of Natural Resources, 2012b) so historical comparisons are difficult to make. A study in 1997 using dredge grabs found immature tubificid oligochaetes made up over 90 percent of the benthos communities at most Sheboygan River sites sampled, and analyses of a subset of these sites determined that there were just two species present: *Limnodrilus hoffmeisteri* and *Limnodrilus cervix* (EVS Environment Consultants Inc. and National Oceanic and Atmospheric Administration, 1998). Our dredge samples at the Sheboygan River AOC in 2012 found degraded diversity and over 80 percent dominance by abundant immature tubificid oligochaetes and adult oligochaetes were primarily of the species *Limnodrilus hoffmeisteri* and *L. cervix*. These results suggest the Sheboygan River benthos community in 2012 had changed little since 1997.

Phytoplankton Community Comparisons to Other Studies

Historical studies in 1938 and 1939 in Lower Green Bay and the Fox River found zooplankton such as rotifers and microcrustaceans were usually present in low numbers (Wisconsin State Committee on Water Pollution, State Board of Health, and Green Bay Metropolitan Sewerage Commission, 1939). Later studies in the 1980s found rotifer abundance higher than that of microcrustaceans in the lower eutrophic portion of Green Bay (Richman, Bailiff, and others, 1984; Richman, Sager, and others, 1984). 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, State Board of Health, and Green Bay Metropolitan Sewerage Commission, 1939) included mostly diatoms and blue-green algae, with blooms of toxin-producer *Aphanizomenon*. Later surveys found plankton communities dominated by blue-green algae and small crustaceans, both with limited 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 portion of Green Bay (Richman, Bailiff, and others, 1984; Richman, Sager, and others, 1984).

The MMSD collected zooplankton and phytoplankton periodically from 1980 through 1997 in the Milwaukee Estuary AOC using methods fairly similar to those used in this study. Zooplankton were collected using an 80-micrometer mesh plankton net with vertical hauls from 1 m off the bottom to the surface, and 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 the Milwaukee Harbor (*MILH*) subsite sampled in 2012 for this study. At the MMSD site, 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 *MILH* in 2012. However, zebra mussel veligers were subdominant (22 to 35 percent) in 2012, and copepods and cladocerans were only minor components of the community. For rotifers, *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, instead, the previously subdominant taxa began to be dominant. *Synchaeta oblonga* was the dominant rotifer in the spring and summer of 2012 at *MILH*, and *Keratella crassa* was dominant in the fall of 2012. 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 appeared grossly similar to 1995–97, with nauplii and Cyclopoid copepodites dominant and Calanoid copepodites subdominant. Harpacticoid copepods, a benthic taxon, were reported first in the 1997 sample in low abundance, and these copepods were present in 2012 also in low abundance. For cladocerans, *Bosmina longirostris* was the dominant cladoceran species in all MMSD samples as well as in 2012 at *MILH*, and *Ceriodaphnia lacustris* and *Diaphanosoma birgei* were subdominant in the summer and fall 2012 samples, respectively.

In the MMSD phytoplankton samples collected near *MILH*, diatoms and green algae were generally the dominant algal group, followed by blue-green algae and (or) cryptophytes, depending on the season. In 2012, diatoms were the dominant group (58 percent) in spring, cryptophytes were dominant (50 percent) in summer, and green algae (37 percent) and blue-green algae (36 percent) were codominant in fall (fig. 8). Diatom taxa were identified beyond phylum in about one third of the MMSD samples and, in those cases, dominant taxa varied by season and year, so comparisons are difficult and not attempted here. Changes in dominance from diatoms to other algal groups can indicate short-term and long-term declines in water quality.

Summary and Conclusions

The non-AOCs selected as comparison sites in this study were the best available with regard to being less impaired, having similar physical characteristics to the AOCs, and being located in the Great Lakes region. When the benthos or plankton community at an AOC is rated as more impaired than one of these non-AOC comparison sites, whether or not the non-AOC(s) have some impairment themselves, it emphasizes the finding of impairment at the AOC. Conversely, a finding of no statistical difference between a community or sample at an AOC community and selected non-AOCs does not mean that the benthos or plankton community at an AOC is unimpaired.

