Prepared in cooperation with the Wisconsin Department of Natural Resources and the U.S. Environmental Protection Agency

An Evaluation of the Zooplankton Community at the Sheboygan River Area of Concern and Non-Area of Concern Comparison Sites in Western Lake Michigan Rivers and Harbors in 2016

Scientific Investigations Report 2017–5131

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

Cover. Sheboygan River, Wisconsin. Photograph by Amanda Bell, U.S. Geological Survey.

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RYAN K. ZINKE, Secretary

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Contents

Figures

Tables

Conversion Factors

International System of Units to U.S. customary units

Multiply	By	To obtain
micrometer (µm)	0.00003937	inch (in.)
meter (m)	3.281	foot (ft)
meter (m)	1.094	yard (yd)
kilometer (km)	0.6214	mile (mi)
square kilometer (km^2)	0.3861	square mile (mi^2)
liter (L)	0.2642	gallon (gal)
gram (g)	0.03527	ounce, avoirdupois (oz)

Datum

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

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

Altitude, as used in this report, refers to distance above the vertical datum.

Supplemental Information

Concentrations of chemical constituents in water are given in milligrams per liter (mg/L).

The mesh opening size for the plankton net is given in micrometers (µm).

Abbreviations

ANOSIM	analysis of similarity
ANOVA	analysis of variance
AOC	Area of Concern
BUI	Beneficial Use Impairment
EPA	U.S. Environmental Protection Agency
GLRI	Great Lake Restoration Initiative
nMDS	nonmetric multidimensional scaling
PAH	polycyclic aromatic hydrocarbon
PCB	polychlorinated biphenyl compound
QA–QC	quality assurance and quality control
SIMPER	similarity percentage
USGS	U.S. Geological Survey
VOC	volatile organic compound
WDNR	Wisconsin Department of Natural Resources

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Abstract

The Great Lakes Areas of Concern (AOCs) are considered to be the most severely degraded areas within the Great Lakes basin, as defined in the Great Lakes Water Quality Agreement and amendments. Among the 43 designated AOCs are four Lake Michigan AOCs in the State of Wisconsin. The smallest of these AOCs is the Sheboygan River AOC, which was designated as an AOC because of sediment contamination from polychlorinated biphenyl compounds (PCBs), polycyclic aromatic hydrocarbons (PAHs), volatile organic compounds (VOCs), and heavy metals. The Sheboygan River AOC has 9 of 14 possible Beneficial Use Impairments (BUIs), which must be addressed to improve overall water-quality, and to ultimately delist the AOC. One of the BUIs associated with this AOC is the “degradation of phytoplankton and zooplankton populations,” which can be removed from the list of impairments when it has been determined that zooplankton community composition and structure at the AOC do not differ significantly from communities at non-AOC comparison sites. In 2012 and 2014, the U.S. Geological Survey collected plankton (phytoplankton and zooplankton) community samples at the Sheboygan River AOC and selected non-AOC sites as part of a larger Great Lakes Restoration Initiative study evaluating both the benthos and plankton communities in all four of Wisconsin’s Lake Michigan AOCs. Although neither richness nor diversity of phytoplankton or zooplankton in the Sheboygan River AOC were found to differ significantly from the non-AOC sites in 2012, results from the 2014 data indicated that zooplankton diversity was significantly lower, and so rated as degraded, when compared to the Manitowoc and Kewaunee Rivers, two non-AOC sites of similar size, land use, and close geographic proximity.

As a follow-up to the 2014 results, zooplankton samples were collected at the same locations in the AOC and non-AOC sites during three sampling trips in spring, summer, and fall 2016. An analysis of similarity indicated no significant difference between the zooplankton community composition and structure in the AOC and non-AOC sites. Zooplankton taxa richness in the AOC was rated as “not degraded” in 2016 because of significantly higher taxa richness values in samples collected from the Sheboygan River AOC, compared with the non-AOC sites as a group (that is, data pooled from both non-AOC sites). Zooplankton diversity in 2016, however, was characterized as “degraded” in the AOC on the basis of significantly lower ($p<0.05$) values in samples collected from the AOC compared with those collected from the non-AOC sites as a group. Annual variation in zooplankton community composition and structure at the Sheboygan River AOC was significantly different among all 3 years sampled, as indicated by an analysis of similarity test. Zooplankton richness was significantly higher in 2014 than in both 2012 and 2016, and diversity was significantly higher in 2012 than in both 2014 and 2016. Postremediation recovery can often be complicated by non-AOC-related stressors such as nutrients, invasive species, and extremes in flow, which could affect the recovery of zooplankton communities in the Sheboygan River AOC. The effect of the stressors on postremediation recovery underscores the importance of sampling multiple years when assessing the effectiveness of remediation activities. The results from this study will be used by the Wisconsin Department of Natural Resources and the U.S. Environmental Protection Agency to determine if restoration efforts have been effective in removing the plankton BUI and to monitor future conditions in the AOC.

Introduction

The Great Lakes Water Quality Agreement, signed by the United States and Canada in 1972, and amendments designated certain sites within the Laurentian Great Lakes as Areas of Concern (AOCs; International Joint Commission, 1987) because of severe environmental degradation (typically the result of anthropogenic pollution). Each AOC was designated with up to 14 Beneficial Use Impairments (BUIs), which include “degradation of phytoplankton and zooplankton populations” (International Joint Commission, 1987). Removal of a BUI is an important step towards the delisting of an AOC. BUI removal can occur when the local, site-specific delisting targets have been met and postremediation monitoring data supports the delisting of the BUI (U.S. Environmental Protection Agency, 2001).

A total of 43 AOCs have been designated (that is, “listed”) along the Great Lakes shorelines. The State of Wisconsin has jurisdiction in five of these AOCs, four of which are located in Lake Michigan harbors and river mouths: Lower Menominee River, Lower Green Bay and Fox River, Sheboygan River, and Milwaukee Estuary. The Sheboygan River was designated an AOC in 1987, primarily because of sediment contamination by polychlorinated biphenyl compounds (PCBs), polycyclic aromatic hydrocarbons (PAHs), volatile organic compounds (VOCs), and heavy metals (Wisconsin Department of Natural Resources, 1989). Substantial remediation efforts in the Sheboygan River AOC have taken place in recent years by the U.S. Environmental Protection Agency’s (EPA) Superfund, Great Lakes Legacy Act, and Great Lakes Restoration Initiative (GLRI) programs, and the multiagency Sheboygan River Priority Watershed Project (Wisconsin Department of Natural Resources, 2015a). In addition, dredging of contaminated sediment was completed in 2013 (Wisconsin Department of Natural Resources, 2014), and the BUI for restrictions on dredging activity was removed in 2014 (Wisconsin Department of Natural Resources, 2015a).

As of 1987, the Sheboygan River was designated as an AOC with a total of nine BUIs, including the degradation of phytoplankton and zooplankton communities. The U.S. Geological Survey (USGS) collected plankton community samples from the Sheboygan River AOC in 2012 and 2014, as part of a larger GLRI study, which was aimed at evaluating the benthos and plankton communities in all four of Wisconsin’s Lake Michigan AOCs (Scudder Eikenberry and others, 2014, 2016b). Although neither phytoplankton nor zooplankton were found to be significantly degraded in the Sheboygan River in 2012 (Scudder Eikenberry and others, 2016a), results from the 2014 data collection indicated that zooplankton diversity was degraded in the Sheboygan River in 2014 compared with the Manitowoc and Kewaunee Rivers, two non-AOC comparison sites of similar size, physical and chemical characteristics, and close geographic proximity. In 2016, the USGS, in cooperation with the Wisconsin Department of Natural Resources (WDNR) and the EPA, conducted a followup study of the zooplankton community at the Sheboygan River AOC and the same non-AOC comparison sites used in the 2012 and 2014 studies.

