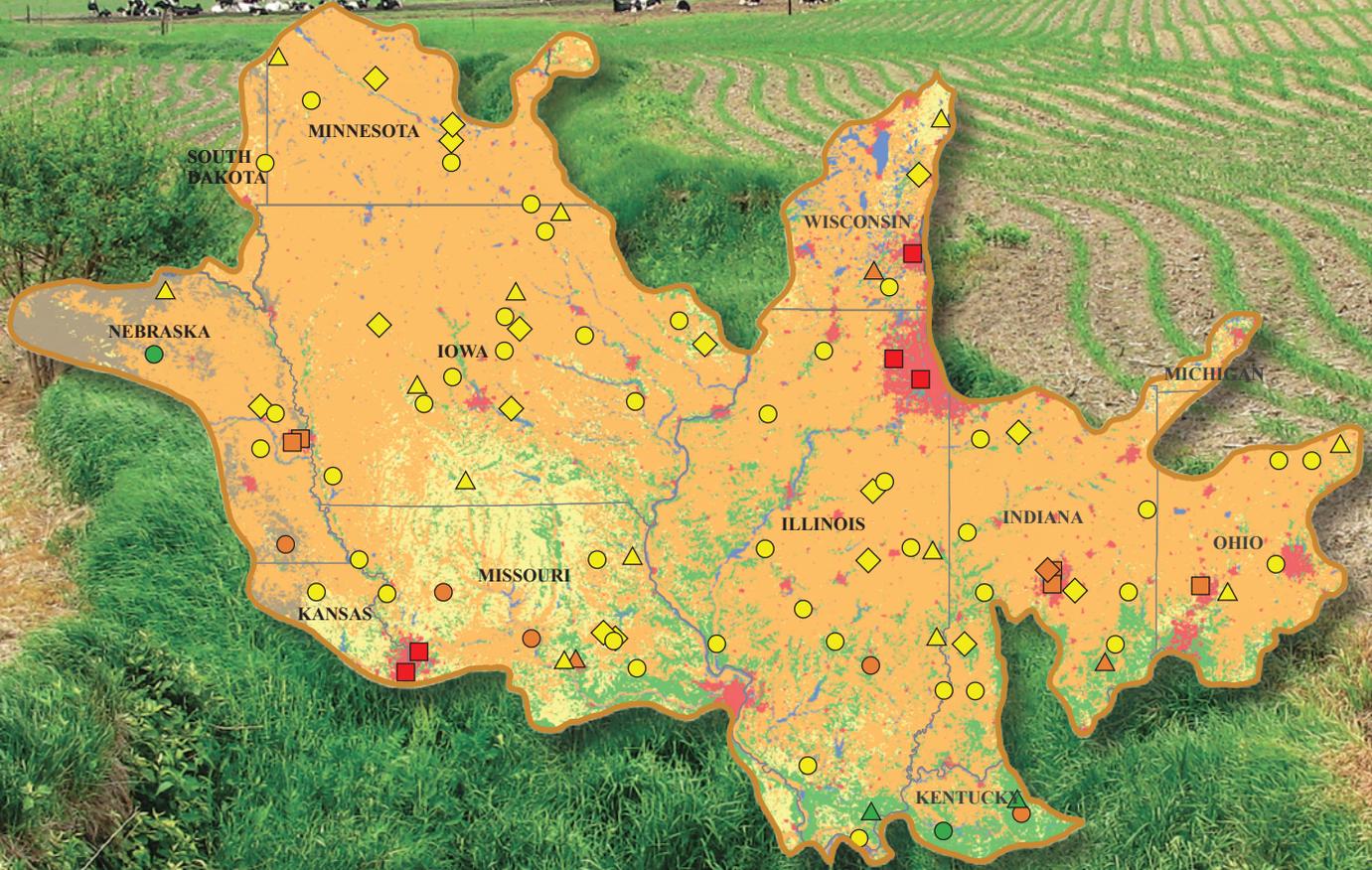


National Water-Quality Program
Prepared in cooperation with the U.S. Environmental Protection Agency

Design and Methods of the Midwest Stream Quality Assessment (MSQA), 2013



Open-File Report 2017-1073

Front cover. Map showing the study area for the Midwest Stream Quality Assessment with sites by selection category and watershed land-use type (see figure 3 for full explanation).

Map background. A farm in northwestern Indiana, 2013. (Photograph by Peter Van Metre, U.S. Geological Survey)

Back cover inset photos. Top right, USGS volunteer servicing pesticide micro-autosampler, July 2013. (Photograph by Shannon Meppelink, U.S. Geological Survey); bottom left, U.S. Geological Survey personnel deploying in-stream fish and frog enclosure experiments. (Photograph by Peter Van Metre, U.S. Geological Survey)

Back cover background. South Fork Iowa River upstream of H Avenue, Hardin County, Iowa. (Photograph by Shannon Meppelink, U.S. Geological Survey)

Design and Methods of the Midwest Stream Quality Assessment (MSQA), 2013

By Jessica D. Garrett, Jeffrey W. Frey, Peter C. Van Metre, Celeste A. Journey,
Naomi Nakagaki, Daniel T. Button, and Lisa Nowell

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RYAN K. ZINKE, Secretary

U.S. Geological Survey

William H. Werkheiser, Acting Director

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Conversion Factors

International System of Units to U.S. customary units

Multiply	By	To obtain
Length		
nanometer (nm)	3.937×10^{-8}	inch (in.)
micron (μm)	3.937×10^{-5}	inch (in.)
millimeter (mm)	0.03937	inch (in.)
centimeter (cm)	0.3937	inch (in.)
meter (m)	3.281	foot (ft)
kilometer (km)	0.6214	mile (mi)
kilometer (km)	0.5400	mile, nautical (nmi)
meter (m)	1.094	yard (yd)
Area		
square meter (m^2)	0.0002471	acre
square meter (m^2)	10.76	square foot (ft^2)
square centimeter (cm^2)	0.1550	square inch (in^2)
hectare (ha)	0.003861	square mile (mi^2)
square kilometer (km^2)	0.3861	square mile (mi^2)
Volume		
microliter (μL)	2.642×10^{-7}	gallon (gal)
milliliter (mL)	0.0002642	gallon (gal)
liter (L)	0.2642	gallon (gal)
cubic meter (m^3)	264.2	gallon (gal)
liter (L)	61.02	cubic inch (in^3)
microliter (μL)	6.102×10^{-5}	cubic foot (ft^3)
Flow rate		
meter per second (m/s)	3.281	foot per second (ft/s)
cubic meter per second (m^3/s)	35.31	cubic foot per second (ft^3/s)
Mass		
gram (g)	0.03527	ounce, avoirdupois (oz)
kilogram (kg)	2.205	pound avoirdupois (lb)

Temperature in degrees Celsius ($^{\circ}\text{C}$) may be converted to degrees Fahrenheit ($^{\circ}\text{F}$) as follows:

$$^{\circ}\text{F} = (1.8 \times ^{\circ}\text{C}) + 32.$$

Temperature in degrees Fahrenheit ($^{\circ}\text{F}$) may be converted to degrees Celsius ($^{\circ}\text{C}$) as follows:

$$^{\circ}\text{C} = (^{\circ}\text{F} - 32) / 1.8.$$

Datum

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

Supplemental Information

Specific conductance is given in microsiemens per centimeter at 25 degrees Celsius ($\mu\text{S}/\text{cm}$ at 25 °C).

Concentrations of chemical constituents in water are given in either milligrams per liter (mg/L) or micrograms per liter ($\mu\text{g}/\text{L}$).

Abbreviations—Continued

Aii	agricultural intensity index
ASR	analytical services request form
AUS	micro-autosampler
CAPEST	California Pesticide Fate Research Laboratory
CAS	Chemical Abstracts Service
CERC	Columbia Environmental Research Center
CFF	caged fish and frog
CGGAR	Crustal Geophysics and Geochemistry Analytical-Research Laboratory
CMERA	Central Mineral and Environmental Resources Analytical Laboratory
CNIT	continuous nitrate and biological response
DOC	dissolved organic carbon
DQI	data quality indicator
EPA	U.S. Environmental Protection Agency
EPT	Ephemeroptera, Plecoptera, and Trichoptera
IBI	Index of Biological Integrity
INT	intensive contaminants monitoring
LC-MS/MS	liquid chromatography/tandem mass spectrometry
LIMS	Laboratory Information Management System
MERC	mercury
MMI	macroinvertebrate multimetric index

Abbreviations—Continued

MSQA	Midwest Stream Quality Assessment
NAWQA	National Water-Quality Assessment
NHDPlus	National Hydrography Dataset Plus
NRCS	Natural Resources Conservation Service
NRP	National Research Program
NRSA	National Rivers and Streams Assessment
NWIS	National Water Information System
NWQL	National Water Quality Laboratory
OPP	Office of Pesticide Programs
PAHs	polycyclic aromatic hydrocarbons
PBIO	EPA NRSA periphyton biomass sample
PCFF	Personal Computer Field Form
PCHL	EPA NRSA periphyton chlorophyll <i>a</i> sample
PERI	EPA NRSA periphyton identification/enumeration sample
POCIS	polar organic compound integrating samplers
RC	row crop
QA	quality assurance
QC	quality control
QWDX	Water Quality Data Exchange
RSQA	Regional Stream Quality Assessment
SED	sediment source
SVOCs	semivolatile organic compounds
TPN	total particulate nitrogen
TWU	toxicity-weighted pesticide use
USGS	U.S. Geological Survey
WSC	Water Science Center
WTOX	water toxicity

Design and Methods of the Midwest Stream Quality Assessment (MSQA), 2013

By Jessica D. Garrett, Jeffrey W. Frey, Peter C. Van Metre, Celeste A. Journey, Naomi Nakagaki, Daniel T. Button, and Lisa Nowell

Abstract

During 2013, the U.S. Geological Survey (USGS) National Water-Quality Assessment Project (NAWQA), in collaboration with the USGS Columbia Environmental Research Center, the U.S. Environmental Protection Agency (EPA) National Rivers and Streams Assessment (NRSA), and the EPA Office of Pesticide Programs assessed stream quality across the Midwestern United States. This Midwest Stream Quality Assessment (MSQA) simultaneously characterized watershed and stream-reach water-quality stressors along with instream biological conditions, to better understand regional stressor-effects relations. The MSQA design focused on effects from the widespread agriculture in the region and urban development because of their importance as ecological stressors of particular concern to Midwest region resource managers.

A combined random stratified selection and a targeted selection based on land-use data were used to identify and select sites representing gradients in agricultural intensity across the region. During a 14-week period from May through August 2013, 100 sites were selected and sampled 12 times for contaminants, nutrients, and sediment. This 14-week water-quality “index” period culminated with an ecological survey of habitat, periphyton, benthic macroinvertebrates, and fish at all sites. Sediment was collected during the ecological survey for analysis of sediment chemistry and toxicity testing. Of the 100 sites, 50 were selected for the MSQA random stratified group from 154 NRSA sites planned for the region, and the other 50 MSQA sites were selected as targeted sites to more evenly cover agricultural and urban stressor gradients in the study area. Of the 50 targeted sites, 12 were in urbanized watersheds and 21 represented “good” biological conditions or “least disturbed” conditions. The remaining 17 targeted sites were selected to improve coverage of the agricultural intensity gradient or because of historical data collection to provide temporal context for the study.

This report provides a detailed description of the MSQA study components, including surveys of ecological conditions, routine water sampling, deployment of passive polar organic compound integrative samplers, and stream sediment sampling at all sites. Component studies that were completed to provide finer scale temporal data or more extensive analysis at selected sites, included continuous water-quality monitoring, daily pesticide sampling, laboratory and in-stream water toxicity testing efforts, and deployment of passive suspended-sediment samplers.

Introduction

In 2013, the U.S. Geological Survey (USGS) National Water-Quality Assessment Project (NAWQA) began its third decade of assessment (Rowe and others, 2010). A major objective of the NAWQA Project is to determine the quality of streams across the Nation. The Regional Stream Quality Assessment (RSQA) component of NAWQA is a primary programmatic approach to accomplishing this objective.

The goals of an RSQA are to characterize multiple environmental stressors and ecological conditions in streams throughout a region and to improve our understanding of the effects of the stressors on the ecology of these streams. Sediment, streamflow variation, and nutrients are essential parts of a natural, healthy stream ecosystem; however, deviation from their natural condition can degrade stream ecosystems (Dubrovsky and others, 2010; Carlisle and others, 2013). Contaminants differ from the other stressors in that most are derived from human activities, and through toxic or endocrine-disrupting effects, contaminants have the potential to adversely affect aquatic life. To efficiently manage water resources, knowing under what conditions an individual stressor or combination of stressors causes an adverse effect on the biological condition of the stream is important

information. All these stressors have the potential to affect the beneficial uses of water resources by humans as well. Findings from these regional studies will provide communities and policymakers information on which human and environmental factors are the most important in controlling stream quality.

The RSQA approach developed by NAWQA attempts to balance the advantages of two spatial scales. Effects of single or multiple stressors on biological condition are perhaps easiest to evaluate in small, watershed-scale studies, where biogeochemical processes and complex environmental interactions can be monitored, or in field or laboratory experiments, where conditions can be manipulated to identify cause(s) of impairment. However, results of such studies are not necessarily applicable to other locations. At the other end of the spatial scale, biological condition and individual stressors can be evaluated over a large geographic area (U.S. Environmental Protection Agency, 2013) to develop databases and empirical models at the regional scale that can describe and predict the occurrence of stressors and their effects on stream biological condition. An RSQA is designed as a 1-year, multistressor assessment done at a regional scale—generally parts of several states—with embedded components or studies to target regionally important topics or focused on processes requiring local or experimental scales. Regionally focused components and smaller scale studies provide a bridge between the large regional or national scale of the program and the ability to study process questions that require smaller study scales.

The Midwest Stream Quality Assessment (MSQA) in 2013 was the first of the NAWQA Cycle 3 RSQA studies. The NAWQA Project collaborated with the USGS Columbia Environmental Research Center (CERC), the U.S. Environmental Protection Agency (EPA) National Rivers and Streams Assessment (NRSA) and the EPA Office of Pesticide Programs (OPP) to assess stream quality across the Midwestern United States. The MSQA combined the regional- and national-scale water-quality assessment approaches of the USGS and the EPA, encompassing parts of 11 States with an area about 600,000 square kilometers (km²). The MSQA monitored 100 stream sampling sites across the Midwestern United States for contaminants, nutrients, sediment, toxicity, ecological communities, water level, and stream habitat during the 2013 crop growing season. Within the RSQA stressor-ecological response framework, the MSQA design targeted effects from widespread agriculture in the region and from urban development.