It is critical to consider a variety of measures when comparing communities at an AOC with one or more less impaired sites because some measures address only a single aspect of the community. Use of both 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 community 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 or blue-green algae replace diatoms. Multivariate statistical analyses such as MDS and ANOSIM may be more sensitive at detecting community change than diversity or richness metrics because multivariate methods test differences based the specific taxa present at each site. However, in this study, the metric comparisons detected differences between AOCs and non-AOCs that the multivariate comparisons often did not, possibly because the multivariate tests highlight different aspects of these communities or because a metric may have had a lower variability and thus smaller differences could be detected statistically. An IBI combines structural and functional measures and may therefore be a more effective measure to use for defining differences or change. At present, there are no zooplankton or phytoplankton IBIs for use in rivermouths or harbors. The benthos IBI for rivermouths and harbors may be more valuable with the addition of functional and tolerance information for oligochaetes due to their importance in these ecosystems and the range in environmental preferences for this large and diverse group of organisms.

With regard to the BUI for benthos at the sampled AOCs, predominant taxa at sites were oligochaetes and chironomid midges. The benthos at the Lower Menominee and Milwaukee Estuary AOCs were rated as degraded overall because the metrics for more than one season or at more than one comparison level were rated as degraded. The Milwaukee Estuary AOC benthos samples were mostly comprised of immature tubificid oligochaetes at the three subsites (*MILR*, *MENO*, and *MILH*) except for the fall sample from *MILH* which, as mentioned earlier, had zebra mussels in greater abundance than oligochaetes. Those oligochaete taxa that were identified to species included generally tolerant ones such as *Limnodrilus hoffmeisteri*.

In the Lower Green Bay and Fox River AOC, the two Fox River subsites (*FOXR* and *FOXD*) sampled in this study were not rated as degraded for benthos in more than one season or event in comparison to non-AOCs. Although abundant in some historical samples, our study found *Hexagenia* sp. larvae only in dredge samples from the Lower Menominee River (*MENI*) and its paired non-AOCs, the Escanaba River (*ESCA*) and Oconto River (*OCON*). Sediment size fraction analyses showed that the substrates in those particular dredge samples had a high proportion of sand (at least 80 percent), unlike the substrate in the Lower Green Bay and Fox River samples collected as part of our study.

AOC zooplankton communities appeared to be less impaired than benthos communities when compared to the non-AOC sites, which may be due to greater effects of historic sediment contamination on benthos communities. Zooplankton at the AOCs were not statistically different from non-AOCs as a group or from non-AOC pairs when compared across all seasons; *FOXR* and *MILR* were rated as degraded overall for zooplankton richness based on comparisons for one season to either non-AOCs as a group or to their non-AOC comparison sites. Zebra mussel veligers were in the top three dominant taxa of the zooplankton community in 2012 in the Milwaukee Estuary AOC for all seasons at *MENO* and for summer at *MILR*.

No AOC was rated as degraded for phytoplankton in comparison to the non-AOCs but samples at some sites contained large percentages of nondiatoms, including blue-green algal taxa that are known to form toxins. Blue-green algae dominated the phytoplankton communities at the Lower Green Bay and Fox River AOC, comprising over 50 percent of the total abundance in spring and up to 87 and 92 percent, respectively, in fall samples. Although over 70 percent of the spring phytoplankton community in the Sheboygan River AOC was green algae, the community transitioned to increasing percentages of diatoms and eventual dominance by diatoms in the fall sample. Phytoplankton communities in the Milwaukee Estuary AOC were diverse, but nondiatoms were dominant at sites in two-thirds of the samples, indicating that the community is impaired. It is important to consider which groups of algal taxa are dominant in making assessments of site integrity because dominance by green or blue-green algae is indicative of water quality impairment due to eutrophication.

Further study after removal of historic sediment contamination at each of these AOCs should show improvements in benthos and plankton communities, although recovery may be complicated by other stresses such as excessive nutrients and invasive taxa. The magnitude of effort required to remediate these sites cannot be understated, and this report provides important information for evaluating the status of these communities in 2012.