The purpose of this study was to provide an assessment of the current state of the zooplankton community composition and structure in the Sheboygan River AOC to inform the decision-making process of the WDNR and the EPA for removal of the BUI for the “degradation of phytoplankton and zooplankton populations.” This was accomplished by determining whether the zooplankton community at the AOC differed significantly from the two less-impaired, non-AOC comparison sites. These results were used to determine whether the zooplankton community at the AOC would be considered degraded in comparison to the zooplankton communities at the non-AOC sites. This report presents an assessment of the status of zooplankton communities at the Sheboygan River AOC and the two non-AOC comparison sites in 2016, and a comparison of the results in this study with those of similar studies conducted by the USGS in 2012 and 2014. The results of this study are intended for use by the WDNR and the EPA to monitor progress in the Sheboygan River AOC and to determine if restoration efforts have been effective enough to remove the plankton BUI.

Description of Study Area

The Sheboygan River AOC and the two non-AOC comparison sites, the Kewaunee and Manitowoc Rivers, are located on the western shore of Lake Michigan in Wisconsin (fig. 1; table 1). The Kewaunee and Manitowoc Rivers were selected as non-AOC comparison sites because they were not within any AOC, and therefore presumed to be less impaired, are nearby harbors along the western shoreline of Lake Michigan, and have similar environmental characteristics. Although no Lake Michigan river mouths or harbors are truly unimpaired, these comparison sites were assumed to have biological communities similar to what would be present at the AOC without the contamination identified during its designation. Sampling locations in the AOC and the two non-AOC comparison sites were the same in 2016 as in 2012 and 2014 (Scudder Eikenberry and others, 2014, 2016b). Because of their proximity to Lake Michigan, the zooplankton communities sampled from these locations may reflect both river and Lake Michigan taxa; however, this is the general nature of aquatic communities at river mouths.

The Sheboygan River AOC has a drainage area of 1,043 square kilometers (km^2), which is the smallest of the Lake Michigan AOCs in Wisconsin. The watershed is predominantly agricultural with clay soils. The Sheboygan River has several legacy contaminant issues, including PCBs, PAHs, heavy metals, and VOCs, which were identified when it was first designated as an AOC in 1987 (Wisconsin Department of Natural Resources, 2015a). The 2016 sampling location was downstream of the dredged areas, near the mouth of the river, and downstream of the 8th Street Bridge; this is the same location that was sampled in 2012 and 2014.

The Kewaunee River is approximately 80 kilometers (km) north of the Sheboygan River, and 40 km north of the Manitowoc River. The Kewaunee River watershed is 354 km^2 in area, which is smaller than the Sheboygan River watershed. Land use is predominantly agricultural, and the watershed has primarily clay soils. The Kewaunee River supports a warm-water sport fishery and has seasonal runs of

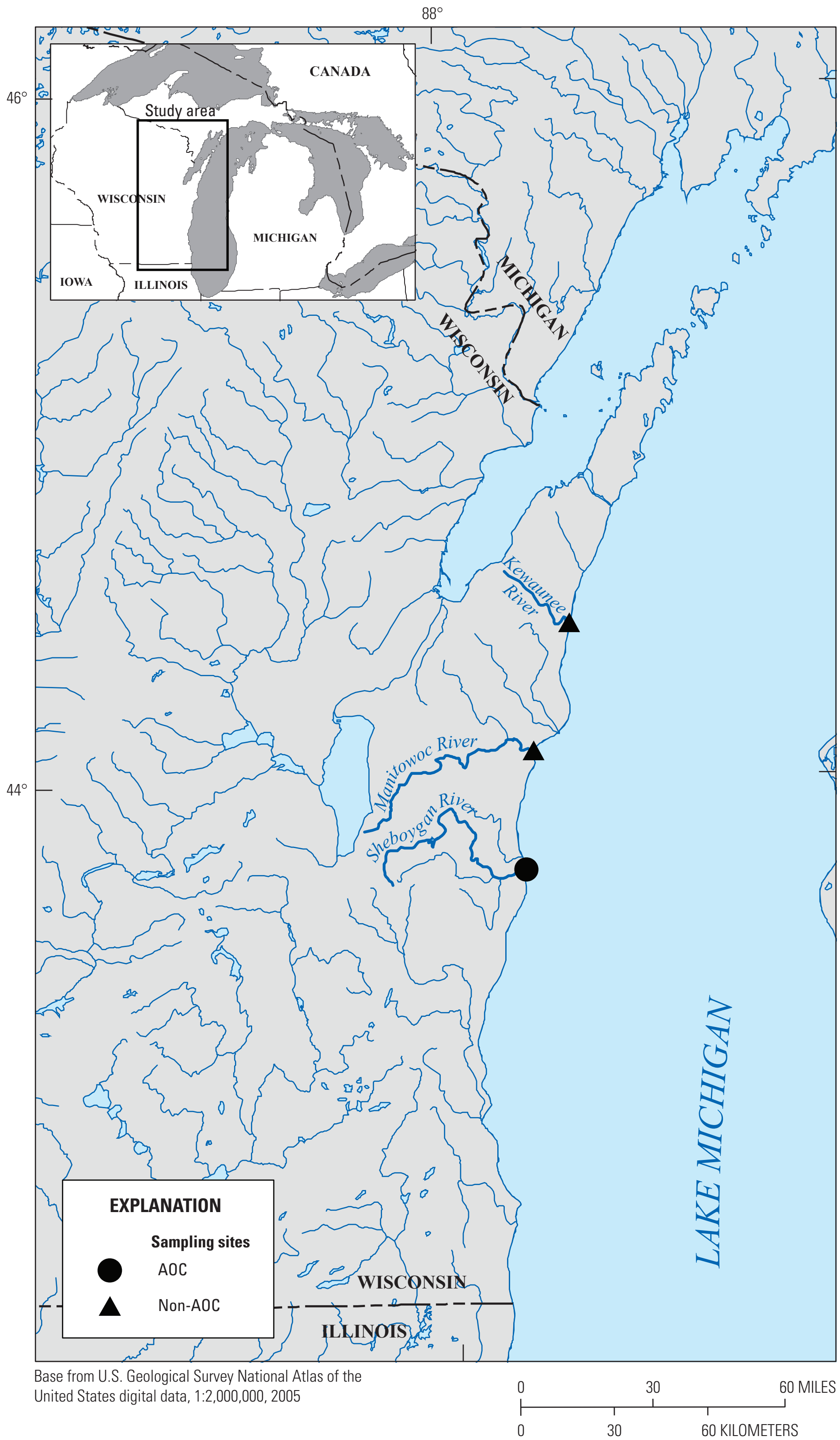

Figure 1. Western Lake Michigan showing the Sheboygan River Area of Concern (AOC) and two non-AOC comparison sites, the Kewaunee and Manitowoc Rivers, from which zooplankton were collected in 2016.

Table 1. Sites sampled for evaluation of the Sheboygan River Area of Concern (AOC) and two non-AOC comparison sites, the Kewaunee and Manitowoc Rivers, in western Lake Michigan in 2016.

[ID, identification number; km², square kilometer; NA, not available; AOC, Area of Concern]

Site name	Abbreviated name	Station ID	Latitude (decimal degrees)	Longitude (decimal degrees)	Drainage area (km^2)
Sheboygan River AOC	SHEB	040860041	43.74887	-87.70352	1,043
Kewaunee River non-AOC	KEWA	NA	44.46073	-87.50205	354
Manitowoc River non-AOC	MANI	040854307	44.0919	-87.66183	1,341

salmon and trout from Lake Michigan. Sediment sampling in 1988 revealed levels of oil and grease, total phosphorus, lead, and chemical oxygen demand that would be considered characteristic of moderately polluted sediments (Wisconsin Department of Natural Resources, Lakeshore Basin Partnership Team, 2001). Potential sources of contamination to the river include nonpoint source runoff from agricultural and urban land uses as well as point source contributions from water-treatment facilities and several industries. The 2016 sampling location was near the State Highway 42 Bridge just upstream of the river mouth; this is the same location that was sampled in 2012 and 2014.