Purpose and Scope

The purpose of this report is to describe the design and methods used in the 2013 MSQA. The MSQA study combined the targeted design commonly used by the USGS NAWQA Project (for example, Coles and others, 2012) and the random stratified design of the EPA NRSA (Olsen and others, 1999; U.S. Environmental Protection Agency, 2013) and included several embedded, focused studies. The selected network of

sites is described relative to the goals for each component of the study. Field data collection and processing methods are described for continuous monitoring of water level and water quality, collection of integrated and discrete water-quality and sediment samples, and assessment of habitat and ecological stream condition. Laboratory analyses are described for water, sediment, and ecological samples, including analyses of integrated and discrete samples for chemical constituents, physical properties, or laboratory organism exposure experiments. Geospatial data were compiled or processed to aid the study design process or to be used in interpreting results and are described in this report. Quality assurance (QA) methods and quality control (QC) samples for the study are summarized in this report. As components within the overall MSQA, special studies that were completed also are described in this report.

The work described in this report represents the combined efforts of study-team members from the USGS Water Science Centers (WSC), NAWQA, CERC, the National Water Quality Laboratory (NWQL) of the USGS and selected other USGS laboratories, the EPA NRSA, the EPA OPP, and State agencies and contractors supporting the NRSA program in the Midwest region. This report does not present or summarize data collected, except certain sample counts. Methods previously published are summarized, with details provided only for notable deviations.

Study Area Description

The MSQA region overlies the Midwestern agricultural region dominated by corn and soybean cultivated crops and comprises parts of 12 States, with an area of about 600,000 km² (fig. 1). The area consists of flat, rich soils that are well suited for row crop (RC) agriculture (Baker and Capel, 2011). The region has a population of nearly 38 million, including Chicago, Illinois; Indianapolis, Indiana; Columbus, Ohio; as well as other cities (GeoLytics, 2013; fig. 1; appendix 1). The MSQA study area is formed by the combination of all or parts of six EPA Level III Ecoregions (8.2.1, 8.2.3, 8.2.4, 8.3.2, 9.2.3, and 9.2.4, excluding the southern part of 9.2.4 in Kansas, Missouri, and Oklahoma [not shown]), plus small parts of surrounding ecoregions where the watersheds of MSQA sites extend across those boundaries. The area covers large parts of Illinois, Indiana, Iowa, southern Minnesota, northern Missouri, and western Ohio, plus portions of northeastern Kansas, western Kentucky, eastern Nebraska, southeastern South Dakota, southeastern Wisconsin, and a small extension into southern Michigan (although no sampling sites were in Michigan) (fig.1).

The region has a severe, midlatitude humid continental climate marked by warm to hot summers and cold to severe winters (Griffith, 2010). The mean annual temperature ranges from approximately 6 to 14 degrees Celsius (°C) with frost-free period from 140 to 220 days. The mean annual precipitation ranges from 730 to 1,320 millimeters (mm), with wetter conditions in the south and drier in the north and west (Griffith, 2010).

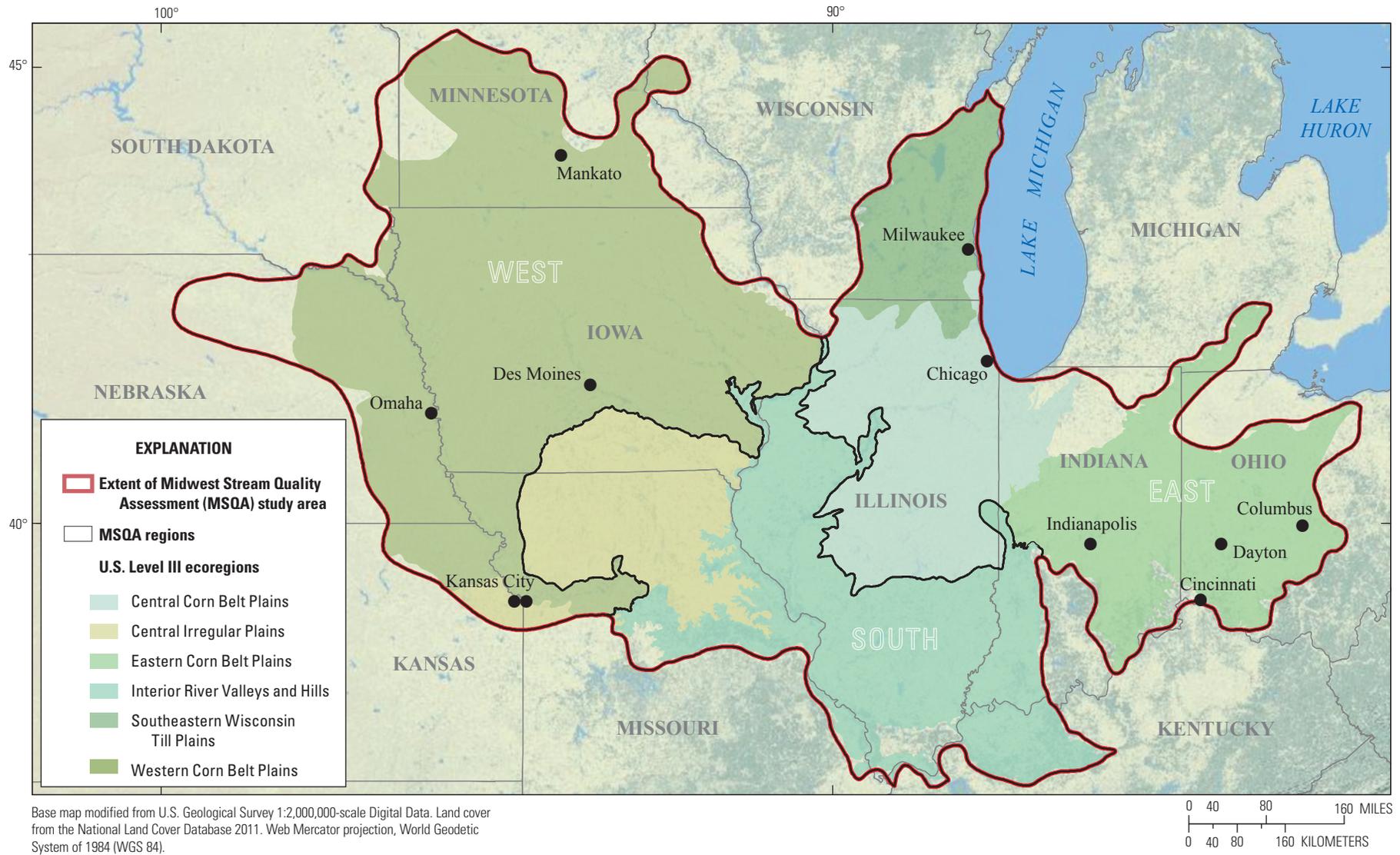


Figure 1. Study area for the Midwest Stream Quality Assessment (MSQA) with ecoregions.

Physiographically, the region ranges from flat valleys to rolling hills with low to moderately graded streams and previously vegetated by forest, prairie, and savanna (Griffith, 2010). Flat to rolling glacial till plains are underlain by carbonates, shale, sandstones, limestone, and some areas of coal. Valley slopes and bluffs rim large river valleys filled with alluvium, loess, and lacustrine deposits. Alfisols, Histosols, and Mollisols are typical, with a mesic soil temperature regime and aquatic or udic soil moisture regime (Griffith, 2010). Streams are low to moderate gradient, and many areas have been channeled and tiled. A few areas have natural lakes, reservoirs, or wetlands. Historic vegetation types included forests containing oak, maple, beech, basswood, hickory, elm, walnut, and poplar; mesic and dry upland prairie containing big bluestem, little bluestem, Indiangrass, prairie dropseed, switchgrass, sideoats grama, and numerous forbs; and savanna (Griffith, 2010). Natural vegetation has been extensively replaced by agriculture.

The Midwest is an economically important agricultural region with environmental conditions that are ideal for agriculture, but intensive large-scale agriculture leads to high inputs of nutrients (Dubrovsky and others, 2010) and pesticides to streams (Gilliom and others, 2006; Thelin and Stone, 2013). As a result, the Midwest contributes substantially to nutrient, suspended sediment, and contaminant loading to the Mississippi River and the Gulf of Mexico (not shown) (Aulenbach and others, 2007; Meade and Moody, 2010; Heimann and others, 2011). The MSQA region is estimated to contribute about 64 percent of the nitrogen load reaching the Gulf of Mexico in a typical year (Robertson and others, 2009) although the region covers only about 20 percent of the Mississippi River watershed area. State and Federal agencies have placed a high priority on assessing the effects of agricultural management practices on water quality and aquatic ecosystems in the Mississippi River Basin (Mississippi River/Gulf of Mexico Watershed Nutrient Task Force, 2008). Crop-land agriculture and extensive animal production dominate much of the region. Crop production is largely corn, soybeans, and other forage and feed grains to support cattle, dairy, hog, and poultry operations (Griffith, 2010).

Previous Studies

The MSQA covers a large geographic area, approximately 600,000 km², that has numerous regional, State, and watershed studies describing stream water quality, ecological condition, and chemical stressors. The high inputs of agricultural chemicals required in RC agriculture are commonly associated with some of the highest concentrations of nutrients (Dubrovsky and others, 2010) and pesticides (Gilliom and others, 2006) in streams in the country. For sites in permeable areas with strong surface connections to groundwater, shallow groundwater also has some of the highest nitrate concentrations nationally (Nolan and Hitt, 2006). Some of the

largest loadings of nutrients to the Gulf of Mexico and Lake Erie (not shown) (Robertson and others, 2009; Robertson and Saad, 2011) are derived from the Corn Belt region. Agricultural management practices such as the large amount of tile drains in the Midwest region (Zucker and Brown, 1998) affect the transport of agricultural chemicals. The drains can greatly improve yields for farmers and help to decrease the amount of constituents transported to streams through overland flow, yet also readily transport soluble constituents such as nitrate (Baker and others, 2006) and orthophosphate to streams (Smith and others, 2015). The EPA periodically leads national and regional stressor-response studies as part of the Wadeable Stream Assessment (U.S. Environmental Protection Agency, 2006) and the NRSA (U.S. Environmental Protection Agency, 2015). In the Temperate Plains region, the Wadeable Stream Assessment and NRSA studies determined that 73–85 percent of the river miles were in poor or fair biological condition based on the macroinvertebrate multimetric index (MMI). Of the stress factors measured for the NRSA streams, conditions for total nitrogen, total phosphorus, and vegetated riparian cover were considered only fair or poor for more than 50 percent of river and stream lengths (U.S. Environmental Protection Agency, 2015) in the Temperate Plains. The MSQA region covers about two-thirds of the EPA Temperate Plains region. A structural equation model for stream quality for two areas (central Nebraska and Indiana-Ohio) within the MSQA region determined that the amount of croplands in the watershed could be used to explain higher dissolved constituents such as nitrate, whereas the particulate forms of constituents—total phosphorus, total nitrogen, suspended sediment—best explained adverse effects on invertebrate communities (Riseng and others, 2011). Increasing agricultural intensity (Waite, 2013) and total nitrogen and total phosphorus (Waite, 2014) accounted for decreases in Ephemeroptera, Plecoptera, and Trichoptera (EPT) richness in several study areas across the country that included multiple sites in Nebraska, Indiana, and Ohio.

Urbanization, even in a region that is dominated by agriculture like the Corn Belt, also can have adverse effects on water quality and ecological communities. In a summary of ecological assessments by the NAWQA Project, Carlisle and others (2013) concluded that urban areas tend to have a greater negative effect on ecological communities than agricultural areas. Relative to streams in agricultural and undeveloped areas, stream sediments in urban areas tend to have higher levels of metals (Mahler and others, 2006), legacy organochlorine compounds (Van Metre and Mahler, 2005; Phillips and others, 2010), polycyclic aromatic hydrocarbons (PAHs) (Li and others, 2003; Van Metre and Mahler, 2005), and insecticides (Nowell and others, 2013). Elevated levels of polychlorinated biphenyls have been reported in water and air in greater Chicago and elevated PAHs have been reported in Minneapolis, Milwaukee, and greater Chicago (fig. 1) (Christensen and others, 1997; Li and others, 2003; Van Metre and Mahler, 2005; Van Metre and Mahler, 2010).

Design of the Midwest Stream Quality Assessment Study

The objectives of the MSQA, for perennial (year-round) streams in the Midwest, are as follows:

1. Assess the status of ecological conditions; the geographic distribution of spring-summer seasonal concentrations of contaminants, nutrients, suspended sediment, and hydrologic condition; and toxicity of sediment and water in wadeable streams in the region.
2. Assess relations among concentrations of contaminants, nutrients, suspended sediment, and flow condition; toxicity of sediment; and ecological conditions in the sampled streams.
3. Identify and evaluate natural and anthropogenic factors affecting the occurrence of stressors and ecological conditions in the sampled watersheds.
4. Develop statistical models to predict concentrations of contaminants, nutrients, and sediment and to predict ecological conditions in perennial streams in the region.
5. Characterize the effects of multiple stressors and ecology in streams spanning the wide range of stressors associated with agriculture and urban land use. Because land uses affect the occurrence of stressors in streams and can adversely affect stream ecosystems, the MSQA study selected sites spanning wide ranges in these land uses to yield sites spanning stressor gradients.

Approach

The design of an RSQA relies on the concept of a water-quality index period during which stream ecology is assumed to be affected by the chemistry, flow condition, and habitat of the streams. The index period is assumed to extend for several weeks to months prior to the ecology assessment period, as indicated by the range in time period that researchers have observed is necessary for stream benthic invertebrate communities to recover from stressor effects (Moulton and others, 2002). Thus, an intensive period of water-quality assessment precedes the ecological survey at stream sampling sites in an RSQA. Another key concept in the design of an RSQA is the gradient approach to site selection, in which sites are targeted for sampling that span gradients in land use and other potential stressor variables. The assumption is that sampling across these gradients will yield data spanning ranges in many specific stressors (for example, contaminants) that will in turn allow us to better understand the effects of those stressors on stream ecology. The strength of this approach, which has generally been used by NAWQA in assessments since its inception in 1991, is that the approach provides datasets that are amenable to statistical analysis of relations between possible explanatory variables (for example, urban land use) and water

quality. Identifying and where possible, quantifying these relations can improve our understanding of the causes of observed water-quality conditions and provide useful information to resource managers and the public.