References

American Public Health Association, American Water Works Association, and Water Environment Federation, 2006, Part 2540 E. Fixed and volatile solids ignited at 550 °C, *in* Eaton, A.D., Rice, E.W., and Baird, R.B., eds., Standard methods for the examination of water and wastewater (20th ed.): p. 2–55—2–61.

Ankley, G.T., Lodge, K., Call, D.J., Balcer, M.D., Brooke, L.T., Cook, P.M., Kreis, R.G., Carlson, A.R., Johnson, R.D., Niemi, G.J., Hoke, R.A., West, C.W., Giesy, J.P., Jones, P.D., and Fuying, Z.C., 1992, Integrated assessment of contaminated sediments in the Lower Fox River and Green Bay, Wisconsin: Ecotoxicology and Environmental Safety, v. 23, no. 1, p. 46–63.

Bahls, L.L., 1993, Periphyton bioassessment methods for Montana streams: Helena, Montana, Department of Health and Environmental Services, 69 p.

Bailey, R.C., Day, K.E., Norris, R.H., and Reynoldson, T.B., 1995, Macroinvertebrate community structure and sediment bioassay results from nearshore areas of North American Great Lakes: Journal of Great Lakes Research, v. 21, no. 1, p. 42–52.

Balch, R.F., Mackenthun, K.M., Van Horn, W.M., and Wisniewski, T.F., 1956, Biological studies of the Fox River and Green Bay: Madison, The Institute of Paper Chemistry and the Wisconsin Committee on Water Pollution, 74 p.

Ball, J.R., Harris, V.A., and Patterson, D.J., 1985, Lower Fox River—De Pere to Green Bay water quality standards review: Madison,Wisconsin Department of Natural Resources—Bureau of Water Resources Management and Bureau of Fish Management, variously paginated.

Bode, R.W., Novak, M.A., Abele, L.E., Heitzman, D.L., and Smith, A.J., 2002, Quality assurance workplan for biological stream monitoring in New York State: Albany, New York State Department of Environmental Conservation, p. 76–102.

Bouyoucos, G.J., 1962, Hydrometer method improved for making particle size analysis of soils: Agronomy Journal, v. 54, no. 5, p. 464–465.

Brinkhurst, R.O., and Gelder, S.R., 1991, Annelida: Oligochaeta and Branchiobdellida, *in* Thorp, J.H., and Covich, A.P., eds., Ecology and classification of North American freshwater invertebrates: San Diego, California, Academic Press, Inc., p. 401–435.

Canfield, T.J., Dwyer, F.J., Fairchild, J.F., Haverland, P.S., Ingersoll, C.G., Kemble, N.E., Mount, D.R., La Point, T.W., Burton, G.A., and Swift, M.C., 1996, Assessing contamination in Great Lakes sediment using benthic invertebrate communities and the sediment quality triad approach: Journal of Great Lakes Research, v. 22, no. 3, p. 565–583.

Cao, Yong, Larsen, D.P., and St-J. Thorne, R., 2001, Rare species in multivariateanalysis for bioassessment: Some considerations: Journal of the North American Benthological Society, v. 20, no. 1, p. 144–153.

Carlisle, D.M., Meador, M.R., Short, T.M., Tate, C.M., Gurtz, M.E., Bryant, W.L., Falcone, J.A., and Woodside, M.D., 2013, The quality of our Nation's waters—Ecological health in the Nation's streams, 1993–2005: U.S. Geological Survey Circular 1391, 120 p.

Clarke, K.R., and Gorley, R.N., 2006, PRIMER v6: Users manual/tutorial: Plymouth, United Kingdom, Primer-E Ltd, 192 p.

Cuffney, T.F., Bilger, M.D., and Haigler, A.M., 2007, Ambiguous taxa: effects on the characterization and interpretation of invertebrate assemblages: Journal of the North American Benthological Society, v. 26, no. 2, p. 286–307.

De Stasio, Bart, 2013, Aquatic Invasive Species Monitoring Project—Summary report for 2006–2012 to the Fox River Navigational System Authority: Appleton, Wisconsin, Lawrence University, Department of Biology, 14 p. plus appendixes, accessed 7 April 2016, at http://foxlocks.org/wp-content/uploads/2016/02/FRNSA-AIS-Project-Summary-Report-final-2006-2012.pdf.