The Manitowoc River is approximately 40 km north of the Sheboygan River and 40 km south of the Kewaunee River. The area of the watershed is 1,341 km^2, which is similar to the size of the Sheboygan River watershed. Land use is predominantly agricultural with clay soils; these characteristics are also similar to those of the AOC. The Manitowoc River has fish consumption advisories in place because of elevated levels of PCBs (Wisconsin Department of Natural Resources, 2015b). There are also multiple water-treatment facilities and industries that discharge to the river. Continued monitoring also takes place at an EPA Superfund site about a mile upstream from the mouth of the Manitowoc River. The sampling location was just upstream of the 10th Street Bridge; this is the same location that was sampled in 2012 and 2014.

Methods

Zooplankton community samples were collected from the Sheboygan River AOC and the two non-AOC comparison sites, the Kewaunee and Manitowoc Rivers. Samples were collected in 2016 at the AOC and non-AOC comparison sites at the same locations as were sampled previously in 2012 and 2014, and sampling methods were similar to those used for zooplankton collection at these same locations (Scudder Eikenberry and others, 2014, 2016b). All data are available in Olds and others (2017).

Sample Collection and Processing

Zooplankton samples were collected once per month from each site during the growing season in late May, mid-July, and late August. Although the August sample was collected approximately 1 month before the beginning of the fall season, for simplicity, the sampling events will hereafter be referred to as spring, summer, and fall seasonal samples. All samples were collected by boat. In-place water-quality measurements were made just before and immediately after each zooplankton sample collection. These measurements of pH, specific conductance, water temperature, and dissolved oxygen were made using a calibrated YSI Inc. multiparameter water-quality sonde deployed at the surface of the water.

Zooplankton collection methods were based on the EPA standard operating procedures (U.S. Environmental Protection Agency, 2010b), with some modification. For each sample, a 63-micrometer (µm)-mesh plankton net was lowered to a 5-meter (m) depth or no more than 0.5 m from the bottom (where maximum depth was less than [<] 5 m) and then slowly raised to the surface (fig. 2*A*). If the water depth was less than 5 m (as was the case for all samples at the Kewaunee River and for most samples in the Sheboygan River), additional tows were taken and composited to provide a total of 5 m of sampled water depth. After each tow, the net was raised and gently rinsed from the outside with garden sprayers filled with tap water (fig. 2*B*). Once organisms were washed from the net into the dolphin bucket, the sample was then transferred to a 500- or 1,000-milliliter (mL) plastic sample bottle (fig. 2*C*). One-half of an Alka Seltzer tablet was added per 500-mL sample to increase carbon dioxide, preventing rotifers from contracting and impeding laboratory identification (Chick and others, 2010). Between 30 and 60 minutes after sample collection, the sample was preserved with sucrose-buffered formalin to a final solution of 4.2 percent sucrose and 4 to 5 percent formalin (Haney and Hall, 1973; Chick and others, 2010). Zooplankton samples were identified and enumerated by EcoAnalysts, Inc. in Moscow, Idaho, using EPA Standard Operating Procedure LG403 (U.S. Environmental Protection Agency, 2010a). For microcrustacean identification, a Folsom plankton splitter and a stereoscopic microscope were used

A

B

C

Figure 2. *A*, A plankton net being lowered into the water, *B*, a plankton net being washed into dolphin collection bucket, and *C*, a sample being transferred from the dolphin collection bucket to the sample bottle.

for identification. For rotifer identification, a Sedgewick-Rafter counting cell and compound microscope were used for identification. Identifications were made to the lowest practical taxonomic level; genus or species levels were preferred.

Data Analysis

Prior to calculating metrics and completing multivariate analyses, zooplankton community data were inspected for "ambiguous taxa" (taxa whose abundances were reported for multiple, related taxonomic levels across the entire dataset). In order to ensure that the values for richness used in comparisons between sites are not artificially inflated by ambiguous taxa, these taxa are generally resolved by distributing the counts of the "parent" (higher level taxonomic rank) to the "children" (lower taxonomic level rank) present within each subsite, taking into account the proportion of counts already assigned to each child, as well as removing the counts for the parent (Cuffney and others, 2007). The only ambiguous taxa found in this study were immature copepod (Copepoda) taxa, consisting of copepod nauplii and cyclopoid (Cyclopoida) and calanoid (Calanoida) copepodites. Where present, immature copepods were kept as unique taxa in the analysis, because they made up a large portion of the zooplankton samples collected, and thus could not easily be distributed to specific children taxa.

Spatial and temporal variation in zooplankton community structure was examined by use of multivariate analysis of taxa relative abundances. All multivariate analyses were completed using PRIMER 6 software routines (Clarke and Gorley, 2006). To calculate diversity in the natural logarithm (ln or $\log_e$), the DIVERSE routine was used. Differences in zooplankton community structure between the AOC and the two non-AOC sites as well as between primary and replicate samples were examined with similarity percentage (SIMPER) analysis. Ordination plots of sites and seasons were performed by nonmetric multidimensional scaling (nMDS). Zooplankton community structure was also compared among the AOC and non-AOC sites by use of analysis of similarity (ANOSIM); this procedure is analogous to an analysis of variance (ANOVA) using similarity matrices. These analyses were also used to compare annual variation in zooplankton communities in 2016 to those previously sampled by the USGS in 2012 and 2014. The above multivariate analyses were performed on a Bray-Curtis similarity matrix (Bray and Curtis, 1957), generated from taxa relative abundances. The Bray-Curtis similarity matrices formed the basis of the SIMPER, nMDS, and ANOSIM analyses. Relative abundances were fourth-root transformed to decrease the influence of common taxa and increase the influence of intermediate and rare taxa so results were not overwhelmed by common taxa (Clarke and Warwick, 2001).

Taxa richness (the total number of unique taxa) and diversity (Shannon diversity index; Shannon, 1948) were also used for zooplankton community comparisons between the

Sheboygan River AOC and the two non-AOC comparison sites. Results from the AOC were compared with results from each non-AOC site individually as well as with results from the two non-AOC sites as a group (that is, data were pooled from both non-AOC sites). ANOVA was used to determine whether significant differences existed between the richness, diversity, and water-quality measurements at the AOC and the non-AOC comparison sites. Significant ANOVA values ($p<0.05$) were followed by Bonferroni postevent univariate statistical tests to determine whether there were significant differences between specific sites or seasons. ANOVA and Bonferroni postevent statistical tests were completed in Data Desk version 7 (Data Description Inc., 2015). The same approach was used to evaluate annual variations in zooplankton taxa richness and diversity, by comparing the data collected in the present study to that collected previously in 2012 and 2014. The term “significant” refers to values with statistical significance of $p<0.05$.

Quality Assurance and Quality Control

Quality assurance and quality control (QA–QC) samples were collected during each sampling period at the Sheboygan River AOC site to evaluate the field variability of zooplankton community taxonomic results. No significant differences were found in richness or in diversity between primary and replicate samples. Results from a SIMPER analysis also indicated minimal variability between primary and replicate samples. For QA–QC purposes, similarities greater than 60 percent were considered to be acceptable (Kelly, 2001). Average similarity between the primary and replicate sample in spring was 84 percent, average similarity in summer was 78 percent, and average similarity in fall was 82 percent. Similarities within each season were greater than 60 percent both with and without the inclusion of veligers (planktonic larvae) of the genus *Dreissena* (dreissenid veligers) and copepod nauplii in the analysis. Because dreissenid veligers and copepod nauplii often dominated the QA–QC samples, analyses were completed with and without them to ensure that these taxa were not influencing the QA–QC results.