The MSQA combined the NAWQA–RSQA approach (chemical index period and targeted gradient site selection) with the EPA–NRSA approach, in which sampling sites are selected using a stratified random approach. The NRSA stratifies all streams across regions of the country by stream order (size) then randomly selects sampling sites within subsets of streams stratified (grouped) by stream order. The strength of the NRSA approach is that it can be used to make statistically valid inferences about stream quality (such as population estimates) and be used to evaluate the relative importance of measured stressors to aquatic ecosystem condition. Another NRSA strength is it allows for the sampling of a large number of sites across the country for a given amount of resources relative to the RSQA approach because it does not include the extensive stressor characterization of an RSQA. NRSA chemical sampling is limited to fewer parameters than RSQA and to collection on the day of ecological sampling. However, that strength also is a limitation, in that the assessment of stressors is limited to fewer parameters all sampled one time on the day of the ecological sampling. Between the two approaches, therefore, is a general tradeoff between the numbers of sites and intensity of stressor characterization. The NRSA and NAWQA approaches were used to achieve the desired mix of sites spanning stressor gradients and including enough random sites in the MSQA region to make statistically valid inferences about the population of streams in the region.

Smaller scale studies embedded within the MSQA provide a bridge between the large regional scale of the program and the ability to study questions that require experimental control or more intensive monitoring than is practical for large numbers of sites. These studies were designed to provide insight into the occurrence or causes of specific stressors or ecological responses. All the specialized studies investigated only a subset of sites but had complementary objectives to the broader MSQA objectives. See the “Major Components of Stressor Sampling and Special Studies” sections for details.

Site Selection

Site selection was driven by the following two primary goals: (1) to make statistically valid inferences about stream quality regionally and (2) to determine the relative importance of multiple stressors to aquatic ecosystem condition. To make statistically valid inferences about stream quality regionally, the first 50 MSQA sites were chosen from potential sites selected for the NRSA program using a Generalized Random Tessellation Stratified survey design and a sample frame based on stream segments from the National Hydrography Dataset Plus (NHDPlus) (U.S. Environmental Protection Agency, 2010; U.S. Environmental Protection Agency and the U.S. Geological Survey, 2012). To determine the relative importance of multiple stressors on aquatic ecosystem condition, 50 additional targeted sites were

selected to achieve full coverage of stressor gradients present in the region. Additional consideration was given to targeted sites with availability of other data such as active streamflow gages or historical water-quality or ecology data from the USGS and other programs.

The process for site selection included evaluation of stream and watershed characteristics derived from geospatial data sources. In particular, variables affecting fate and transport of contaminants or related to stream ecological condition were considered at the stream segment (Omernik, 1987; U.S. Environmental Protection Agency and the U.S. Geological Survey, 2005, 2012; Gronberg, 2012; McKay and others, 2012; and U.S. Environmental Protection Agency, 2013), riparian reach (Kuchler, 1964; Homer and others, 2015), and watershed (U.S. Department of Agriculture, 1995; U.S. Department of Agriculture National Agricultural Statistics Service, 2014; Wolock, 1997, 2003; Nakagaki and others, 2007; Maupin and Ivahnenko, 2011; Nakagaki and others, 2012; U.S. Environmental Protection Agency and the U.S. Geological Survey, 2012; Prism Climate Group, 2013; Robertson and Saad, 2013; Nowell and others, 2014; Baker and Stone, 2015; and Homer and others, 2015) scales. The “Selection of Random Sites” and “Selection of Targeted Sites” sections describe the methods used to achieve the dual-agency (USGS MSQA and EPA NRSA) goals. The “Description of Selected Network” section describes how well the full set of sites selected achieved the conflicting goals.

Geospatial Datasets

A geospatial database was developed for the MSQA containing stream characteristics for potential sites (for example, active streamflow gages) and for all NHDPlus reaches in the region. Characteristics for each potential stream site were determined for the 100-meter (m) riparian buffer for the individual stream reach, the upstream accumulated riparian area for the watershed, and the whole watershed. The MSQA geospatial database contained about 220,000 records (segments), with each record representing a potential sampling location in the study area. All stream segments within the study area were identified using the NHDPlus Version 1 Dataset (U.S. Environmental Protection Agency and the U.S. Geological Survey, 2005), with later computations also linked to NHDPlus Version 2 (McKay and others, 2012; U.S. Environmental Protection Agency and the U.S. Geological Survey, 2012). The NHDPlus integrates the National Hydrography Dataset with the National Elevation Dataset and the Watershed Boundary Dataset to produce a stream network based on medium resolution National Hydrography Dataset (1:100,000 scale) for the conterminous United States. The median length of stream segments in the NHDPlus was 1.5 kilometers, indicating the approximate spatial resolution of the watersheds relative to any location on a stream. Because the NRSA random site selection (see “Selection of Random Sites” section) also was done from the NHDPlus stream network, the MSQA database contains geospatial data for all

potential NRSA sites in the region, and for past USGS and State monitoring sites in the region.

Geospatial variables, such as agricultural intensity index (Aii) and natural vegetation in the riparian area, were considered for sites selected to represent a wide distribution of stressor gradients in the region. Select geospatial variables affecting fate and transport of agrochemicals or ecological condition, or both, are listed in table 1. Typically, a national grid for each type of geospatial data was overlaid on the NHDPlus stream network, and the geospatial data were processed by taking the mean of the grid cells that overlapped the relevant watershed area for each NHDPlus stream segment in the study area. Select key geospatial variables used in site selection are described in the following sections (see also “Site Selection” and “Selection of Targeted Sites” sections). Additional variables are described in appendix 1.

Agricultural Intensity Index

A watershed-scale Aii was based on the following two equally weighted attributes: the percentage of RC and the toxicity-weighted pesticide use (TWU) within the basin (fig. 2). The percentage of RC values consisted of the percentage of the watershed area composed of cultivated crops (category 82) from the 2011 National Land Cover Database (Homer and others, 2015; U.S. Geological Survey, 2014). The TWU was derived by estimating the agricultural use of individual pesticides in the basin, normalizing (dividing) each pesticide use estimate by the relative toxicity of that pesticide to cladocerans (an order of invertebrates commonly used in toxicity testing; for example, *Daphnia magna*), summing those toxicity-normalized use estimates for all pesticides applied in the basin, and dividing by basin area to express TWU as areal intensity. Agricultural use values were 2009 high county-level pesticide use estimates, in kilograms, from Stone (2013) and based on Thelin and Stone (2013), and relative toxicity values were median toxicity concentrations described by Nowell and others (2014).

The Aii was calculated as follows: percentage of RC was normalized to the maximum in the region and scaled to the 0–50 range, the logarithm of TWU was normalized to the maximum value in the region (because TWU is log-normally distributed) and scaled to the 0–50 range, and the two scaled values were summed to get an Aii that potentially ranges from 0 to 100. The Aii correlated strongly to percentage of RC and TWU. Mutually exclusive bins (ranges in Aii) were identified to represent very low agricultural intensity (Aii less than 25), low agricultural intensity (Aii of 25 to less than 50), medium agricultural intensity (Aii of 50 to less than 75), and high agricultural intensity (Aii of 75 and greater) (fig. 2).

The rationale for using the Aii was to determine a single index for agricultural sources of chemical stressors. Whereas percentage of RC tends to be high for basins where agrochemicals such as nutrients and herbicides are widely applied, the TWU tends to be high where pesticides are applied in large quantities or are highly toxic to invertebrates (such as insecticides), or both. The percentage of RC, therefore, reflects nutrient stressors and the TWU reflects pesticide stressors.

Table 1. Geospatial variables affecting fate and transport of agrochemicals and ecological condition.

[NHD, National Hydrography Dataset; MSQA, Midwest Stream Quality Assessment; NLCD, National Land Cover Database; ≤, less than or equal to; AAPFCO, Association of American Plant Food Control Officials; kg/km², kilograms per square kilometer; NPDES, National Pollutant Discharge Elimination System]

Variable	Description	Data source citation
Stream segment		
Ecoregion	Level III and IV Ecoregion that covers a majority of the drainage area	Omernik, 1987.
Segment length	Initial length used for buffering to create the riparian-zone boundaries was estimated based on NHD (Version 1) watershed area; ecological sample reach length based on average wetted width	U.S. Environmental Protection Agency and the U.S. Geological Survey, 2005; U.S. Environmental Protection Agency, 2013.
Strahler stream order	NHDPlus Version 1 and Version 2 Strahler Stream Order	U.S. Environmental Protection Agency and the U.S. Geological Survey, 2005; U.S. Environmental Protection Agency and the U.S. Geological Survey, 2012; McKay and others, 2012.
State	Maximizing the range of values covered for some important variables (for example, soil impervious layer, base-flow index, tile drains, and soil types) was accomplished by dispersing the final MSQA sites geographically across states in the region	Gronberg, 2012.
Riparian reach scale		
Natural vegetation	Percentage of NLCD 2011 sum of classes for forest, shrub, herbaceous, and wetlands, 100-meter riparian buffer only	Homer and others, 2015; Kuchler, 1964.
Row crop	Percentage of NLCD 2011 class cultivated crops, 100-meter riparian buffer in reach only	Homer and others, 2015.
Urban development	Percentage of NLCD 2011 sum of classes high-, medium-, and low-intensity developed, 100-meter riparian buffer of reach only	Homer and others, 2015.
Canopy	Percentage riparian canopy cover, 100-meter riparian buffer of reach only	Homer and others, 2015.
Watershed scale		
Base-flow index	Accumulated base-flow index, percentage of streamflow that is from base flow	Wolock, 2003.
Mean annual precipitation	Mean annual rainfall, during 33-year period (1981–2013)	Prism Climate Group, 2013.
Cumulative drainage area	Cumulative drainage area, accumulated for upstream reaches	U.S. Environmental Protection Agency and the U.S. Geological Survey, 2012.
Mean sand/silt/clay in soil	Accumulated mean percent of soil by size texture	Wolock, 1997.
Soil restrictive layer ≤ 25 centimeters	Accumulated fraction (percentage) of soils in basins with a soil restrictive layer ≤ 25 centimeters	Nakagaki and others, 2012.
Nitrogen and phosphorus loads from fertilizer/manure	Nitrogen and phosphorus from fertilizer and manure (initial estimates used during site selection from Census of Agriculture 1997 and AAPFCO, based on county-wide sales and percent 1992 agricultural land cover in watershed, kg/km ²)	U.S. Department of Agriculture National Agricultural Statistics Service, 1997; Nakagaki and others, 2007.
Nitrogen and phosphorus from NPDES facilities	Nitrogen and phosphorus loads to surface-water, accumulated for upstream reaches, in kg/km ² . Initial estimates used during site selection were based on 1997 and 2002 data, respectively	Robertson and Saad, 2013; Maupin and Ivahnenko, 2011.

Table 1. Geospatial variables affecting fate and transport of agrochemicals and ecological condition.—Continued

[NHD, National Hydrography Dataset; MSQA, Midwest Stream Quality Assessment; NLCD, National Land Cover Database; ≤, less than or equal to; AAPFCO, Association of American Plant Food Control Officials; kg/km², kilograms per square kilometer; NPDES, National Pollutant Discharge Elimination System]

Variable	Description	Data source citation
Watershed scale—Continued		
Urban	Accumulated percentage of NLCD 2011 class, developed—high, medium, and low intensity	Homer and others, 2015.
Row crop	Accumulated percentage of NLCD 2011 class, cultivated crops	Homer and others, 2015.
Agricultural intensity index	Based on percentage of row crops (%RC) and the toxicity-weighted pesticide use within the basin	Homer and others, 2015; Nowell and others, 2014; Baker and Stone, 2015.
Mean slope	The percent rise, or slope; accumulated for upstream reaches	U.S. Environmental Protection Agency and the U.S. Geological Survey, 2012.
Tile drainage	Accumulated area, in percent, of land that is under tile drainage	U.S. Department of Agriculture, 1995.

Agricultural intensity index

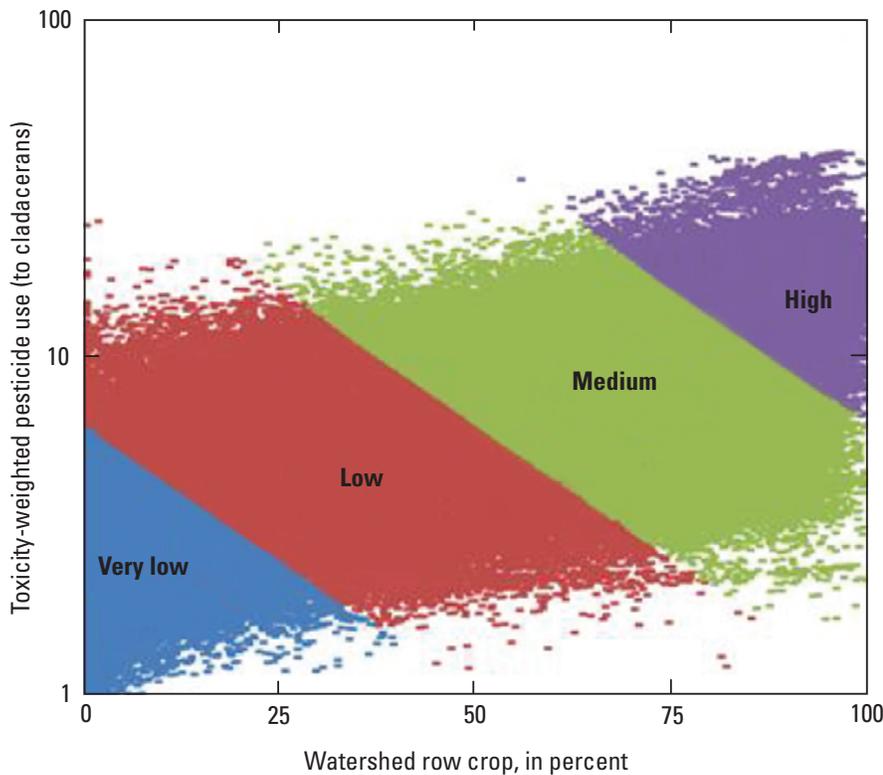


Figure 2. Percentage of row crop and area-normalized toxicity weighted pesticide use (towards cladocerans) in the watershed for all watersheds in the study area. These two parameters were used to calculate the agricultural intensity index (Aii) and four categories of Aii are identified.