De Stasio, B.T., Jr., Schrimpf, M.B., and Cornwell, B.H., 2014, Phytoplankton communities in Green Bay, Lake Michigan, after invasion by dreissenid mussels: increased dominance by cyanobacteria: Diversity, v. 6, no. 4, p. 681–704.

EVS Environment Consultants, Inc., and National Oceanic and Atmospheric Administration, 1998, Sheboygan River and Harbor—Aquatic ecological risk assessment, Volume 1 of 3: Seattle, Washington, 135 p., accessed April 7, 2016, at http://docs.lib.noaa.gov/rescue/NOAA_E_DOCS/E_Library/ORR/general/sheboygan_v1.pdf.

Federal Water Pollution Control Administration, 1968, Water quality investigations Lake Michigan Basin—Biology: Chicago, U.S. Department of the Interior–Federal Water Pollution Control Administration, Great Lakes Region, 40 p., available at http://nepis.epa.gov/.

Flotemersch, J.E., Stribling, J.B., and Paul, M.J., 2006, Concepts and approaches for the bioassessment of non-wadable streams and rivers: Cincinnati: U.S. Environmental Protection Agency, Report EPA–600–R–06–127, variously paged.

Foster, A.M., Fuller, Pam., Benson, Amy, Constant, S., Raikow, D., Larson, J., and Fusaro, A., eds., 2013, *Corbicula fluminea*, USGS Nonindigenous Aquatic Species Database: Gainesville, Florida, U.S. Geological Survey, accessed January 17, 2014, at http://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=92. Revision Date: 5/10/2012.

Gannon, J.E., and Stemberger, R.S., 1978, Zooplankton (especially crustaceans and rotifers) as indicators of water quality: Transactions of the American Microscopical Society, v. 97, no. 1, p. 16–35.

Grapentine, L.C., 2009, Determining degradation and restoration of benthic conditions for Great Lakes Areas of Concern: Journal of Great Lakes Research, v. 35, no. 1, p. 36–44.

Howmiller, R.P., and Beeton, A.M., 1971, Biological evaluation of environmental quality, Green Bay, Lake Michigan: Journal of the Water Pollution Control Federation, v. 43, no. 1, p. 123–133.

Integrated Paper Services Inc., 2000, A macrointertebrate study of the depositional "soft" substrates of the Lower Fox River, Wisconsin—1999: Integrated Paper Services, Inc., Monitoring Study Series Report No. 4., 127 p.

International Joint Commission United States and Canada, 1987, Protocol amending the 1978 agreement between the United States of America and Canada on Great Lakes water quality as amended on October 16, 1983: 75 p.

Karner, Dawn, 2005, ESS BIO METHOD 2035: Phytoplankton identification and enumeration: Madison,Wisconsin State Laboratory of Hygiene, Environmental Health Division, 11 p.

Kelly, M.G., 2001, Use of similarity measures for quality control of benthic diatom samples: Water Research, v. 35, no. 11, p. 2784–2788.

Kennedy-Parker, DeWayne, 2011, ESS BIO METHOD 151.1, Revision 4: Chlorophyll a, fluorescence: Madison, Wisconsin State Laboratory of Hygiene, Environmental Health Division, Inorganic Chemistry Department, 15 p.

Kennedy, C.R., 1966, The life history of *Limnodrilus hoffmeisteri Clap.* (Oligocheta: Tubificidae) and its adaptive significance: Oikos, v. 17, p. 158–168.

Lange-Bertalot, H., 1979, Pollution tolerance of diatoms as a criterion for water quality estimation: Nova Hedwigia Beiheft, v. 64, p. 285–304.

Larson, J.H., Trebitz, A.S., Steinman, A.D., Wiley, M.J., Mazur, M.C., Pebbles, Victoria, Braun, H.A., and Seelbach, P.W., 2013, Great Lakes rivermouth ecosystems: Scientific synthesis and management implications: Journal of Great Lakes Research, v. 39, no. 4, p. 513–524.

Lenat, D.R., 1993, A biotic Index for the southeastern United States: derivation and list of tolerance values, with criteria for assigning water-quality ratings: Journal of the North American Benthological Society, v. 12, no. 3, p. 279–290.