Physical and Chemical Comparisons Between the Sheboygan River AOC and non-AOC Sites

Physical and chemical characteristics were determined by six in-place water-quality measurements per location, across the spring, summer, and fall seasons (table 2). Specific conductance was the only water-quality parameter that differed significantly between sites ($p<0.05$). With or without replicates included, average specific conductance was significantly higher at the Sheboygan River AOC when compared with the Kewaunee River ($p<0.05$) but not when compared with the Manitowoc River. As might be expected, all four water-quality parameters (water temperature, pH, specific conductance, and dissolved oxygen concentration) significantly differed between seasons ($p<0.05$). Temperature was highest in summer compared with spring and fall, pH and dissolved oxygen were highest in fall compared with spring and summer, and specific conductance was higher in spring compared with fall.

Table 2. Means and standard deviations of in-place water-quality measurements in the Sheboygan River Area of Concern (AOC) and two non-AOC comparison sites in western Lake Michigan in 2016.

[Samples collected per site (n) = 6 across spring, summer, and fall seasons. Samples were collected using a YSI Inc. multiparameter water-quality sonde. ID, identification number; °C, degree Celsius; µS/cm at 25 °C, microsiemens per centimeter at 25 degrees Celsius; mg/L, milligram per liter; AOC, Area of Concern]

Site name	Sample type	Water temperature (°C)		pH		Specific conductance (µS/cm at 25 °C)		Dissolved oxygen (mg/L)	
		Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
Sheboygan River AOC	Primary	20.6	1.95	8.28	0.379	552	62.4	8.08	4.16
Sheboygan River AOC	Replicate	20.8	1.85	8.24	0.421	552	69.5	8.06	4.26
Kewaunee River non-AOC	Primary	19.2	2.39	8.17	0.373	420	60.4	9.18	2.71
Manitowoc River non-AOC	Primary	20.6	2.24	8.38	0.291	504	52.6	8.36	2.42

Zooplankton Community Comparisons Between the Sheboygan River AOC and Selected Non-AOC Sites

The differences between zooplankton communities in the Sheboygan River AOC and the two non-AOC comparison sites were evaluated using multivariate statistics with relative abundances of zooplankton taxa and univariate statistics with biological metrics. All biological community data are available at Olds and others (2017).

Zooplankton Community Comparisons in 2016

Rotifers accounted for more than 50 percent of the taxa among all community samples collected from the Sheboygan River AOC, the Kewaunee River, and the Manitowoc River across the spring, summer, and fall seasons, and approximately 30 percent were invasive dreissenid veligers (immature zebra or quagga mussels; based on taxonomic data collected by the USGS in 2012 and 2014 at these sites, dreissenid veligers in these samples are most likely to be immature zebra mussels). The dominant zooplankton taxa in freshwater systems are generally rotifers, microcrustaceans (such as cladocerans and copepods), and protozoans. Rotifers are generally outcompeted by microcrustaceans because rotifers have lower clearance rates and smaller size requirements for food particles (Wallace and Snell, 1991); however, rotifers respond better to increased temperatures because of their short development times and high population growth rates, allowing them to take advantage of new environmental conditions better than microcrustaceans can. These characteristics can result in rotifers being more abundant in freshwater systems that are subjected to an increased amount of anthropogenic disturbance, such as the Great Lakes AOCs.

In the Sheboygan River AOC, the zooplankton community in spring was dominated by rotifers, with a majority of the community composed by *Synchaeta sp.* (fig. 3). A very small percentage of the sample was comprised of copepods, and no dreissenid veligers were found. In summer, the community at the AOC was largely composed of dreissenid veligers, followed by a smaller percentage of rotifers (mainly *Synchaeta sp.* and *Polyarthra sp.*) and copepods (mainly nauplii). In fall, the majority of the community in the AOC was dreissenid veligers, with smaller percentages of rotifers (mainly *Synchaeta sp.* and *Polyarthra sp.*), copepods (mainly nauplii), and cladocerans (mainly *Bosmina longirostris*). Most *Synchaeta* species have a strong seasonal pattern to their distribution (Stemberger, 1979). In 2014 and 2016, the rotifer *Synchaeta* was identified to genus only but *Synchaeta oblonga* was the only species of *Synchaeta* found at the Sheboygan River site in 2012 in July and August (Scudder Eikenberry and others, 2014, 2016b). Stemberger (1979) noted that this rotifer taxon is more commonly found in fall through spring when temperatures are cooler. Rotifer taxa *Polyarthra remata* and *P. vulgaris* were found in the Sheboygan River in 2012 and *P. major* was also found in 2014 (Scudder Eikenberry and others, 2014, 2016b). *P. vulgaris*, which was found in all three seasons in 2012, is considered to be pollution tolerant (Gannon and Stemberger, 1978).

In contrast to the Sheboygan River AOC, zooplankton community samples from the Kewaunee River were dominated by rotifers during each season (fig. 3). The rotifers *Polyarthra sp.* and *Brachionus sp.* made up most of the community at the Kewaunee River in spring. The rotifers *Conochilus unicornis* and *Keratella sp.* made up the majority of the community in summer, and the rotifers *Polyarthra sp.* and *Keratella sp.* made up most of the community in fall. The rotifers *Brachionus sp.* are considered to be a useful indicator of eutrophic conditions, and *Conochilus unicornis* is a common open-water (limnetic) rotifer that usually peaks in summer (Stemberger, 1979). *Keratella* may be the most common genus of freshwater limnetic rotifer and at least three species often occur simultaneously in the Great Lakes. The cladoceran *Bosmina longirostris* is found worldwide and can be one of the most abundant crustaceans in the Great Lakes in fall because it prefers cool, well-oxygenated waters (Balcer and others, 1984).

The compositions of zooplankton communities in the Manitowoc River showed similar patterns to those at the Sheboygan River AOC. The Manitowoc River spring sample was also dominated by rotifers (mainly *Synchaeta sp.* and *Brachionus sp.*), and approximately a quarter of the sample was composed of dreissenid veligers (fig. 3). In summer, a majority of the Manitowoc River community sample was composed of dreissenid veligers, followed by a smaller percentage of copepods (mainly nauplii) and rotifers (mainly *Brachionus sp. and Polyarthra sp.*). In fall, nearly half of the community was composed of rotifers (mainly *Polyarthra sp.*, *Keratella sp.*, and *Conochilus unicornis*) and smaller percentages of copepods (mainly nauplii), dreissenid veligers, and cladocerans (mainly *Bosmina longirostris*).

Ordination by nMDS is used to represent samples in two or three dimensions, where similar objects plot close together and dissimilar objects plot far apart. These ordination plots (fig. 4) are useful for visualizing similarities and differences between zooplankton communities among the AOC and non-AOC comparison sites. Stress values indicate the how well relations between objects are represented in the nMDS plot, with an optimal stress value of <0.2. The relations between communities were well represented by the nMDS plot, as indicated by a low two-dimensional stress value (0.06). In general, the samples from the zooplankton community at the Sheboygan River AOC grouped more closely with those at the Manitowoc River (they are similar to one another) than those at the Kewaunee River (they have more differences). In spring and summer, the zooplankton communities at the Sheboygan