Riparian Land Cover

Riparian land-cover characteristics were considered in addition to watershed characteristics in site selection. Riparian natural vegetation was determined by summing the following land-cover percentages within the 100-m riparian buffer zone: deciduous, evergreen, and mixed forest; shrub/scrubland; grassland; woody wetland; and emergent herbaceous wetland from the National Land Cover Database 2011 (categories 41–43, 52, 71, 90, 95). Similarly, land cover within the 100-m buffer zone was considered for urban and agricultural land-use categories such as imperviousness and cultivated crops.

Including riparian land cover in site selection criteria is important because riparian natural vegetation may mitigate the effects of agricultural stressors on ecological condition in streams. Riparian buffer vegetation has been determined to effectively reduce the transport of nutrients, pesticides, and suspended sediment by slowing overland flow carrying suspended sediment and attached pesticides and nutrients, especially phosphorus and total forms of nitrogen (Baker and others, 2006); enhancing denitrification (Cooper, 1990); and increasing uptake of nutrients by vegetation (Cooper, 1990; Jordan and others, 1993). Streambank erosion and soil loss in Iowa streams were greater for streams with riparian RC and pasture than for streams with riparian forest (Zaimes and others, 2006). In addition, riparian woodlands shade small streams, decreasing water temperature and photosynthesis; are an energy source to the base of the food chain (coarse organic particulate matter in the form of leaf litter); and create instream structure (fallen woody debris) that increases hydraulic complexity and provides stable habitat substrates for periphyton algae and invertebrate animals to colonize (Gurnell and others, 1995). Several studies have indicated that biological community condition improved with increasing riparian buffers or when agricultural land within the riparian buffer was taken out of production and (typically) planted with native grasses (Christensen and others, 2012).

Selection of Random Sites

The NRSA selected sites randomly from stratified groups of stream lines in the NHDPlus dataset, with stratification by stream category (table 2) and State. The NRSA stream categories with an “09” (Streams09, Rivers09) designation means the site was sampled by NRSA during the 2008–9 national survey; all other sites were newly selected for the 2013–14 survey. Rivers in the NRSA RiversMajor category included rivers fifth order and greater as described by Benke and Cushing (2005), and typically are nonwadeable; this category was not used for MSQA. Following the NRSA study design for 2013–14, 154 initially selected “base” sites that were on first- to fourth-order streams were within the MSQA area. A list of alternate or “over” sites also was selected for each stream category.

Fifty of the MSQA sites were selected from the 154 NRSA sites planned for the region. The random MSQA sites were selected so as to maintain the same proportions in

Table 2. Stream categories used by the National Rivers and Streams Assessment (NRSA) survey design for 2013–14.

[>, greater than]

Stream category	Strahler stream order	Description
Streams09	1–4	Streams sampled in 2008–9 NRSA.
SmallStreams	1–2	New small streams selected.
LargeStreams	3–4	New large streams selected.
Rivers09	>4	Rivers sampled in 2008–09 NRSA.
RiversMajor	>4	New major rivers selected.
RiversOther	>4	New additional rivers selected.

each State and stream category as the 154 NRSA sites in the region. For example, 50 MSQA random sites is 32 percent of the 154 NRSA sites in the region; therefore, of 8 Streams09 NRSA sites in Iowa, 3 sites (32 percent of 8 sites is 2.6) were selected for MSQA. Because the scope of the MSQA study included only wadeable streams (typically, less than about 2,000 km² in the Midwest), the NRSA base sites with stream order greater than five were excluded, with additional sites distributed to the small and large streams categories. That is, for each NRSA base site in the rivers categories with stream order greater than five, an alternate slot was assigned to either the small or large streams category.

The process for confirmation of candidates for the 50 random MSQA sites, overlapping the NRSA selection, evaluated site suitability for NRSA and MSQA objectives. Sites were evaluated for sampling, initially using online mapping tools and secondly with a field reconnaissance visit. Possible reasons for rejecting a random candidate site included lack of appropriate access, safety concerns, lack of permission from the landowner(s), or a likelihood of the stream going dry during the 14-week MSQA sampling period. If a site was rejected, it was replaced by the next alternate in the appropriate State and stream category, first from the base list, and then from the over list, following NRSA methods. In most cases, the difference in MSQA criteria and NRSA criteria was the requirement for flow through the 14-week sampling period, which led to a few streams in small watersheds being rejected for the MSQA even though the streams would have met NRSA criteria. The NRSA site suitability requirements are based on conditions on the single date of the ecological assessment, with a need for at least 50 percent of the reach to contain water but not necessarily flow.

Selection of Targeted Sites

These 50 targeted sites met one of the following four goals: (1) to represent urban land use in a metropolitan area within the region; (2) to represent reference or “least disturbed” conditions; (3) when combined with random sites, to more evenly cover agricultural stressor gradients in the study

area; or (4) to provide a temporal context for the 2013 study year. Geospatial data for stream site, reach, and watershed characteristics used for selection of targeted sites are described in the “Geospatial Datasets” section.

Because urbanization accounts for a relatively small portion of the area of the Midwest, the sites selected using the random design tend to have relatively little watershed urban land use (table 3). All 50 random MSQA sites had less than 8 percent developed urban (summing low, medium, and high density urban categories) and less than 14 percent total urban land use, which includes urban open space such as urban parks and golf courses. To represent urban streams, therefore, targeted sites were selected in moderately to heavily urbanized watersheds in the following seven metropolitan areas across the region: Chicago, Illinois; Cincinnati and Dayton, Ohio; Indianapolis, Indiana; Kansas City, Missouri, and Kansas City, Kansas; Milwaukee, Wisconsin; and Omaha, Nebraska. Residential and commercial settings were selected (areas of heavy industry were avoided), with a preference for recent urban development. Where possible, the two sites in a metropolitan area included one watershed that was undergoing urbanization (active conversion of agricultural lands to urban) and one that was relatively stable and fully developed. Targeting these urban sites was done to provide a detailed characterization of the chemistry, toxicity, and ecology of urban streams in the Midwest and to allow comparison to reference and agricultural settings in the region.

The remaining nonurban sites were selected on the basis of watershed and riparian land use and historical or concurrent water-quality monitoring or ecology monitoring, or both, for the site. Dispersing targeted sites geographically across the region was done to help ensure, along with the 50 random sites, wide ranges for other important variables; for example, soil impervious layer, base-flow index, tile drains, and soil type.

As a component of the nonurban targeted sites, reference sites were selected to represent “good” biological or “least disturbed” conditions. These sites were selected based on previous ecological sampling and geospatial screening and were distributed among three subregions—West, South, and East—each a single or combination of Level III Ecoregions (Omernik, 1987; Omernik, 1995). Sites with historical ecological survey data indicating good biological condition were identified based on suggestions from multiple agencies and sources, with relative ecological condition based on metrics such as MMI, EPT richness, and index of biological integrity (IBI). Additional candidate reference sites were identified using geospatial data. These geospatial candidate sites were perennial streams (based on watershed area or historical flow data) with relatively little urban development and low Aii in the watershed and with riparian areas with a low percentage of RC and high percentage of riparian natural vegetation. Other considerations in the selection of reference sites included avoidance of sites downstream from point sources or near large rivers or reservoirs, and positive weight was given

for sites with streamflow gages in protected areas and also for sites selected by the EPA as 2013 NRSA reference sites. Because the study area is dominated by RC agriculture, no pristine reference sites are available; thus, these sites should be considered “least developed” sites and not true reference sites.

Other targeted sites were selected to improve coverage of the agricultural intensity gradient, to include sites with extensive historical and temporal monitoring data, and to include sites of particular importance to other agencies and programs (table 3, site selection type of “other”). Three NAWQA “trend sites” were included that have been monitored frequently for more than 20 years to provide historical context to the 2013 MSQA sampling (South Fork Iowa River NE of New Providence, Iowa; Sugar Creek at Co Rd 400 S at New Palestine, Indiana; and Maple Creek near Nickerson, Nebraska). Sites also were selected to overlap with U.S. Department of Agriculture monitoring programs, which included the Natural Resources Conservation Service (NRCS) Mississippi River Basin Initiative, the NRCS National Water Quality Initiative, and the Agricultural Research Service.

Description of Selected Network

The 100 sites selected are distributed spatially across the study area and reasonably cover the agricultural land-use gradients and provide an indication of conditions in urban watersheds in the region (table 3, fig. 3). Land use within the 100 MSQA watersheds reflects the land use within the region, with the average watershed being 54 percent RC, 11 percent pasture and hay, 8 percent urban, and most of the remainder woodlands and grasslands. In spite of the difficulty in finding reference sites in the region, the 100 MSQA sites should be representative of stressors and ecological conditions for wadeable streams including some of the least developed or disturbed watersheds in the region.

Because of the MSQA requirement for flowing stream conditions throughout the index sampling period, climatic conditions during the study, particularly during site reconnaissance, had some effect on site selection. Relative to the climatic normals, rainfall was, on average, typical during the study, though parts of the study area reported antecedent drought conditions. Average rainfall was typical across the region during the index sampling period from May to early August 2013, with an average of 5 percent higher precipitation from May through July 2013 compared to long-term (1981–2013) averages for each sampling site (Prism Climate Group, 2013; appendix 1, table 3). Many sites had drought conditions in the preceding 12 months; however, from May 2012 through April 2013, precipitation was on average 14 percent less than the long-term site averages. Drought conditions were more severe (20 percent lower than average precipitation) and the shift to wetter conditions more prominent (index period was 10 percent wetter than the long-term average) in the western part of the MSQA region.

Table 3. Description of site, reach, and watershed characteristics of selected sites assessed as part of the U.S. Geological Survey (USGS) National Water-Quality Assessment (NAWQA) Midwest Stream Quality Assessment (MSQA) in 2013.

[NWIS, USGS National Water Information System database; latitude and longitude from NWIS based on North American Datum of 1983 (NAD 83); mi², square mile; m, meter; land use based on 2011; Aii, agricultural intensity index, which was not calculated for two sites selected just outside the initial study area; antecedent (5/2012 to 4/2013) and index period (5/2013 to 7/2013) precipitation are expressed as percentage (%) difference from long-term monthly means; MERC, mercury; INT, intensive contaminants monitoring; TPN, total particulate nitrogen; CNIT, continuous nitrate and biological response; AUS, micro-autosampler; WTOX, water toxicity; CFF, caged fish and frogs; SEDS, sediment source; --, not applicable; Ill., Illinois; Ind., Indiana; Ky, Kentucky; Wis., Wisconsin; Minn., Minnesota; Nebr., Nebraska; Mo, Missouri]

Map identifier (fig. 3)	Station name	NWIS station number	State	Subregion	Site selection type
OH_Massies	Massies Creek at Wilberforce, Ohio	03241500	Ohio	East	Reference—good
OH_Mill	Mill Creek at Carthage, Ohio	03259000	Ohio	East	Urban
OH_Wolf	Wolf Creek at Dayton, Ohio	03271000	Ohio	East	Urban
IL_MidVermilion	Middle Fork Vermilion River above Oakwood, Ill.	03336645	Illinois	South	Reference—good
IL_Spoon	Spoon River near St. Joseph, Ill.	03336890	Illinois	East	Random
IN_Raccoon	Big Raccoon Creek at Ferndale, Ind.	03340900	Indiana	South	Random
IN_Williams	Williams Creek at 96th Street, Indianapolis, Ind.	03351072	Indiana	East	Urban
IN_Fall	Fall Creek at 16th Street at Indianapolis, Ind.	03352875	Indiana	East	Urban
IN_Eagle	Eagle Creek at Zionsville, Ind.	03353200	Indiana	East	Other
KY_Richland	Richland Creek at Carbondale Road near Richland, Ky.	03383782	Kentucky	South	Random
IL_Lusk	Lusk Creek near Eddyville, Ill.	03384450	Illinois	South	Reference—good
IL_Massac.WQ	Massac Creek at Metropolis, Ill.	03611200	Illinois	South	Random
WI_Tisch	Tisch Mills Creek at Tisch Mills, Wis.	040852508	Wisconsin	East	Reference—good
WI_Otter	Otter Creek at Willow Road near Plymouth, Wis.	040857005	Wisconsin	East	Other
WI_Lincoln	Lincoln Creek at Sherman Boulevard at Milwaukee, Wis.	040869416	Wisconsin	East	Urban
WI_HoneyWau	Honey Creek at Wauwatosa, Wis.	04087119	Wisconsin	East	Urban
MN_Florida	Florida Creek at 171st Avenue near Marietta, Minn.	05299770	Minnesota	West	Reference—geospatial
MN_Threemile	Threemile Creek at 210th Avenue near Ghent, Minn.	05315295	Minnesota	West	Random
MN_WBeaver	West Fork Beaver Creek at 320 Street near Bechyn, Minn.	0531656290	Minnesota	West	Other
MN_Maple	Maple River at Highway 30 near Mapleton, Minn.	05320410	Minnesota	West	Random

Table 3. Description of site, reach, and watershed characteristics of selected sites assessed as part of the U.S. Geological Survey (USGS) National Water-Quality Assessment (NAWQA) Midwest Stream Quality Assessment (MSQA) in 2013–Continued.