Marchant, Richard, 2002, Do rare species have any place in multivariate analysis for bioassessment?: Journal of the North American Benthological Society, v. 21, no. 2, p. 311–313.

Merritt, R. W. and K. W. Cummins. 1996. An introduction to the aquatic insects of North America, third edition: Dubuque, Iowa, Kendall/Hunt Publishing Company, 862 p.

Michigan Department of Environmental Quality, 2008, Guidance for delisting Michigan's Great Lakes Areas of Concern, Report OGL–OO2: Lansing, Michigan Department of Environmental Quality, Office of the Great Lakes, 65 p.

Nalepa, T.F., Schloesser, D.W., Pothoven, S.A., Hondrop, D.W., Fanslow, D.L., Tuchman, M.L., and Fleischer, G.W., 2001, First finding of the amphipod *Echinogammarus ischnus* and the mussel *Dreissena bugensis* in Lake Michigan: Journal of Great Lakes Research, v. 27, no. 3, p. 384–391.

Ohio Environmental Protection Agency, 2008, Delisting targets for Ohio Areas of Concern: Columbus, Ohio, Ohio Environmental Protection Agency Report, 85 p.

Reynoldson, T.B., 1987, Interactions between sediment contaminants and benthic organisms: Hydrobiologia, v. 149, no 1, p. 53–66.

Richman, Sumner, Bailiff, M.D., Mackey, L.J., and Bolgrien, D.W., 1984, Zooplankton standing stock, species composition and size distribution along a trophic gradient in Green Bay, Lake Michigan: Verhandlung Internationale Vereinigung Limnologie, v. 22, p. 475–487.

Richman, Sumner, Sager, P.E., Banta, G., Harvey, R.T., and De Stasio, B.T., Jr., 1984, Phytoplankton standing stock, size distribution, species composition and productivity along a trophic gradient in Green Bay, Lake Michigan: Verhandlung Internationale Vereinigung Limnologie, v. 22, no. 1, p. 460–469.

Rodriguez, Pilar, and Reynoldson, T.B., 2011, The pollution biology of aquatic Oligochaetes: London, Springer, 265 p.

Scudder Eikenberry, B.C., Bell, A.H., Burns, D.J., and Templar, H.A., 2014, Benthos and plankton community data for selected rivers and harbors along Wisconsin's Lake Michigan shoreline, 2012: U.S. Geological Survey Data Series 824, 30 p. plus 8 appendixes, available at https://pubs.er.usgs.gov/publication/ds824.

Shannon, C.E., 1948, A mathematical theory of communication: Bell System Technical Journal, v. 27, no. 3, p. 379–423.

Surber, E.W., and Cooley, H.L., 1952, Bottom fauna studies of Green Bay, Wisconsin, in relation to pollution: Washington, D.C., U.S. Public Health Service Division of Water Pollution Control and Wisconsin State Committee on Water Pollution, 7 p.

Thorp, J.H., and Covich, A.P., 1991, Ecology and classification of North American freshwater invertebrates: San Diego, Academic Press, Inc., 911 p.

U.S. Environmental Protection Agency, 1994, Assessment of Benthic Invertebrate Community Structure, Chapter 7, in ARCS assessment guidance document: Chicago, Illinois, Great Lakes National Program Office—U.S. Environmental Protection Agency Report 905–B94–002, p. 131–175.

U.S. Environmental Protection Agency, 2010a, Standard operating procedure for benthic invertebrate field sampling (LG406), *in* Sampling and analytical procedures for GLNPO's Open Lake Water Quality Survey of the Great Lakes: Chicago, U.S. Great Lakes National Program Office-Environmental Protection Agency, Report EPA 905–R–05–001, 9 p., accessed November 2, 2011, at https://www3.epa.gov/greatlakes/monitoring/sop/.

U.S. Environmental Protection Agency, 2010b, Standard operating procedure for benthic invertebrate laboratory analysis (LG407), *in* Sampling and analytical procedures for GLNPO's Open Lake Water Quality Survey of the Great Lakes: Chicago, Great Lakes National Program Office-U.S. Environmental Protection Agency, Report EPA 905–R–001, 12 p., accessed October 22, 2012, at https://www3.epa.gov/greatlakes/monitoring/sop/.