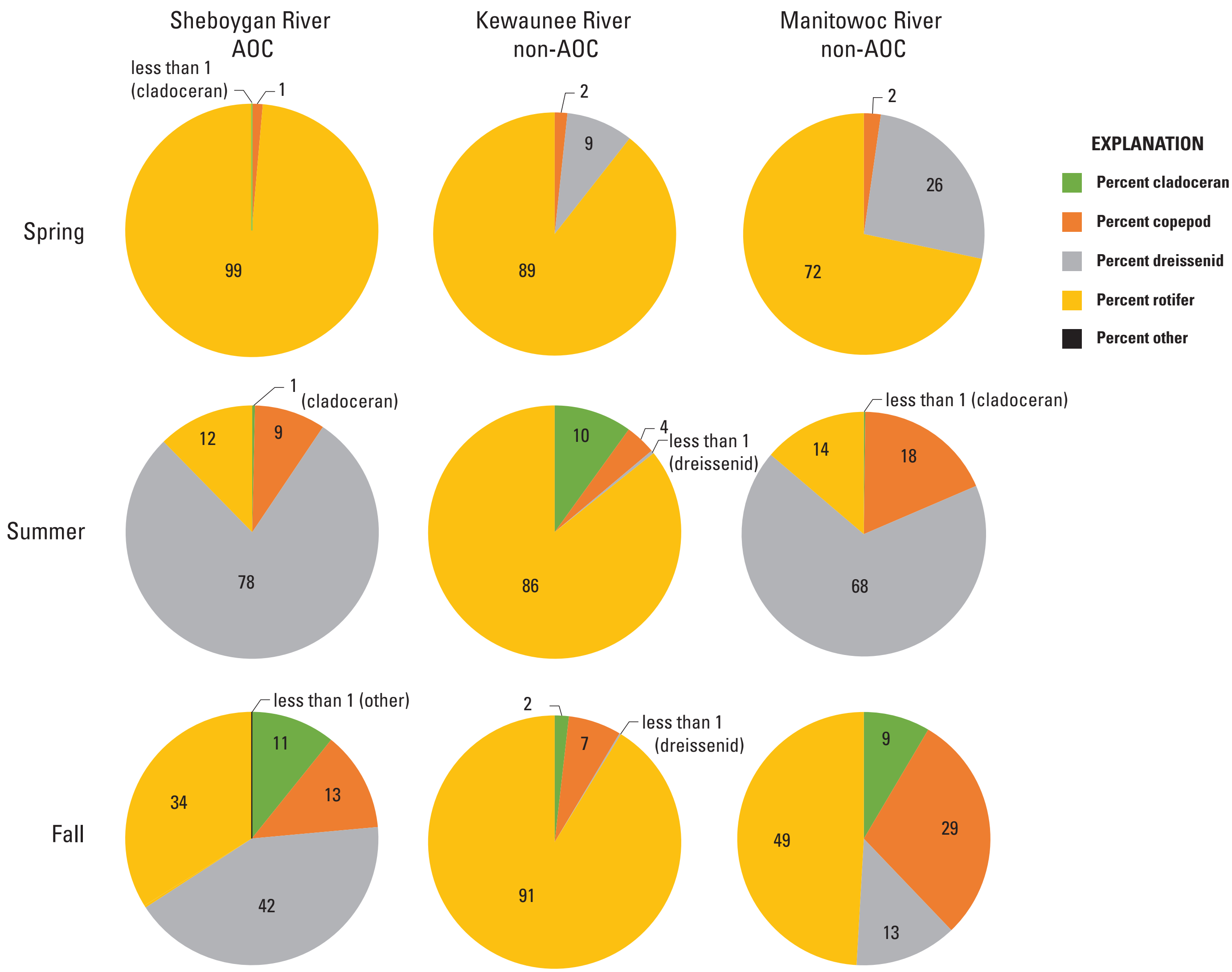

Figure 3. The percentage of each type of zooplankton taxa in samples collected at the Sheboygan River Area of Concern (AOC) and two non-AOC comparison sites, the Kewaunee and Manitowoc Rivers, in western Lake Michigan in 2016.

AOC and the Manitowoc River were more similar to each other than to those at the Kewaunee River. The zooplankton community at the Kewaunee River in summer was most similar to the zooplankton communities at the Kewaunee and Manitowoc Rivers from fall. The zooplankton community at the Sheboygan River AOC in fall was more different than the other groups, plotting further away from all other samples. The replicate community samples collected at the Sheboygan River AOC within each season were very similar to each other, plotting closely together.

The Sheboygan River AOC and the Manitowoc River zooplankton communities generally had higher percentages of dreissenid veligers, which is a potential reason for the similarities between the communities at these sites. Despite some differences, especially between the Sheboygan River AOC and the Kewaunee River, a one-way ANOSIM test indicated that there was no significant difference between the communities at the Sheboygan River AOC and the two non-AOC comparison sites at $p<0.05$ (global $R=0.093$), regardless of if dreissenid veligers and copepod nauplii were included in the analysis and if replicate samples were used in the analysis. The ANOSIM global R value is an indication of how different the communities being compared are. A global R value closer to 0 indicates that the differences are not clear, and a global R value closer to 1 indicates that there are very clear differences between the communities. A significant p-value ($p<0.05$) indicates a high confidence in the global R value result. Although the differences between the communities

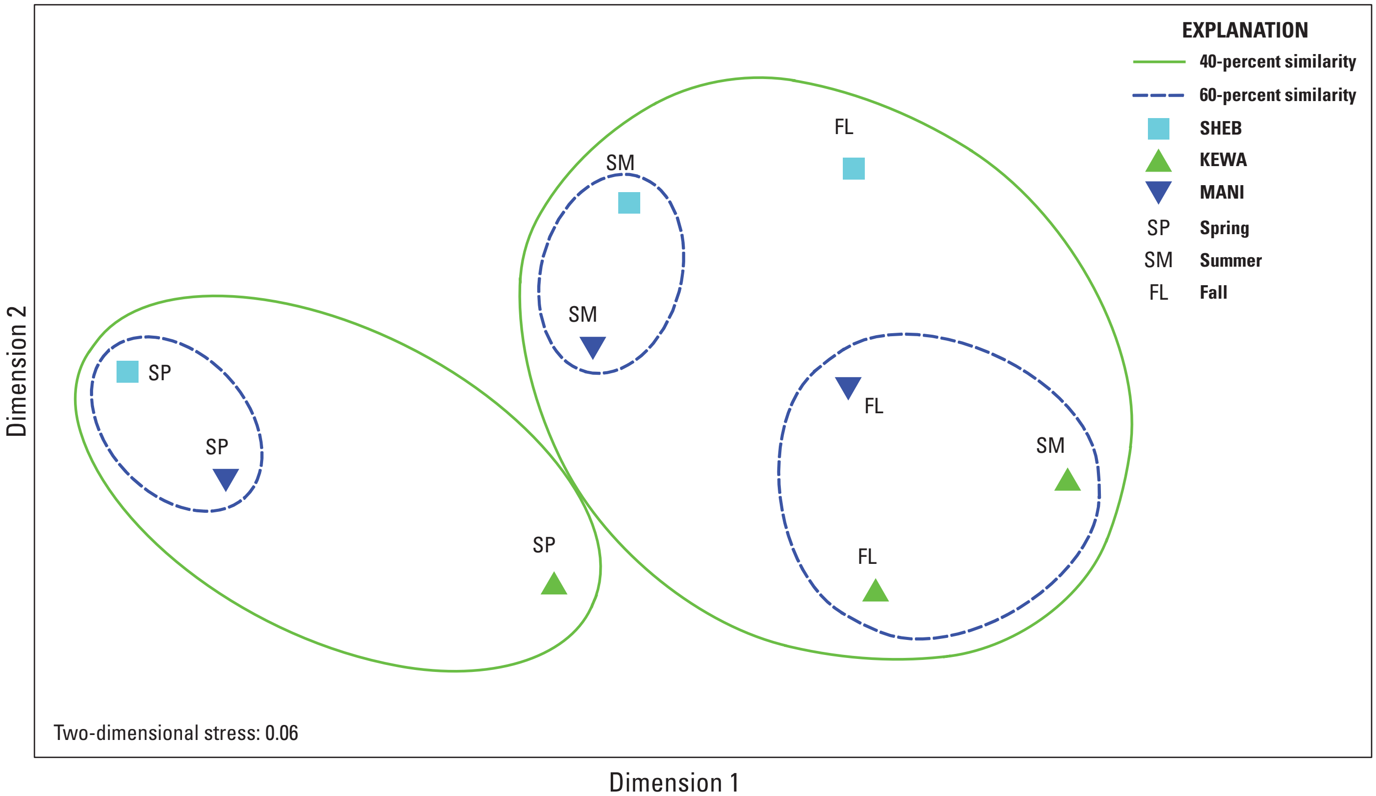

Figure 4. Zooplankton communities collected at the Sheboygan River (SHEB) Area of Concern (AOC) and two non-AOC comparison sites, the Kewaunee (KEWA) and Manitowoc (MANI) Rivers, in western Lake Michigan in 2016.

were not significant at $p<0.05$, a one-way SIMPER test found that samples from the AOC and the non-AOC comparison sites were on average 53 percent dissimilar. Rotifers (mainly *Polyarthra sp.*, *Synchaeta sp.*, *Keratella sp.*, *Brachionus sp.*, and *Conochilus unicornis*) accounted for a majority (60 percent) of this dissimilarity, copepods (mainly immature nauplii and copepodites), cladocerans (*Bosmina longirostris* and *Diaphanosoma sp.*), and dreissenid veligers accounted for much of the remainder. Similar results were found when the analysis was run with and without dreissenid veligers, copepod nauplii, and replicate samples.