[NWIS, USGS National Water Information System database; latitude and longitude from NWIS based on North American Datum of 1983 (NAD 83); mi², square mile; m, meter; land use based on 2011; Aii, agricultural intensity index, which was not calculated for two sites selected just outside the initial study area; antecedent (5/2012 to 4/2013) and index period (5/2013 to 7/2013) precipitation are expressed as percentage (%) difference from long-term monthly means; MERC, mercury; INT, intensive contaminants monitoring; TPN, total particulate nitrogen; CNIT, continuous nitrate and biological response; AUS, micro-autosampler; WTOX, water toxicity; CFF, caged fish and frogs; SEDS, sediment source; --, not applicable; Ill., Illinois; Ind., Indiana; Ky, Kentucky; Wis., Wisconsin; Minn., Minnesota; Nebr., Nebraska; Mo, Missouri]

Map identifier (fig. 3)	Level III Ecoregion name	Latitude	Longitude	Drainage area (mi ²)	Sampled reach length (m)	Land use (percentage)				Aii
						Developed/ Urban	Urban	Forest	Cultivated crops	
OH_Massies	Eastern Corn Belt Plains	39.72236	-83.8822	63	480	1.38	6.0	4.8	83.3	72.7
OH_Mill	Eastern Corn Belt Plains	39.20199	-84.47115	115	600	50.83	80.4	13.7	3.2	8.8
OH_Wolf	Eastern Corn Belt Plains	39.76667	-84.23667	69	600	22.43	40.9	9.0	38.3	45.8
IL_MidVermilion	Interior River Valleys and Hills	40.13694	-87.74583	432	1,200	2.78	6.6	5.2	84.9	79.7
IL_Spoon	Central Corn Belt Plains	40.16417	-88.02750	41	220	1.99	7.1	0.1	91.7	84.0
IN_Raccoon	Interior River Valleys and Hills	39.71115	-87.07128	222	760	0.95	5.8	13.0	72.8	69.2
IN_Williams	Eastern Corn Belt Plains	39.92694	-86.17222	16	360	36.77	62.3	4.5	16.7	30.4
IN_Fall	Eastern Corn Belt Plains	39.78889	-86.17778	317	800	18.09	31.9	6.5	55.4	60.4
IN_Eagle	Eastern Corn Belt Plains	39.94639	-86.26028	106	600	5.49	14.5	5.5	69.9	69.4
KY_Richland	Interior River Valleys and Hills	37.26634	-87.60116	12	160	0.70	2.3	66.8	8.2	20.8
IL_Lusk	Interior Plateau	37.47259	-88.54769	43	320	0.50	3.1	82.1	2.9	--
IL_Massac.WQ	Interior River Valleys and Hills	37.19160	-88.70881	38	280	1.01	7.1	36.2	15.4	30.9
WI_Tisch	Southeastern Wisconsin Till Plains	44.32754	-87.63675	16	280	2.60	5.0	9.0	17.9	34.1
WI_Otter	Southeastern Wisconsin Till Plains	43.78889	-87.92139	10	150	4.52	6.7	16.6	42.6	49.9
WI_Lincoln	Southeastern Wisconsin Till Plains	43.09714	-87.96725	13	360	81.20	93.8	1.9	0.0	1.3
WI_HoneyWau	Southeastern Wisconsin Till Plains	43.04383	-88.00511	10	180	88.31	99.6	0.3	0.0	0.0
MN_Florida	Western Corn Belt Plains	44.90222	-96.30928	116	160	0.10	3.9	0.9	54.1	64.1
MN_Threemile	Western Corn Belt Plains	44.49025	-95.87744	63	150	0.39	4.5	0.8	76.0	78.6
MN_WBeaver	Western Corn Belt Plains	44.69041	-95.03530	92	240	0.90	5.5	1.1	84.9	90.4
MN_Maple	Western Corn Belt Plains	43.90733	-94.04094	195	800	1.20	6.3	0.6	87.9	85.5

Table 3. Description of site, reach, and watershed characteristics of selected sites assessed as part of the U.S. Geological Survey (USGS) National Water-Quality Assessment (NAWQA) Midwest Stream Quality Assessment (MSQA) in 2013.—Continued

[NWIS, USGS National Water Information System database; latitude and longitude from NWIS based on North American Datum of 1983 (NAD 83); mi², square mile; m, meter; land use based on 2011; Aii, agricultural intensity index, which was not calculated for two sites selected just outside the initial study area; antecedent (5/2012 to 4/2013) and index period (5/2013 to 7/2013) precipitation are expressed as percentage (%) difference from long-term monthly means; MERC, mercury; INT, intensive contaminants monitoring; TPN, total particulate nitrogen; CNIT, continuous nitrate and biological response; AUS, micro-autosampler; WTOX, water toxicity; CFF, caged fish and frogs; SEDS, sediment source; --, not applicable; Ill., Illinois; Ind., Indiana; Ky, Kentucky; Wis., Wisconsin; Minn., Minnesota; Nebr., Nebraska; Mo, Missouri]

Map identifier (fig. 3)	Precipitation			Gaged	MSQA subset components and special studies							
	Long-term annual mean, 1981–2013	Antecedent, % difference	Index period, % difference		MERC	INT	TPN	CNIT	AUS	WTOX	CFF	SEDS
OH_Massies	1,030	-13.6	5.2	Yes	Yes	Yes	--	--	--	--	--	--
OH_Mill	1,053	-9.0	6.0	Yes	--	Yes	--	--	--	--	--	--
OH_Wolf	1,027	-13.6	-7.4	Yes	--	Yes	--	--	--	--	--	--
IL_MidVermilion	972	-0.8	-7.7	Yes	Yes	--	--	--	--	--	--	--
IL_Spoon	991	2.0	-5.8	No	Yes	--	--	--	--	--	--	--
IN_Raccoon	1,085	5.6	-16.8	No	Yes	--	--	--	--	--	--	--
IN_Williams	1,066	4.8	-19.7	Yes	--	Yes	--	--	--	--	--	--
IN_Fall	1,075	3.5	-25.7	Yes	--	Yes	--	--	--	--	--	--
IN_Eagle	1,062	5.4	-16.7	Yes	--	Yes	--	Yes	Yes	Yes	--	--
KY_Richland	1,252	-3.2	22.2	No	--	--	--	--	--	--	--	--
IL_Lusk	1,254	-14.2	-3.4	Yes	Yes	--	--	Yes	--	--	--	--
IL_Massac.WQ	1,243	-15.9	11.0	No	Yes	--	--	--	--	--	--	--
WI_Tisch	786	7.0	-10.0	No	Yes	Yes	--	--	--	--	--	--
WI_Otter	866	4.9	1.3	Yes	--	Yes	--	--	--	--	--	--
WI_Lincoln	842	10.3	27.6	Yes	--	Yes	--	--	Yes	Yes	--	--
WI_HoneyWau	878	7.9	24.8	Yes	--	Yes	--	--	Yes	Yes	--	--
MN_Florida	665	-21.4	22.8	No	Yes	--	--	--	--	--	--	--
MN_Threemile	693	-9.9	-3.9	No	Yes	--	--	--	--	--	--	--
MN_WBeaver	711	-12.7	0.0	No	--	Yes	--	--	--	--	--	--
MN_Maple	820	-18.7	28.1	No	Yes	--	--	--	--	--	--	--

Table 3. Description of site, reach, and watershed characteristics of selected sites assessed as part of the U.S. Geological Survey (USGS) National Water-Quality Assessment (NAWQA) Midwest Stream Quality Assessment (MSQA) in 2013.—Continued

[NWIS, USGS National Water Information System database; latitude and longitude from NWIS based on North American Datum of 1983 (NAD 83); mi², square mile; m, meter; land use based on 2011; Aii, agricultural intensity index, which was not calculated for two sites selected just outside the initial study area; antecedent (5/2012 to 4/2013) and index period (5/2013 to 7/2013) precipitation are expressed as percentage (%) difference from long-term monthly means; MERC, mercury; INT, intensive contaminants monitoring; TPN, total particulate nitrogen; CNIT, continuous nitrate and biological response; AUS, micro-autosampler; WTOX, water toxicity; CFF, caged fish and frogs; SEDS, sediment source; --, not applicable; Ill., Illinois; Ind., Indiana; Ky, Kentucky; Wis., Wisconsin; Minn., Minnesota; Nebr., Nebraska; Mo, Missouri]

Map identifier (fig. 3)	Station name	NWIS station number	State	Subregion	Site selection type
MN_LeSueur	Le Sueur River near Rapidan, Minn.	05320500	Minnesota	West	Other
MN_Sevenmile	Sevenmile Creek below footbridge in Park near Kasota, Minn.	05325148	Minnesota	West	Other
IA_Johns	Johns Creek near Worthington, Iowa	05418180	Iowa	West	Random
IA_Maquoketa	North Fork Maquoketa River near Fulton, Iowa	05418400	Iowa	West	Other
IA_Wapsipinicon	Wapsipinicon River at McIntire, Iowa	05420520	Iowa	West	Reference—good
WI_Scuppernong	Scuppernong River near Palmyra, Wis.	05426400	Wisconsin	East	Reference—geospatial
IA_IowaH	South Fork Iowa River at H Avenue near Buckeye, Iowa	05451112	Iowa	West	Random
IA_IowaProv	South Fork Iowa River at NE of New Providence, Iowa	05451210	Iowa	West	Other
IA_OldMan	Old Mans Creek at Kansas Avenue SW near Iowa City, Iowa	05455095	Iowa	West	Random
MN_Cedar	Cedar River at 100th Street near Lyle, Minn.	05457200	Minnesota	West	Random
IA_Cedar	Cedar River at Lancer Avenue at Osage, Iowa	05457520	Iowa	West	Random
IA_Maynes	Maynes Creek near Hampton, Iowa	05458800	Iowa	West	Reference—good
IA_Wolf	Wolf Creek near Dysart, Iowa	05464220	Iowa	West	Random
IA_Indian	Unnamed Tributary to East Branch Indian Creek near Zearing, Iowa	05471090	Iowa	West	Random
IA_BeaverB	Beaver Creek at Bouton, Iowa	05481820	Iowa	West	Random
IA_NRaccoon	North Raccoon River near Sac City, Iowa	05482300	Iowa	West	Other
IA_BeaverG	Beaver Creek at Glendon, Iowa	05483341	Iowa	West	Random
IA_Raccoon	Middle Raccoon River near Bayard, Iowa	05483450	Iowa	West	Reference—geospatial
IA_Walnut	Walnut Creek near Vandalia, Iowa	05487550	Iowa	West	Other
IN_Yellow	Yellow River at Knox, Ind.	05517000	Indiana	East	Other

Table 3. Description of site, reach, and watershed characteristics of selected sites assessed as part of the U.S. Geological Survey (USGS) National Water-Quality Assessment (NAWQA) Midwest Stream Quality Assessment (MSQA) in 2013–Continued.

[NWIS, USGS National Water Information System database; latitude and longitude from NWIS based on North American Datum of 1983 (NAD 83); mi², square mile; m, meter; land use based on 2011; Aii, agricultural intensity index, which was not calculated for two sites selected just outside the initial study area; antecedent (5/2012 to 4/2013) and index period (5/2013 to 7/2013) precipitation are expressed as percentage (%) difference from long-term monthly means; MERC, mercury; INT, intensive contaminants monitoring; TPN, total particulate nitrogen; CNIT, continuous nitrate and biological response; AUS, micro-autosampler; WTOX, water toxicity; CFF, caged fish and frogs; SEDS, sediment source; --, not applicable; Ill., Illinois; Ind., Indiana; Ky, Kentucky; Wis., Wisconsin; Minn., Minnesota; Nebr., Nebraska; Mo, Missouri]

Map identifier (fig. 3)	Level III Ecoregion name	Latitude	Longitude	Drainage area (mi ²)	Sampled reach length (m)	Land use (percentage)				Aii
						Developed/ Urban	Urban	Forest	Cultivated crops	
MN_LeSueur	Western Corn Belt Plains	44.10972	-94.04167	1,110	--	1.48	6.7	1.4	82.5	82.9
MN_Sevenmile	Western Corn Belt Plains	44.26223	-94.02907	36	150	0.63	4.7	3.4	83.3	81.6
IA_Johns	Western Corn Belt Plains	42.39717	91.06225	7	200	3.07	7.4	0.6	84.6	70.5
IA_Maquoketa	Western Corn Belt Plains	42.16433	-90.72933	505	1,200	1.89	6.0	10.2	59.1	55.7
IA_Wapsipinicon	Western Corn Belt Plains	43.44328	-92.61125	29	280	0.81	5.4	2.7	84.6	81.6
WI_Scuppernong	Southeastern Wisconsin Till Plains	42.88611	-88.54139	25	320	0.98	3.7	28.5	26.4	34.8
IA_IowaH	Western Corn Belt Plains	42.43617	-93.34675	102	600	1.48	6.9	0.4	90.4	82.7
IA_IowaProv	Western Corn Belt Plains	42.31508	-93.15206	224	600	1.15	6.7	1.9	85.7	79.9
IA_OldMan	Western Corn Belt Plains	41.60839	-91.63844	193	520	2.61	7.1	2.7	63.0	58.9
MN_Cedar	Western Corn Belt Plains	43.51422	-93.00286	543	1,280	2.92	9.1	1.4	80.7	78.6
IA_Cedar	Western Corn Belt Plains	43.25372	-92.81203	862	2,920	2.57	8.4	1.5	81.8	79.5
IA_Maynes	Western Corn Belt Plains	42.68083	-93.20325	71	600	1.14	6.2	2.0	87.5	82.8
IA_Wolf	Western Corn Belt Plains	42.25153	-92.29889	299	800	1.18	6.6	1.3	86.6	80.8
IA_Indian	Western Corn Belt Plains	42.10661	-93.35081	8	150	0.42	7.0	0.3	92.0	84.4
IA_BeaverB	Western Corn Belt Plains	41.84925	-94.02828	203	440	1.38	7.3	0.1	87.9	81.4
IA_NRaccoon	Western Corn Belt Plains	42.35475	-94.99033	700	880	1.71	7.2	0.3	87.4	85.9
IA_BeaverG	Western Corn Belt Plains	41.58925	-94.40161	28	440	0.47	3.3	20.4	26.8	43.0
IA_Raccoon	Western Corn Belt Plains	41.77908	-94.49286	375	880	2.02	7.6	2.3	80.5	75.5
IA_Walnut	Western Corn Belt Plains	41.53703	-93.25897	20	240	1.14	5.1	2.5	64.9	65.4
IN_Yellow	Central Corn Belt Plains	41.30278	-86.62056	435	1,200	3.39	9.1	11.6	70.3	71.1

Table 3. Description of site, reach, and watershed characteristics of selected sites assessed as part of the U.S. Geological Survey (USGS) National Water-Quality Assessment (NAWQA) Midwest Stream Quality Assessment (MSQA) in 2013.—Continued