U.S. Environmental Protection Agency, 2010c, Standard operating procedure for phytoplankton analysis (LG401), *in* Sampling and analytical procedures for GLNPO's Open Lake Water Quality Survey of the Great Lakes: Chicago, Great Lakes National Program Office-U.S. Environmental Protection Agency, Report EPA 905–R–001, 44 p,. accessed August 22, 2011, at https://www3.epa.gov/greatlakes/monitoring/sop/.

U.S. Environmental Protection Agency, 2010d, Standard operating procedure for phytoplankton sample collection and preservation field procedures (LG400), *in* Sampling and analytical procedures for GLNPO's Open Lake Water Quality Survey of the Great Lakes: Chicago, Great Lakes National Program Office-U.S. Environmental Protection Agency, Report EPA 905–R–001, 7 p., accessed November 2, 2011, at https://www3.epa.gov/greatlakes/monitoring/sop/.

U.S. Environmental Protection Agency, 2010e, Standard operating procedure for zooplankton analysis (LG403), *in* Sampling and analytical procedures for GLNPO's Open Lake Water Quality Survey of the Great Lakes: Chicago, Great Lakes National Program Office-U.S. Environmental Protection Agency, Report EPA 905–R–001, 20 p., accessed October 22, 2012, at https://www3.epa.gov/greatlakes/monitoring/sop/.

U.S. Environmental Protection Agency, 2010f, Standard operating procedure for zooplankton sample collection and preservation and Secchi depth measurement field procedures (LG402), *in* Sampling and analytical procedures for GLNPO's Open Lake Water Quality Survey of the Great Lakes: Chicago, Great Lakes National Program Office-U.S. Environmental Protection Agency, Report EPA 905–R–001, 9 p., Accessed November 2, 2011, at https://www3.epa.gov/greatlakes/monitoring/sop/.

U.S. Geological Survey, 1989, Solids, volatile-on-ignition, total-in-bottom-material, gravimetric, *in* Fishman, M.J., and Friedman, L.C., eds., Techniques of water-resources investigations of the United States Geological Survey (3d ed.): Washington, D.C., U.S. Geological Survey, p. 451.

U.S. Policy Committee, 2001, Restoring United States Areas of Concern: Delisting principles and guidelines: Chicago, U.S. Environmental Protection Agency, 27 p., accessed May 5, 2014, at https://www.epa.gov/sites/production/files/2015-08/documents/aoc-delisting-principles-guidelines-20011206.pdf.

Van Dam, Herman, Mertens, Adrienne, and Sinkeldam, Jos, 1994, A coded checklist and ecological indicator values of freshwater diatoms from the Netherlands: Netherlands Journal of Aquatic Ecology, v. 28, no. 1, p. 117–133.

Wallace, R.L., and Snell, T.W., 1991, Rotifera, *in* Thorp, J.H., and Covich, A.P., eds., Ecology and classification of North American freshwater invertebrates: New York, Academic Press, Inc., p. 187–248.

Weigel, B.M., and Dimick, J.J., 2011, Development, validation, and application of a macroinvertebrate-based Index of Biotic Integrity for nonwadeable rivers of Wisconsin: Journal of the North American Benthological Society, v. 30, no. 3, p. 665–679.

Wentworth, C.K., 1922, A scale of grade and class terms for clastic sediments: Journal of Geology, v. 30, no. 5, p. 377–392.

Wisconsin Department of Natural Resources, 1990, Paying for the past—Investing in the future: Madison, Wisconsin Department of Natural Resources, p. 16.

Wisconsin Department of Natural Resources, 1993, Lower Green Bay Remedial Action Plan 1993 update—for the Lower Green Bay and Fox River Area of Concern: Madison, Wisconsin Department of Natural Resources, 152 p. plus 10 appendixes, accessed March 8, 2016, at http://dnr.wi.gov/topic/greatlakes/documents/LGB-FR1993RAPupdate.pdf.