The mean (plus or minus standard deviation) of zooplankton richness (fig. 5; table 3) across the three seasons at the Sheboygan River AOC was 16.0 (±6.0). In comparison, mean zooplankton richness at the Kewaunee River was 13.3 (±5.0), and mean richness at the Manitowoc River was 11.3 (±5.5). An ANOVA followed by a Bonferroni test, with primary and replicate samples included, indicated that richness at the Sheboygan River AOC was significantly higher than richness at the Manitowoc River ($p<0.05$) but was not significantly different from the Kewaunee River. Richness at the Sheboygan River AOC increased from the spring through the fall season. Overall, however, richness at the Sheboygan River AOC was significantly higher than at the two non-AOC comparison sites as a group ($p\leq0.01$), indicating that richness in the AOC can be rated as “not degraded.”

Mean zooplankton diversity across seasons was 1.06 (±0.88) at the Sheboygan River AOC, 1.42 (±0.45) at the Manitowoc River, and 1.66 (±0.09) at the Kewaunee River (fig. 5; table 3). Although diversity at the Sheboygan River AOC was not significantly different from either the Manitowoc or Kewaunee rivers when examined individually, diversity at the Sheboygan River AOC was significantly lower than the non-AOC comparison sites as a group ($p<0.05$), and therefore rated as “degraded” in comparison to the non-AOC sites. A finding of no significant difference between a community at the Sheboygan River AOC and the two non-AOC comparison sites does not necessarily mean that there is no impairment at the AOC; therefore, when a metric at the AOC is found to be significantly lower than the non-AOC sites (rated “degraded”), despite the probable impairments present in the non-AOC sites themselves, this emphasizes the finding of degradation at the AOC.

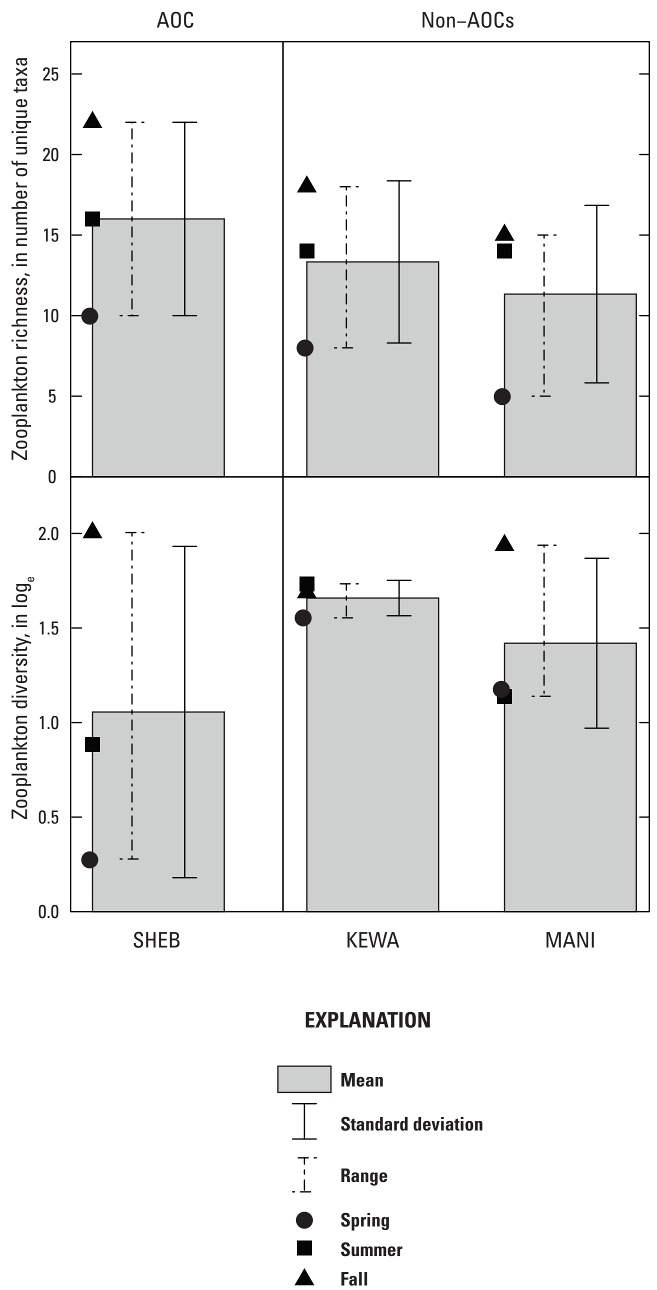

Figure 5. Zooplankton richness and diversity metrics collected at the Sheboygan River (SHEB) Area of Concern (AOC) and two non-AOC comparison sites, the Kewaunee (KEWA) and Manitowoc (MANI) Rivers, in western Lake Michigan in 2016.

Table 3. Richness and diversity values for zooplankton samples collected at the Sheboygan River Area of Concern (AOC) and two non-AOC comparison sites, the Kewaunee and Manitowoc Rivers, in western Lake Michigan in 2016.

Site name	Season	Richness[1]	Diversity[2]
Sheboygan River AOC	Spring	10	0.28
	Summer	16	0.88
	Fall	22	2.00
Sheboygan River AOC replicate sample	Spring	10	0.60
	Summer	16	0.79
	Fall	20	1.69
Kewaunee River non-AOC	Spring	8	1.55
	Summer	14	1.73
	Fall	18	1.69
Manitowoc River non-AOC	Spring	5	1.18
	Summer	14	1.14
	Fall	15	1.94

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

[2]Shannon diversity, calculated as natural logarithm Shannon diversity index, is from Shannon (1948).

Zooplankton Community Comparisons in the Sheboygan River AOC in 2016 Compared With Previous Years

Annual shifts in the zooplankton community composition and structure at the Sheboygan River AOC were evident by the variations of dominant taxa present each year, similarities and differences demonstrated by multivariate analysis, and changes in richness and diversity values for 2012, 2014, and 2016. Rotifers were the dominant taxa in the spring zooplankton samples collected all 3 years at the Sheboygan River AOC (fig. 6). The dominant rotifer species in spring 2012, 2014, and 2016 were *Brachionus bidentate*, *Euchlanis dilatata,* and *Synchaeta*, respectively. In spring 2012, a small percentage of the zooplankton community was composed of dreissenid veligers (primarily *Dreissena polymorpha*; zebra mussel), whereas in 2014 and 2016, no veligers of this invasive species were found in spring samples. In summer samples, rotifers again dominated in 2012 (mainly *Brachionus calyciflorus* and *Synchaeta oblonga*) and 2014 (mainly *Synchaeta sp.*). In summer 2016, however, dreissenid veligers dominated the summer zooplankton community, whereas no dreissenids were found in summer 2012 and only 1 percent of the community in 2014 was composed of dreissenid veligers. In fall 2012, the zooplankton community at the Sheboygan River AOC was again dominated by rotifers (mainly *Brachionus angularis*,