[NWIS, USGS National Water Information System database; latitude and longitude from NWIS based on North American Datum of 1983 (NAD 83); mi², square mile; m, meter; land use based on 2011; Aii, agricultural intensity index, which was not calculated for two sites selected just outside the initial study area; antecedent (5/2012 to 4/2013) and index period (5/2013 to 7/2013) precipitation are expressed as percentage (%) difference from long-term monthly means; MERC, mercury; INT, intensive contaminants monitoring; TPN, total particulate nitrogen; CNIT, continuous nitrate and biological response; AUS, micro-autosampler; WTOX, water toxicity; CFF, caged fish and frogs; SEDS, sediment source; --, not applicable; Ill., Illinois; Ind., Indiana; Ky, Kentucky; Wis., Wisconsin; Minn., Minnesota; Nebr., Nebraska; Mo, Missouri]

Map identifier (fig. 3)	Station name	NWIS station number	State	Subregion	Site selection type
IL_Salt	Salt Creek at Western Springs, Ill.	05531500	Illinois	East	Urban
WI_HoneyDD	Honey Creek at County Highway DD near Burlington, Wis.	05544989	Wisconsin	East	Random
IL_Poplar	Poplar Creek at Elgin, Ill.	05550500	Illinois	East	Urban
IL_Indian	Indian Creek near Fairbury, Ill.	05554300	Illinois	East	Other
IL_Sangamon	Sangamon River at Monticello, Ill.	05572000	Illinois	East	Other
IL_Clear	Clear Creek near Jeisyville, Ill.	05575550	Illinois	East	Random
IL_Becks	Beck Creek at Herrick, Ill.	05592195	Illinois	South	Random
IL_Galum	Galum Creek near Pyatts, Ill.	05599100	Illinois	South	Random
NE_Papillion	Little Papillion Creek at Ak-Sar-Ben at Omaha, Nebr.	06610765	Nebraska	West	Urban
NE_WPapillion	West Papillion Creek at Millard, Nebr.	06610785	Nebraska	West	Urban
NE_Maple	Maple Creek near Nickerson, Nebr.	06800000	Nebraska	West	Other
NE_Wahoo	Wahoo Creek at Ithaca, Nebr.	06804000	Nebraska	West	Random
IA_Nishnabotna	Unnamed Tributary to West Nishnabotna River near Randolph, Iowa	06808495	Iowa	West	Random
KS_Tomahawk	Tomahawk Creek near Overland Park, Kans.	06893350	Kansas	West	Urban
MO_Rock	Rock Creek at Kentucky Road in Independence, Mo.	06893620	Missouri	West	Urban
IA_Long	Long Creek at 137th Street near Van Wert, Iowa	06897937	Iowa	South	Reference—good
KY_Caney	Caney Creek near Caneyville, Ky.	372629086330400	Kentucky	South	Random
KY_Pond	Pond Run at Highway 110 near Falls of Rough, Ky.	373514086371200	Kentucky	South	Reference—good
MO_Moreau	North Moreau Creek near Jefferson City, Mo.	383158092192001	Missouri	South	Reference—good
IL_Allison	Allison Ditch near Vincennes, Ind.	384224087353601	Illinois	South	Random

Table 3. Description of site, reach, and watershed characteristics of selected sites assessed as part of the U.S. Geological Survey (USGS) National Water-Quality Assessment (NAWQA) Midwest Stream Quality Assessment (MSQA) in 2013–Continued.

[NWIS, USGS National Water Information System database; latitude and longitude from NWIS based on North American Datum of 1983 (NAD 83); mi², square mile; m, meter; land use based on 2011; Aii, agricultural intensity index, which was not calculated for two sites selected just outside the initial study area; antecedent (5/2012 to 4/2013) and index period (5/2013 to 7/2013) precipitation are expressed as percentage (%) difference from long-term monthly means; MERC, mercury; INT, intensive contaminants monitoring; TPN, total particulate nitrogen; CNIT, continuous nitrate and biological response; AUS, micro-autosampler; WTOX, water toxicity; CFF, caged fish and frogs; SEDS, sediment source; --, not applicable; Ill., Illinois; Ind., Indiana; Ky, Kentucky; Wis., Wisconsin; Minn., Minnesota; Nebr., Nebraska; Mo, Missouri]

Map identifier (fig. 3)	Level III Ecoregion name	Latitude	Longitude	Drainage area (mi ²)	Sampled reach length (m)	Land use (percentage)				Aii
						Developed/ Urban	Urban	Forest	Cultivated crops	
IL_Salt	Central Corn Belt Plains	41.82583	-87.90028	115	980	74.39	91.7	3.4	0.2	0.6
WI_HoneyDD	Southeastern Wisconsin Till Plains	42.71950	-88.30944	24	360	2.91	5.7	18.5	54.4	62.3
IL_Poplar	Central Corn Belt Plains	42.02611	-88.25556	35	340	54.33	69.2	8.5	5.4	11.5
IL_Indian	Central Corn Belt Plains	40.72278	-88.53000	68	480	2.10	5.4	1.7	91.9	83.9
IL_Sangamon	Central Corn Belt Plains	40.03083	-88.58889	550	880	3.95	8.5	2.4	86.5	81.1
IL_Clear	Central Corn Belt Plains	39.54639	-89.43500	14	320	1.27	4.3	0.4	95.3	85.0
IL_Becks	Interior River Valleys and Hills	39.21556	-89.02056	97	400	2.97	9.3	24.1	48.7	54.5
IL_Galum	Interior River Valleys and Hills	37.94444	-89.37972	162	480	2.43	6.3	17.2	38.0	43.5
NE_Papillion	Western Corn Belt Plains	41.24528	-96.02028	50	440	37.14	47.4	1.9	31.1	24.8
NE_WPapillion	Western Corn Belt Plains	41.20736	-96.12761	59	400	40.11	53.5	1.2	35.0	34.4
NE_Maple	Western Corn Belt Plains	41.56117	-96.54083	368	520	0.62	4.6	1.7	81.0	69.2
NE_Wahoo	Western Corn Belt Plains	41.14750	-96.53778	273	320	1.24	5.2	2.5	74.7	56.9
IA_Nishnabotna	Western Corn Belt Plains	40.87350	-95.59458	5	150	2.65	9.1	3.5	80.2	70.1
KS_Tomahawk	Central Irregular Plains	38.90611	-94.64000	21	400	64.07	86.3	2.8	3.6	--
MO_Rock	Western Corn Belt Plains	39.11194	-94.47222	10	340	66.60	94.2	4.8	0.1	1.4
IA_Long	Central Irregular Plains	40.83814	-93.85717	98	640	1.21	4.4	17.2	26.6	41.6
KY_Caney	Interior River Valleys and Hills	37.44129	-86.55121	72	480	0.78	4.5	59.6	3.9	16.5
KY_Pond	Interior River Valleys and Hills	37.58501	-86.61873	5	150	0.04	2.0	81.9	3.8	8.9
MO_Moreau	Interior River Valleys and Hills	38.53564	-92.31893	336	520	2.04	6.6	21.2	12.4	17.5
IL_Allison	Interior River Valleys and Hills	38.71777	-87.59435	14	320	1.07	5.0	8.8	78.5	68.8

Table 3. Description of site, reach, and watershed characteristics of selected sites assessed as part of the U.S. Geological Survey (USGS) National Water-Quality Assessment (NAWQA) Midwest Stream Quality Assessment (MSQA) in 2013.—Continued

[NWIS, USGS National Water Information System database; latitude and longitude from NWIS based on North American Datum of 1983 (NAD 83); mi², square mile; m, meter; land use based on 2011; Aii, agricultural intensity index, which was not calculated for two sites selected just outside the initial study area; antecedent (5/2012 to 4/2013) and index period (5/2013 to 7/2013) precipitation are expressed as percentage (%) difference from long-term monthly means; MERC, mercury; INT, intensive contaminants monitoring; TPN, total particulate nitrogen; CNIT, continuous nitrate and biological response; AUS, micro-autosampler; WTOX, water toxicity; CFF, caged fish and frogs; SEDS, sediment source; --, not applicable; Ill., Illinois; Ind., Indiana; Ky, Kentucky; Wis., Wisconsin; Minn., Minnesota; Nebr., Nebraska; Mo, Missouri]

Map identifier (fig. 3)	Precipitation			Gaged	MSQA subset components and special studies							
	Long-term annual mean, 1981–2013	Antecedent, % difference	Index period, % difference		MERC	INT	TPN	CNIT	AUS	WTOX	CFF	SEDS
IL_Salt	953	-3.4	-0.5	Yes	--	Yes	--	--	--	--	--	--
WI_HoneyDD	877	2.0	25.9	No	Yes	--	--	--	--	--	--	--
IL_Poplar	951	-7.2	0.3	Yes	--	Yes	--	--	--	--	--	--
IL_Indian	906	-3.6	-5.3	Yes	--	Yes	--	--	--	--	--	--
IL_Sangamon	980	-0.9	0.4	Yes	--	Yes	--	--	--	--	--	--
IL_Clear	968	-3.5	22.2	No	Yes	--	--	--	--	--	--	--
IL_Becks	1,040	2.7	17.7	No	Yes	--	--	--	--	--	--	--
IL_Galum	1,123	-16.4	16.3	No	Yes	--	--	--	--	--	--	--
NE_Papillion	773	-27.3	-16.6	Yes	--	Yes	Yes	--	--	Yes	--	--
NE_WPapillion	771	-28.5	-12.2	Yes	--	Yes	Yes	--	--	--	--	--
NE_Maple	738	-36.1	-11.7	Yes	--	Yes	Yes	--	--	Yes	--	--
NE_Wahoo	778	-32.4	-10.6	Yes	Yes	--	Yes	--	--	--	--	--
IA_Nishnabotna	840	-27.8	-8.0	No	Yes	--	Yes	--	--	--	--	--
KS_Tomahawk	1,032	-24.0	-13.2	Yes	--	Yes	Yes	--	--	--	--	--
MO_Rock	1,079	-22.3	-12.0	Yes	--	Yes	--	--	--	--	--	--
IA_Long	919	-17.9	-10.3	No	Yes	--	Yes	--	--	--	--	--
KY_Caney	1,322	-6.7	22.9	Yes	--	--	--	--	--	--	--	--
KY_Pond	1,262	-7.9	12.6	No	--	--	--	--	--	--	--	--
MO_Moreau	1,105	-24.6	-12.5	No	Yes	Yes	--	--	--	--	Yes	--
IL_Allison	1,167	-11.2	40.3	No	Yes	--	--	--	--	--	--	--

Table 3. Description of site, reach, and watershed characteristics of selected sites assessed as part of the U.S. Geological Survey (USGS) National Water-Quality Assessment (NAWQA) Midwest Stream Quality Assessment (MSQA) in 2013.—Continued

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Map identifier (fig. 3)	Station name	NWIS station number	State	Subregion	Site selection type
IN_Prairie	Prairie Creek at County Road N 100 W near Capehart, Ind.	384240087103901	Indiana	South	Random
MO_Loutre	Loutre River near Montgomery City, Mo.	385638091364601	Missouri	South	Random
IL_Wabash	Little Wabash River near Mason, Ill.	385730088324401	Illinois	South	Random
IN_Otter1	Otter Creek at N County Road 560 E near Butlerville, Ind.	390033085300301	Indiana	East	Reference—good
MO_Moniteau	Moniteau Creek near Rocheport, Mo.	390200092341701	Missouri	South	Reference—geospatial
MO_Perche	Perche Creek near Columbia, Mo.	390227092234101	Missouri	South	Reference—geospatial
IN_Busseron	West Fork Busseron Creek at State Route 48 near Wilfred, Ind.	391110087194401	Indiana	South	Other
IN_Otter2	Otter Creek at W County Road 750 N near Napoleon, Ind.	391114085205801	Indiana	East	Random
IL_Cole	Cole Creek near Hardin, Ill.	391136090341101	Illinois	South	Random
MO_Skull	Skull Lick Creek near Mexico, Mo.	391308091550901	Missouri	South	Random
MO_Fish	Fish Branch near Mexico, Mo.	391443091534001	Missouri	South	Other
MO_Bear	Bear Creek near Gilliam, Mo.	391504093003301	Missouri	West	Random
IL_Mill	Mill Creek near Choctaw, Ill.	391601087414801	Illinois	South	Reference—geospatial
MO_Goodwater	Goodwater Creek near Centralia, Mo.	391815009203901	Missouri	South	Other
IN_Nineveh	Nineveh Creek at Stone Arch Road near Nineveh, Ind.	392158086035901	Indiana	East	Reference—geospatial
KS_French	French Creek at Parallel Road, Onaga, Kans.	393358096130000	Kansas	West	Reference—good
MO_Contrary	Contrary Creek near St. Joseph, Mo.	394151094531501	Missouri	West	Random
IN_Lick	Lick Creek at N County Road 250 W near Harrisburg, Ind.	394253085111101	Indiana	East	Random
MO_Brushy	Brushy Creek near Cameron, Mo.	394254094092301	Missouri	South	Random
KS_Muddy	Muddy Creek at 145 th Street near Wetmore, Kans.	394306095484300	Kansas	West	Random

Table 3. Description of site, reach, and watershed characteristics of selected sites assessed as part of the U.S. Geological Survey (USGS) National Water-Quality Assessment (NAWQA) Midwest Stream Quality Assessment (MSQA) in 2013–Continued.