Wisconsin Department of Natural Resources, 1994, Milwaukee Estuary Remedial Action Plan—Progress through January 1994: Madison, Wisconsin Department of Natural Resources, variously paged, accessed March 8, 2016, http://dnr.wi.gov/topic/greatlakes/documents/MilwaukeeEstuaryRAP1994.pdf.

Wisconsin Department of Natural Resources, 1995, Sheboygan River Remedial Action Plan: Madison, Wisconsin Department of Natural Resources, p. 271, accessed March 98, 2016, at http://dnr.wi.gov/topic/greatlakes/documents/SheboyganRiverRAP1995.pdf.

Wisconsin Department of Natural Resources, 1996, Lower Menominee River Remedial Action Plan Update—February 1996, PUBL–WR–410–96: Madison: Wisconsin Department of Natural Resources, 168 p., accessed March 8, 2016, at http://www.michigan.gov/documents/deq/deq-water-ogl1996-L_Menominee_-RAP_342547_7.pdf.

Wisconsin Department of Natural Resources, 2009, Lower Green Bay and Fox River Area of Concern beneficial use impairment delisting targets: Madison, Wisconsin Department of Natural Resources, 29 p., accessed December 5, 2013, at http://dnr.wi.gov/topic/greatlakes/documents/LGB-FRFinalReport.pdf.

Wisconsin Department of Natural Resources, 2011, Draft Stage 2 Remedial Action Plan for the Milwaukee Estuary Area of Concern: Madison, Wisconsin Department of Natural Resources, Office of the Great Lakes, 80 p., accessed October 25, 2013, at http://dnr.wi.gov/topic/greatlakes/documents/Stage2RAPMilwaukee.pdf.

Wisconsin Department of Natural Resources, 2012a, Remedial Action Plan update for the Lower Green Bay and Fox River Area of Concern: Madison, Wisconsin Department of Natural Resources, p. 72, accessed October 25, 2013, at http://dnr.wi.gov/topic/greatlakes/documents/RAP-UpdateLGBFR2012final.pdf.

Wisconsin Department of Natural Resources, 2012b, Remedial Action Plan update for the Sheboygan River Area of Concern: Madison, Wisconsin Department of Natural Resources, p. 72, accessed October 5, 2013, at http://dnr.wi.gov/topic/greatlakes/documents/RAP-UpdateSHEB2012final.pdf.

Wisconsin Department of Natural Resources, 2013a, Healing our rivers & harbors—Wisconsin's Great Lakes Areas of Concern: Madison, Wisconsin Department of Natural Resources, 16 p.

Wisconsin Department of Natural Resources, 2013b, Remedial Action Plan update for the Lower Green Bay and Fox River Area of Concern: Madison, Wisconsin Department of Natural Resources, 63 p., accessed January 29, 2014, at http://dnr.wi.gov/topic/greatlakes/documents/LGB-FR2013FinalRAPupdate.pdf.

Wisconsin Department of Natural Resources and Michigan Department of Environmental Quality, 2011, Stage 2 Remedial Action Plan for the Lower Menominee River Area of Concern, 81 p., accessed April 7, 2016, at http://dnr.wi.gov/topic/greatlakes/documents/Stage2RAPLowerMenomineeRiver.pdf.

Wisconsin Department of Natural Resources and Michigan Department of Natural Resources, 1990, Lower Menominee River Remedial Action Plan—Stage One Report, PUBL WR-246 90: Madison, Wisconsin Department of Natural Resources, 211 p.

Wisconsin Department of Natural Resources and Milwaukee River Basin Land and Water Partners Team, 2001, The state of the Milwaukee River Basin: Madison, Wisconsin Department of Natural Resources, p. 108.

Wisconsin State Committee on Water Pollution, State Board of Health, and Green Bay Metropolitan Sewerage Commission, 1939, Investigation of the pollution of the Fox and East Rivers and of Green Bay in the vicinity of the city of Green Bay: Madison, State of Wisconsin, 242 p.

Witt, J.D.S., Hebert, P.D.N., and Morton, W.B., 1997, *Echinogammarus ischnus*: another crustacean invader in the Laurentian Great Lakes basin: Canadian Journal of Fisheries and Aquatic Sciences, v. 54, p. no. 2, 264–268.

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