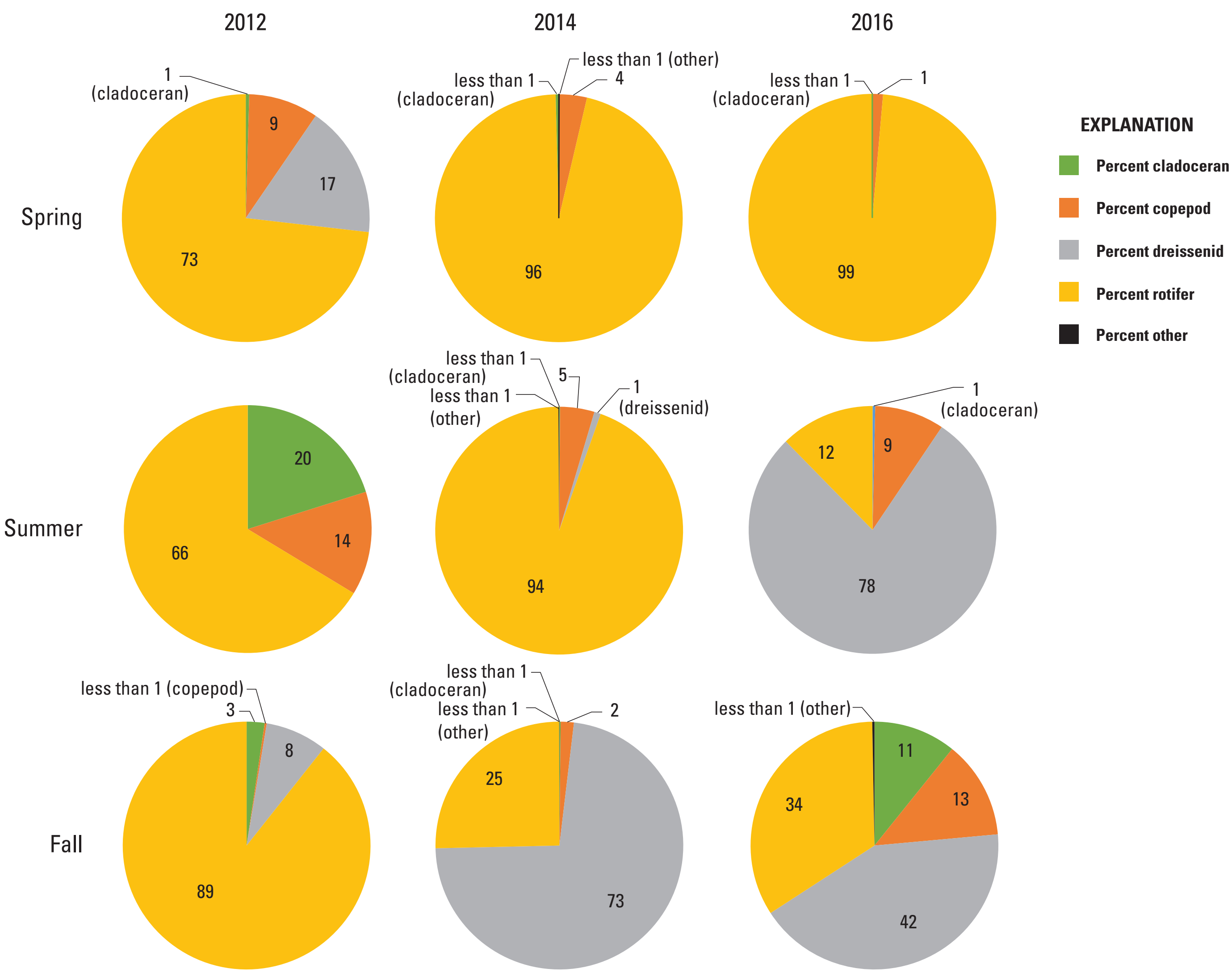

Figure 6. The percentage of each type of zooplankton taxa in samples collected at the Sheboygan River Area of Concern in western Lake Michigan in 2012, 2014, and 2016.

Brachionus caudatus, and *Synchaeta oblonga*). In fall 2014, the zooplankton community shifted to a majority *Dreissena polymorpha* veligers and only 25 percent rotifers. The percentage of dreissenid veligers decreased from 73 percent in fall 2014 to 42 percent in fall 2016. The fall 2016 zooplankton community was also composed of a smaller percentage of rotifers, copepods, and cladocerans.

The nMDS ordination plot of zooplankton communities in the Sheboygan River AOC in all 3 years had low two-dimensional stress (0.08), meaning that the relations between the communities were well represented by the plot. The primary and replicate samples collected at the Sheboygan River AOC each year in spring, summer, and fall generally plotted very closely together, indicating they were similar to each other (fig. 7). This suggested that the samples each year had good quality control and could be incorporated into statistical analysis. Community composition and structure were more similar in 2014 and 2016 than in 2012, as evidenced by the positions of the samples in the nMDS ordination plot. Summer and fall samples within 2014 and 2016 were also more similar than the spring samples in each of those years. A one-way ANOSIM, including replicate samples, indicated a significant difference between zooplankton communities among all years (R=0.94, p<0.01), and pairwise testing indicated significant differences between 2012 and 2014 (R=0.97, p<0.01), 2012 and 2016 (R=1.0, p<0.01), and 2014 and 2016 (R=0.88, p<0.01).