[NWIS, USGS National Water Information System database; latitude and longitude from NWIS based on North American Datum of 1983 (NAD 83); mi², square mile; m, meter; land use based on 2011; Aii, agricultural intensity index, which was not calculated for two sites selected just outside the initial study area; antecedent (5/2012 to 4/2013) and index period (5/2013 to 7/2013) precipitation are expressed as percentage (%) difference from long-term monthly means; MERC, mercury; INT, intensive contaminants monitoring; TPN, total particulate nitrogen; CNIT, continuous nitrate and biological response; AUS, micro-autosampler; WTOX, water toxicity; CFF, caged fish and frogs; SEDS, sediment source; --, not applicable; Ill., Illinois; Ind., Indiana; Ky, Kentucky; Wis., Wisconsin; Minn., Minnesota; Nebr., Nebraska; Mo, Missouri]

Map identifier (fig. 3)	Level III Ecoregion name	Latitude	Longitude	Drainage area (mi ²)	Sampled reach length (m)	Land use (percentage)				Aii
						Developed/ Urban	Urban	Forest	Cultivated crops	
IN_Prairie	Interior River Valleys and Hills	38.70952	-87.18541	123	440	1.12	6.6	10.5	66.3	63.1
MO_Loutre	Interior River Valleys and Hills	38.94376	-91.61281	117	280	0.69	4.1	32.9	27.0	33.3
IL_Wabash	Interior River Valleys and Hills	38.95833	-88.54556	475	480	4.13	11.1	19.1	58.7	59.1
IN_Otter1	Eastern Corn Belt Plains	39.00915	-85.50075	62	800	0.61	3.4	54.5	34.6	42.2
MO_Moniteau	Interior River Valleys and Hills	39.03325	-92.57112	126	320	0.43	4.8	40.8	13.2	21.8
MO_Perche	Interior River Valleys and Hills	39.04129	-92.39483	178	240	1.06	5.1	47.8	11.7	33.9
IN_Busseron	Interior River Valleys and Hills	39.18633	-87.32920	14	150	1.57	5.8	17.4	62.8	55.5
IN_Otter2	Eastern Corn Belt Plains	39.18431	-85.35087	1	150	0.65	4.6	19.3	74.5	59.3
IL_Cole	Interior River Valleys and Hills	39.19333	-90.56972	11	240	1.11	4.2	43.4	35.6	46.5
MO_Skull	Central Irregular Plains	39.21886	-91.91906	29	240	1.13	4.5	19.6	49.1	49.0
MO_Fish	Central Irregular Plains	39.24537	-91.89452	17	150	0.22	2.8	2.8	77.1	55.8
MO_Bear	Western Corn Belt Plains	39.25114	-93.00928	8	150	6.66	13.8	13.9	57.0	52.8
IL_Mill	Interior River Valleys and Hills	39.26694	-87.69667	104	320	2.24	6.8	32.2	53.6	52.8
MO_Goodwater	Central Irregular Plains	39.30429	-92.05239	28	180	3.44	7.9	3.8	66.9	58.9
IN_Nineveh	Eastern Corn Belt Plains	39.36611	-86.06641	6	200	0.10	4.0	35.4	31.7	34.9
KS_French	Western Corn Belt Plains	39.56611	-96.21669	28	280	0.43	3.6	11.0	36.3	30.9
MO_Contrary	Western Corn Belt Plains	39.69756	-94.88753	26	150	1.02	5.8	29.1	36.1	35.0
IN_Lick	Eastern Corn Belt Plains	39.71435	-85.18515	3	200	0.16	5.2	13.2	79.6	72.5
MO_Brushy	Central Irregular Plains	39.71513	-94.15640	36	200	7.27	12.1	11.8	31.9	26.1
KS_Muddy	Western Corn Belt Plains	39.71835	-95.81182	25	200	0.30	4.1	7.9	33.2	34.8

Table 3. Description of site, reach, and watershed characteristics of selected sites assessed as part of the U.S. Geological Survey (USGS) National Water-Quality Assessment (NAWQA) Midwest Stream Quality Assessment (MSQA) in 2013.—Continued

[NWIS, USGS National Water Information System database; latitude and longitude from NWIS based on North American Datum of 1983 (NAD 83); mi², square mile; m, meter; land use based on 2011; Aii, agricultural intensity index, which was not calculated for two sites selected just outside the initial study area; antecedent (5/2012 to 4/2013) and index period (5/2013 to 7/2013) precipitation are expressed as percentage (%) difference from long-term monthly means; MERC, mercury; INT, intensive contaminants monitoring; TPN, total particulate nitrogen; CNIT, continuous nitrate and biological response; AUS, micro-autosampler; WTOX, water toxicity; CFF, caged fish and frogs; SEDS, sediment source; --, not applicable; Ill., Illinois; Ind., Indiana; Ky, Kentucky; Wis., Wisconsin; Minn., Minnesota; Nebr., Nebraska; Mo, Missouri]

Map identifier (fig. 3)	Precipitation			Gaged	MSQA subset components and special studies							
	Long-term annual mean, 1981–2013	Antecedent, % difference	Index period, % difference		MERC	INT	TPN	CNIT	AUS	WTOX	CFF	SEDS
IN_Prairie	1,202	–11.0	14.2	No	Yes	--	--	--	--	--	--	--
MO_Loutre	1,078	–22.2	6.5	No	Yes	--	--	--	--	--	Yes	--
IL_Wabash	1,072	–1.1	33.3	No	Yes	--	--	--	--	--	--	--
IN_Otter1	1,167	–17.0	–10.3	No	Yes	--	--	Yes	--	--	--	--
MO_Moniteau	1,074	–17.2	0.6	No	Yes	--	--	--	--	--	Yes	--
MO_Perche	1,066	–17.4	–2.9	No	Yes	--	--	--	--	--	Yes	--
IN_Busseron	1,127	–9.2	36.1	No	--	--	--	--	--	--	--	--
IN_Otter2	1,151	–18.0	–12.5	No	Yes	--	--	--	--	--	--	--
IL_Cole	1,022	–14.7	31.5	No	Yes	--	--	--	--	--	--	--
MO_Skull	1,058	–16.8	–8.8	No	Yes	--	--	--	--	--	Yes	--
MO_Fish	1,056	–17.7	–8.1	No	--	Yes	--	--	--	Yes	Yes	--
MO_Bear	1,038	–15.2	–8.6	No	Yes	--	--	--	--	--	Yes	--
IL_Mill	1,109	–11.0	33.8	No	Yes	--	--	--	--	--	--	--
MO_Goodwater	1,035	–16.3	–6.2	No	--	Yes	--	--	Yes	Yes	Yes	--
IN_Nineveh	1,131	–13.2	–23.5	No	Yes	--	--	Yes	--	--	--	--
KS_French	867	–30.8	–0.5	No	Yes	--	Yes	--	--	--	--	--
MO_Contrary	939	–38.3	–13.8	No	Yes	--	--	--	--	--	--	--
IN_Lick	1,096	–11.1	–26.9	No	Yes	--	--	--	--	--	--	--
MO_Brushy	978	–28.4	–3.7	No	Yes	--	--	--	--	--	--	--
KS_Muddy	863	–29.8	7.3	No	Yes	--	Yes	--	--	--	--	--

Table 3. Description of site, reach, and watershed characteristics of selected sites assessed as part of the U.S. Geological Survey (USGS) National Water-Quality Assessment (NAWQA) Midwest Stream Quality Assessment (MSQA) in 2013.—Continued

[NWIS, USGS National Water Information System database; latitude and longitude from NWIS based on North American Datum of 1983 (NAD 83); mi², square mile; m, meter; land use based on 2011; Aii, agricultural intensity index, which was not calculated for two sites selected just outside the initial study area; antecedent (5/2012 to 4/2013) and index period (5/2013 to 7/2013) precipitation are expressed as percentage (%) difference from long-term monthly means; MERC, mercury; INT, intensive contaminants monitoring; TPN, total particulate nitrogen; CNIT, continuous nitrate and biological response; AUS, micro-autosampler; WTOX, water toxicity; CFF, caged fish and frogs; SEDS, sediment source; --, not applicable; Ill., Illinois; Ind., Indiana; Ky, Kentucky; Wis., Wisconsin; Minn., Minnesota; Nebr., Nebraska; Mo, Missouri]

Map identifier (fig. 3)	Station name	NWIS station number	State	Subregion	Site selection type
IN_Sugar	Sugar Creek at County Road 400 S at New Palestine, Ind.	394340085524601	Indiana	East	Other
OH_Darby	Big Darby Creek at Prairie Oaks near Lake Darby, Ohio	395942083151401	Ohio	East	Random
MO_Squaw	Squaw Creek Ditch near Squaw Creek Wildlife Area	400227095151501	Missouri	West	Random
MO_Fabius	Little Fabius River near Fabius, Mo.	400240092081201	Missouri	South	Random
MO_NFabius	North Fabius River near Monticello, Mo.	400502091403401	Missouri	South	Reference—good
IL_Herget	Herget Drainage Ditch near Kilbourne, Ill.	400856089562001	Illinois	South	Random
NE_Turkey	Turkey Creek near Steinauer, Nebr.	401143096134301	Nebraska	West	Random
IN_Pine	Big Pine Creek at County Road N 125 E near Williamsport, Ind.	401849087161401	Indiana	East	Random
IN_Limberlost	Limberlost Creek at County Road N 250 E near Bryant, Ind.	403242084553201	Indiana	East	Random
IL_NVermilion	North Fork Vermilion River near Wing, Ill.	404917088222701	Illinois	East	Random
OH_Honey	Unnamed Tributary to Honey Creek near Willard, Ohio	410133082465301	Ohio	East	Random
OH_Sandusky	Sandusky River at CR6 near McClutchenville, Ohio	410150083125701	Ohio	East	Random
OH_Vermilion	Vermilion River at SR18 near Clarksfield, Ohio	411146082244001	Ohio	East	Reference—geospatial
IN_Hodge	Hodge Ditch Stream at County Road N 400 W near Wheatfield, Ind.	411439087065601	Indiana	East	Random
IL_Green	Green River near Hooppole, Ill.	412911089540101	Illinois	East	Random
NE_Bell	Bell Creek near Arlington, Nebr.	413012096210001	Nebraska	West	Random
NE_Elkhorn	Elkhorn River near Oakdale, Nebr.	420444097543301	Nebraska	West	Random
IL_Stillman	Stillman Creek at Stillman Valley, Ill.	420626089101201	Illinois	East	Random
NE_Howe	Howe Creek near Lindy, Nebr.	424048097483601	Nebraska	West	Reference—geospatial
SD_Pipestone	Pipestone Creek near South Dakota/Minn. State Line	435420096290500	South Dakota	West	Random

Table 3. Description of site, reach, and watershed characteristics of selected sites assessed as part of the U.S. Geological Survey (USGS) National Water-Quality Assessment (NAWQA) Midwest Stream Quality Assessment (MSQA) in 2013–Continued.

[NWIS, USGS National Water Information System database; latitude and longitude from NWIS based on North American Datum of 1983 (NAD 83); mi², square mile; m, meter; land use based on 2011; Aii, agricultural intensity index, which was not calculated for two sites selected just outside the initial study area; antecedent (5/2012 to 4/2013) and index period (5/2013 to 7/2013) precipitation are expressed as percentage (%) difference from long-term monthly means; MERC, mercury; INT, intensive contaminants monitoring; TPN, total particulate nitrogen; CNIT, continuous nitrate and biological response; AUS, micro-autosampler; WTOX, water toxicity; CFF, caged fish and frogs; SEDS, sediment source; --, not applicable; Ill., Illinois; Ind., Indiana; Ky, Kentucky; Wis., Wisconsin; Minn., Minnesota; Nebr., Nebraska; Mo, Missouri]

Map identifier (fig. 3)	Level III Ecoregion name	Latitude	Longitude	Drainage area (mi ²)	Sampled reach length (m)	Land use (percentage)				Aii
						Developed/ Urban	Urban	Forest	Cultivated crops	
IN_Sugar	Eastern Corn Belt Plains	39.72778	-85.87944	93	520	2.15	8.0	5.1	81.8	77.3
OH_Darby	Eastern Corn Belt Plains	39.99507	-83.25379	229	840	3.20	8.4	8.3	73.1	56.4
MO_Squaw	Western Corn Belt Plains	40.04087	-95.25416	110	280	1.17	6.7	4.6	65.1	48.3
MO_Fabius	Central Irregular Plains	40.04456	-92.13672	35	320	0.54	3.7	11.2	36.2	39.7
MO_NFabius	Central Irregular Plains	40.08376	-91.67599	453	680	1.01	4.4	16.6	30.7	36.6
IL_Herget	Interior River Valleys and Hills	40.14889	-89.93889	15	400	1.82	5.2	29.9	57.0	63.6
NE_Turkey	Western Corn Belt Plains	40.18944	-96.22398	62	200	0.29	3.8	10.1	15.1	16.9
IN_Pine	Eastern Corn Belt Plains	40.31744	-87.29176	326	440	2.70	5.5	6.4	83.9	80.9
IN_Limberlost	Eastern Corn Belt Plains	40.54527	-84.93408	31	360	0.56	4.7	6.7	85.3	76.8
IL_NVermilion	Central Corn Belt Plains	40.82139	-88.37417	220	880	2.98	6.1	0.3	92.9	84.7
OH_Honey	Eastern Corn Belt Plains	41.02586	-82.78153	10	1,000	1.04	4.8	17.9	74.6	58.3
OH_Sandusky	Eastern Corn Belt Plains	41.03055	-83.21579	771	1,800	2.61	7.9	9.0	76.4	59.5
OH_Vermilion	Eastern Corn Belt Plains	41.19604	-82.41104	130	730	2.02	7.3	26.5	54.1	45.4
IN_Hodge	Central Corn Belt Plains	41.24278	-87.12154	45	400	3.49	5.7	16.7	70.8	83.0
IL_Green	Central Corn Belt Plains	41.48639	-89.90028	520	1,360	2.13	6.0	5.7	85.9	77.5
NE_Bell	Western Corn Belt Plains	41.49312	-96.34804	167	320	0.58	3.8	0.7	88.3	83.2
NE_Elkhorn	Western Corn Belt Plains	42.06927	-97.93312	2,451	1,400	0.41	2.7	0.4	17.9	30.3
IL_Stillman	Central Corn Belt Plains	42.10722	-89.17000	31	200	1.32	4.3	5.4	82.7	73.4
NE_Howe	Western Corn Belt Plains	42.69596	-97.78838	56	200	0.57	4.3	0.5	52.5	54.3
SD_Pipestone	Western Corn Belt Plains	43.90556	-96.48472	172	320	1.27	6.3	0.5	81.0	79.8

Table 3. Description of site, reach, and watershed characteristics of selected sites assessed as part of the U.S. Geological Survey (USGS) National Water-Quality Assessment (NAWQA) Midwest Stream Quality Assessment (MSQA) in 2013.—Continued

[NWIS, USGS National Water Information System database; latitude and longitude from NWIS based on North American Datum of 1983 (NAD 83); mi², square mile; m, meter; land use based on 2011; Aii, agricultural intensity index, which was not calculated for two sites selected just outside the initial study area; antecedent (5/2012 to 4/2013) and index period (5/2013 to 7/2013) precipitation are expressed as percentage (%) difference from long-term monthly means; MERC, mercury; INT, intensive contaminants monitoring; TPN, total particulate nitrogen; CNIT, continuous nitrate and biological response; AUS, micro-autosampler; WTOX, water toxicity; CFF, caged fish and frogs; SEDS, sediment source; --, not applicable; Ill., Illinois; Ind., Indiana; Ky, Kentucky; Wis., Wisconsin; Minn., Minnesota; Nebr., Nebraska; Mo, Missouri]