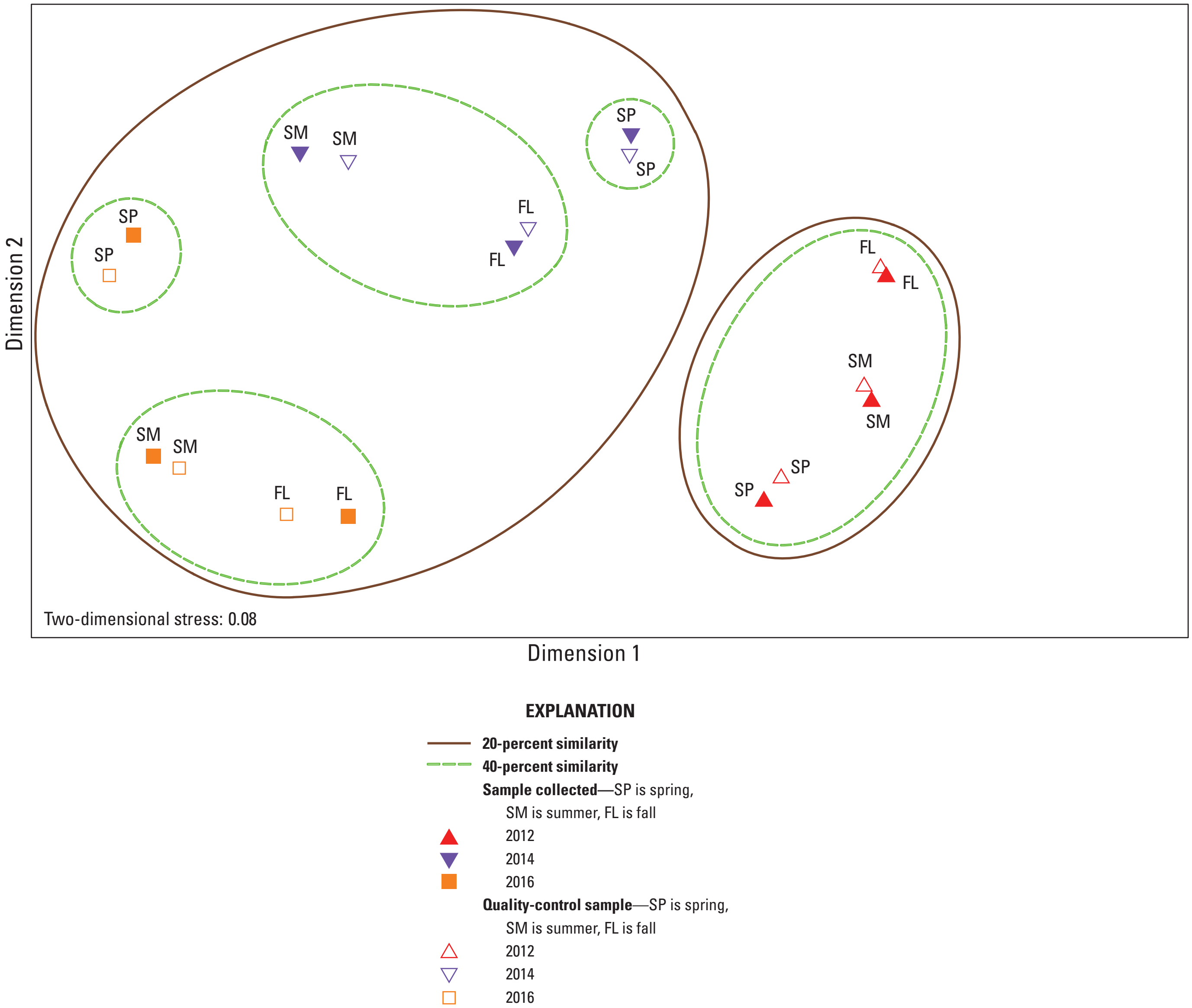

Figure 7. Zooplankton communities collected at the Sheboygan River Area of Concern in western Lake Michigan in 2012, 2014, and 2016.

Zooplankton richness and diversity at the Sheboygan River AOC in 2016 did not show improvement in comparison to the previous years sampled. ANOVA and Bonferroni postevent testing indicated that richness values in 2016 were significantly lower than in 2014 ($p<0.01$) but not lower than in 2012. Richness in 2012 was also significantly lower than in 2014 ($p<0.05$). Mean richness was 20 (±3) in 2012 and 27 (±9) in 2014, compared with 16 (±6) in 2016. Diversity values in the AOC in 2016 were not significantly different from diversity in 2014, but diversity in both of these years was significantly lower than diversity in 2012 ($p<0.05$ for 2016 and $p<0.01$ for 2014). Mean diversity was 2.07 (±0.19) in 2012 and 1.14 (±0.55) in 2014, compared with 1.05 (±0.88) in 2016. In summer 2012, Wisconsin experienced a heat wave and drought, which is likely to have influenced some of the differences in zooplankton community composition and structure at the AOC when comparing the years that were sampled.

Summary and Conclusions

In late May, mid-July, and late August 2016, the U.S. Geological Survey (USGS), in cooperation with the Wisconsin Department of Natural Resources (WDNR) and the U.S. Environmental Protection Agency (EPA), collected zooplankton community samples at the Sheboygan River Area of Concern (AOC) and selected non-AOC sites as a follow-up to a larger Great Lakes Restoration Initiative study in 2012 and 2014, which evaluated both the benthos and plankton communities in all four of Wisconsin's Lake Michigan AOCs. This report describes study areas and field sampling methods and provides data collected and analyzed for characterization of zooplankton communities during the three seasonal sampling events in 2016 at the Sheboygan River AOC in Sheboygan, Wisconsin, and at the two non-AOC comparison sites, the Kewaunee River in Kewaunee, Wisc., and the Manitowoc River in Manitowoc, Wisc. Although no river mouth or harbor in the Great Lakes basin is completely unimpaired by anthropogenic influences, the Kewaunee and Manitowoc Rivers were selected as the best possible non-AOC comparison sites for the Sheboygan River AOC because of similar environmental characteristics and close geographic proximity. Zooplankton communities sampled in this study may reflect both river and Lake Michigan taxa because of their proximity to the lake; however, this is the overall nature of aquatic communities at river mouths.

During each sampling event, in addition to collecting samples from the Sheboygan River at one primary location, samples were also collected at one nearby replicate location to serve as replicates for comparison between the sites. In addition to data on relative abundance and distribution of zooplankton taxa at these sites, ancillary data for in-place water-quality characteristics (water temperature, pH, specific conductance, and dissolved oxygen) were collected concurrently with zooplankton samples. The data collection descriptions and interpretations in this report are part of a study designed to assess the status of the zooplankton communities in the Sheboygan River AOC in comparison to those at the two non-AOC sites to evaluate the related Beneficial Use Impairments (BUIs) at the AOC. Standard statistical analyses with biological metrics and multivariate statistical analyses on relative abundance data were used to characterize the condition of the Sheboygan River AOC compared with that of the non-AOC sites. If the zooplankton communities in the AOC are rated as not significantly degraded in comparison with the communities at the presumptively less impaired non-AOC sites of similar environmental characteristics, then the WDNR and the EPA may decide that the BUI can be removed as a step toward delisting the AOC. However, when an AOC is found to be "degraded" in comparison to the non-AOC comparison sites, despite the probable impairments present in the non-AOC sites themselves, this underscores the degradation at the AOC. On the other hand, a finding of no significant difference between a community at the AOC and non-AOC comparison sites does not necessarily mean that there is no impairment at the AOC.

In general, the nMDS ordination plot of zooplankton samples in 2016 showed the zooplankton communities from the Sheboygan River AOC samples were most similar to the communities from the Manitowoc River samples, which could be because of the closer proximity and more similar water temperatures among the Sheboygan River and the Manitowoc River as well as similar percentages of invasive dreissenid veligers in several of the samples. Overall, however, there was no significant difference between the communities at the AOC and the non-AOC comparison sites, and the communities were on average nearly 50 percent similar. Among sampling years, the communities in 2012, 2014, and 2016 were significantly different from one another, making it difficult to gage the progress of the AOC over a relatively short study period. Different environmental conditions among the years may have contributed to the differences. For example, the 2012 samples were collected before the sediment remediation was completed in the Sheboygan River, as well as in the midst of heat and drought in Wisconsin, both of which could be likely reasons that the 2012 zooplankton samples formed a distinct group that plotted away from the other years on the nMDS ordination plot. The samples collected in 2014 and 2016 were in postremediation conditions and in years that had similar weather conditions; the community samples from these years plotted more closely together, indicating that they were more similar to each other than to the 2012 samples.

Species richness and diversity metrics in the AOC were statistically compared with these metrics in the non-AOC sites, as well as with results from the USGS studies at the Sheboygan River AOC in 2012 and 2014. In 2016, species richness in the AOC was significantly higher than in the Manitowoc River but not significantly different from that in the Kewaunee River, and overall, richness in the AOC was significantly higher than in the non-AOC sites as a group. These results indicate that richness in the Sheboygan River AOC was not degraded in 2016 when compared with the non-AOC sites; however, comparing these results with previous results from 2012 and 2014 tell a different story. Richness values in 2016 were significantly lower than in 2014, but not 2012, indicating that although richness values in the AOC were overall greater in 2016 than the non-AOC sites, the zooplankton communities may still be in the process of improvement following the extensive remediation in the Sheboygan River AOC.

Diversity values in the AOC in 2016 were not significantly different from those for the Kewaunee and Manitowoc Rivers when examined individually, but diversity at the AOC was significantly lower than the non-AOC sites as a group. Across all years sampled in the AOC, diversity in 2014 and 2016 did not differ significantly; however, diversity values in these years were significantly lower than in 2012. These results indicate that zooplankton diversity in the Sheboygan River AOC was degraded compared with the non-AOC sites and more time may be needed for the zooplankton communities to recover following remedial actions taken in the AOC.

Postremediation recovery can often be complicated by non-AOC-related stressors such as nutrients and invasive species, which could result in slow recovery of zooplankton communities in the Sheboygan River AOC. Dredging of contaminated sediment in the AOC was completed in 2013, and the zooplankton communities in 2016 were likely still in the process of recovering from the removal of contaminants and disturbance from dredging activities. Additionally, invasive dreissenid veligers (immature zebra mussels) were found each year and were often in high abundance in zooplankton community samples in the AOC. This exotic member of the zooplankton community is likely contributing to the Sheboygan River AOC's reduced diversity in comparison with the Kewaunee and Manitowoc Rivers.

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