Map identifier (fig. 3)	Precipitation			Gaged	MSQA subset components and special studies							
	Long-term annual mean, 1981–2013	Antecedent, % difference	Index period, % difference		MERC	INT	TPN	CNIT	AUS	WTOX	CFF	SEDS
IN_Sugar	1,106	-0.3	-30.5	Yes	--	Yes	Yes	--	Yes	Yes	--	Yes
OH_Darby	982	-7.0	-3.5	No	Yes	--	--	--	--	--	--	--
MO_Squaw	899	-28.5	-11.0	No	Yes	--	--	--	--	--	--	--
MO_Fabius	1,011	-10.5	-4.4	No	Yes	--	--	--	--	--	--	--
MO_NFabius	977	-11.0	-4.3	Yes	Yes	--	--	--	--	--	--	--
IL_Herget	956	-9.4	31.5	No	Yes	--	--	--	--	--	--	--
NE_Turkey	816	-29.9	10.9	No	Yes	--	Yes	--	--	--	--	--
IN_Pine	973	2.6	-8.5	No	Yes	--	--	--	--	--	--	--
IN_Limberlost	984	3.7	-16.1	No	Yes	--	--	--	--	--	--	--
IL_NVermilion	958	-8.0	-11.2	No	Yes	--	--	--	--	--	--	--
OH_Honey	1,075	-1.8	45.8	No	Yes	--	--	--	--	--	--	--
OH_Sandusky	977	-2.3	22.6	No	Yes	--	--	--	--	--	--	--
OH_Vermilion	1,007	-0.7	32.2	No	--	--	--	--	--	--	--	--
IN_Hodge	978	-4.7	-1.4	No	Yes	--	--	--	--	--	--	--
IL_Green	923	-6.6	-6.2	No	Yes	--	--	--	--	--	--	--
NE_Bell	766	-28.3	-19.9	No	Yes	--	Yes	--	Yes	--	--	--
NE_Elkhorn	639	-49.5	-4.6	Yes	Yes	--	Yes	--	--	--	--	--
IL_Stillman	899	-11.5	3.9	No	Yes	--	--	--	--	--	--	--
NE_Howe	687	-48.1	3.1	No	Yes	--	Yes	--	--	--	--	--
SD_Pipestone	669	-11.7	-1.3	No	Yes	--	--	--	--	--	--	--

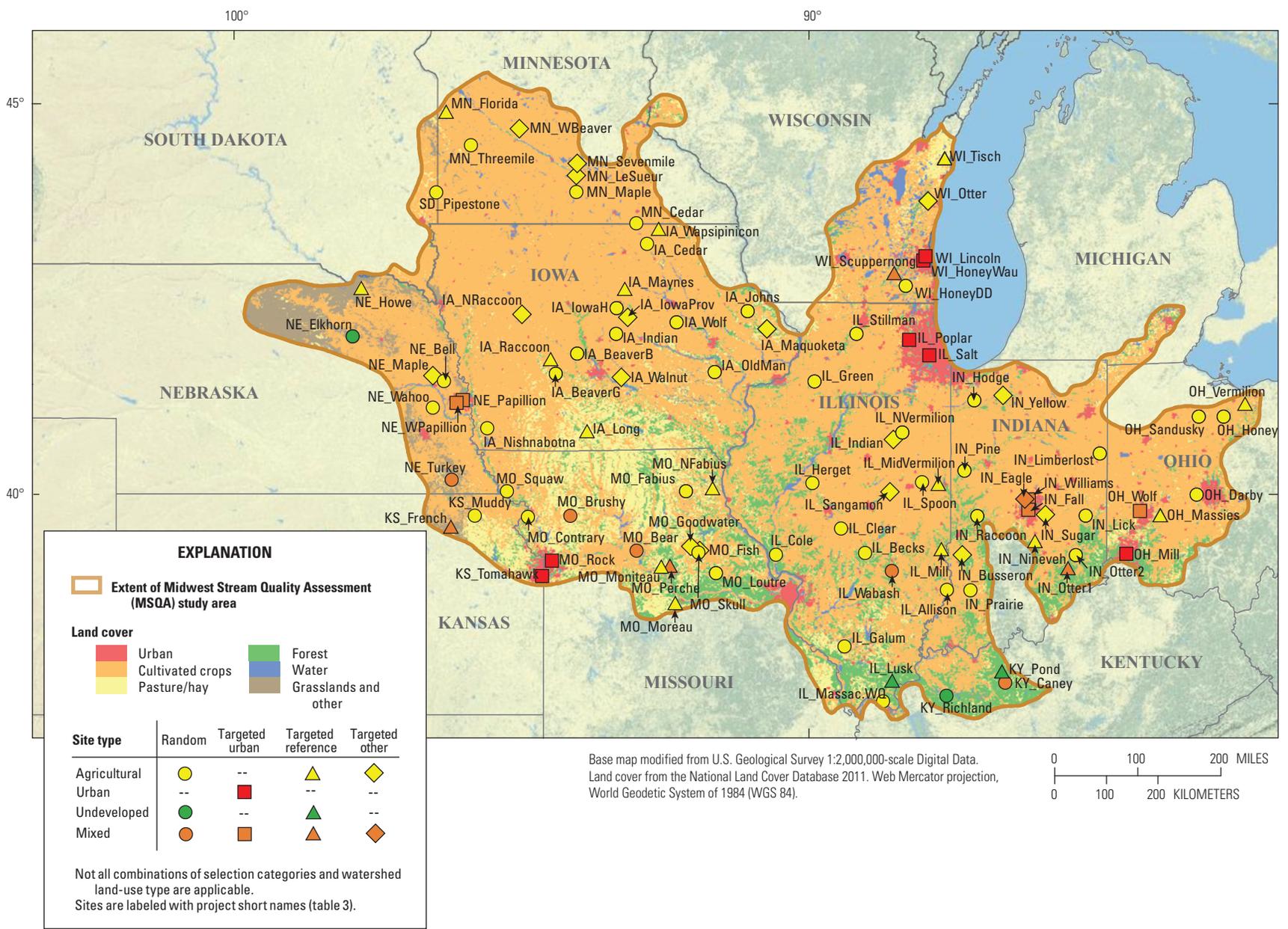


Figure 3. Study area for the Midwest Stream Quality Assessment with sites by selection category and watershed land-use type.

Random Network

Sites selected reasonably covered the NRSA selection groups, in similar proportions to the random selection, so that statistical inferences would be valid. The MSQA random sites were used for the NRSA, creating a potential for skewing the site selection for NRSA, because additional constraints of the MSQA objectives required selection of alternate sites. Overall, the MSQA random site selection did favor mid-sized streams. Large sites were excluded as potentially not wadeable, and low-stream order sites (small streams category) commonly were excluded because of the lack of flow during the drought condition of the reconnaissance period.

Of the 50 random sites in the MSQA study, 38 were initially selected “base” sites and 12 were alternate “over” sites.

Despite the additional constraints of the MSQA objectives, the random sites selected generally met the target goals of similar relative portion of sites by State and by stream category (table 4). The NRSA large streams category was overrepresented in the MSQA random selection because of additional sites chosen in place of nonwadeable reaches from the rivers categories. Though the portion of selected sites in the small streams category was balanced in regard to the NRSA goal, relatively more sites in this category were alternate “over” sites (table 4, appendix 1). Many sites in the small streams category were determined to be dry or not flowing during the site reconnaissance period because of antecedent drought conditions, resulting in more frequent rejection of initial “base” sites in the small streams category than other categories.

Table 4. Distribution of sites balancing project goals for the National Rivers and Streams Assessment (NRSA) and the Midwest Stream Quality Assessment (MSQA).

[Noteable deviations from the target portion of sites from the NRSA base (goal) are in **bold**; --, no data]

Category	NRSA base sites	MSQA random sites	MSQA targeted sites	All MSQA sites	Portion of sites in each category		
					NRSA base (goal)	MSQA probabilistic (achieved)	All MSQA
Counts by State							
Illinois	38	12	7	19	0.25	0.24	¹ 0.19
Indiana	20	7	8	15	0.13	0.14	0.15
Iowa	28	9	8	17	0.18	0.18	0.17
Kansas	3	1	2	3	0.02	0.02	0.03
Kentucky	7	2	1	3	0.05	0.04	0.03
Michigan	0	0	0	0	0.00	0.00	0.00
Minnesota	8	3	4	7	0.05	0.06	0.07
Missouri	22	7	7	14	0.14	0.14	0.14
Nebraska	11	4	4	8	0.07	0.08	0.08
Ohio	9	3	4	7	0.06	0.06	0.07
South Dakota	3	1	0	1	0.02	0.02	0.01
Wisconsin	5	1	5	6	0.03	0.02	¹ 0.06
Counts by NRSA category							
Streams09	41	15	0	15	0.27	0.30	³ 0.15
SmallStreams	21	9	14	23	0.14	0.18	³ 0.23
LargeStreams	22	14	30	44	0.14	² 0.28	³ 0.44
Rivers09	32	5	0	5	0.21	² 0.10	³ 0.05
RiversMajor	19	0	0	0	0.12	² 0.00	³ 0.00
RiversOther	19	7	6	13	0.12	0.14	0.13
All sites	154	50	50	100	--	--	--

¹Exceptions were made to maintaining balance with total number of sites per State in Illinois because of a limit of total number of sites logistically possible for water chemistry sampling and in Wisconsin because of urban sites.

²NRSA base sites with stream order greater than 5 were excluded, with additional sites distributed to the Small and Large streams categories.

³Overall MSQA site selection favored stream over river sites because of the constraint of the wadeable ecological assessment, and large over small streams because of the constraint of streamflow through the entire assessment confounded by drought conditions during the reconnaissance period.

Targeted Network

The targeted sites were used to fill in the parts of the land-use gradient that were not represented with random sites, as shown in figures 4A, 4B, 5A, and 5B.

The 12 targeted urban sites have developed urban land use ranging from 18 to 88 percent and total urban from 32 to 100 percent (table 3, appendix 1). Agricultural land use differs substantially in these 12 watersheds, with percentage of RC ranging from 0 to 55 percent. Because of the presence of urban and agricultural land uses, 5 of the 12 “urban” sites are classified as “mixed” land-use settings using previous NAWQA approaches to categorize site types (Stone and others, 2014). The results from these sites are expected to provide a characterization of the chemistry, toxicity, and ecology of urban streams in the region.

Because true reference conditions do not exist in the region, efforts to find them yielded only limited results in terms of land-use setting (figs. 4A, 4B, 5A, and 5B). Full sampling results are necessary to determine if the approaches for selecting reference sites were successful at providing a set of sites with “good” water quality and biology. Lack of undeveloped reference sites is indicated by the fairly uniform distribution of sites across Aii bins 2, 3, and 4, but under representation of sites in Aii bin 1 (figs. 4A and 4B). For sites with medium to high agricultural intensity (Aii greater than 50), the selected sites represent a wide range of RC agriculture within the riparian buffer (fig. 4B)—important because riparian RC is expected to adversely affect ecological conditions in streams. Sampling a range of other potentially important variables affecting chemical transport to streams was ensured by having the 100 MSQA sites distributed geographically across the region; for example, soil impervious layer, base-flow index, tile drains, and soil characteristics.

Major Components of Stressor Sampling

The 100 sites were classified into major sampling groups, according to the sampling regime addressing broad objectives, and small subsets of sites for special studies addressing research questions for specific stressors or responses. At all 100 sites, the core sampling regime was consistent to assess stressors in water and streambed sediment and to characterize biological communities and habitat, referred to as BASIC group sampling. Within the 100 sites, three major groups of MSQA sites were selected for sampling for mercury (MERC group), broader coverage of contaminants (intensive contaminants monitoring [INT group]), and investigation of a QC issue related to measurement of total particulate nitrogen (TPN group) in streams. Overlap between several of the groups is shown in the sampling plan in figure 6, and the membership of sites in the MSQA subset component and special studies is listed in table 3. Additional smaller groups

of sites were selected for special studies, which are described in a later section. Special study groups included continuous nitrate and biological response (CNIT), micro-autosampler for pesticides (AUS), water toxicity (WTOX), caged fish and frog (CFF), and sediment source (SED).

The objectives of the contaminant sampling and analyses of the BASIC, MERC, and INT groups are the same, except that they apply to different lists of contaminants—assess the status of contaminants, assess relations between contaminant concentrations and toxicity and ecological condition, and evaluate natural and anthropogenic factors in the watersheds affecting contaminant concentrations.

Basic Group Sampling

All 100 sites were sampled for water chemistry (12 times), bed sediment chemistry (once), and ecology (once). The BASIC group water constituents were suspended-sediment concentration, nutrients, chloride and sulfate, dissolved organic carbon (DOC), glyphosate by immunoassay method, and dissolved pesticides; bed sediment constituents were major and trace elements, radionuclides, pesticides, and grain size; ecological-survey constituents were phytoplankton chlorophyll *a*, habitat, and ecological communities (algae, invertebrates, and fish). In addition, polar organic compound integrating samplers (POCIS) were deployed in all 100 streams for about 5 weeks; these samplers accumulate polar (relatively water-soluble) contaminants from the water column during the deployment period.

Mercury Group Sampling

A subset of 71 sites (MERC group, table 3) had water samples collected for analysis of total and methyl mercury during every other water-quality sampling visit of the 12 visits. The samples were collected to provide supporting information relative to mercury analysis in fish tissue plugs obtained at the time of ecological sampling for these sites. The 71 MERC sites were the 50 random sites and the 21 targeted least ecologically disturbed sites.

Intensive Contaminants Monitoring

To assess the status of contaminants relative to toxicity, ecological condition, and watershed factors, a primary objective of the INT group sampling was to more fully characterize chemical mixtures at the urban sites and for a subset of intensive agricultural sites. A subset of 27 sites (table 3) included sample analysis for glyphosate by liquid chromatography/tandem mass spectrometry (LC-MS/MS) and hormones in water on every other visit. Additionally, bed sediment collected during the ecological assessment was tested for halogenated organic compounds and PAHs.

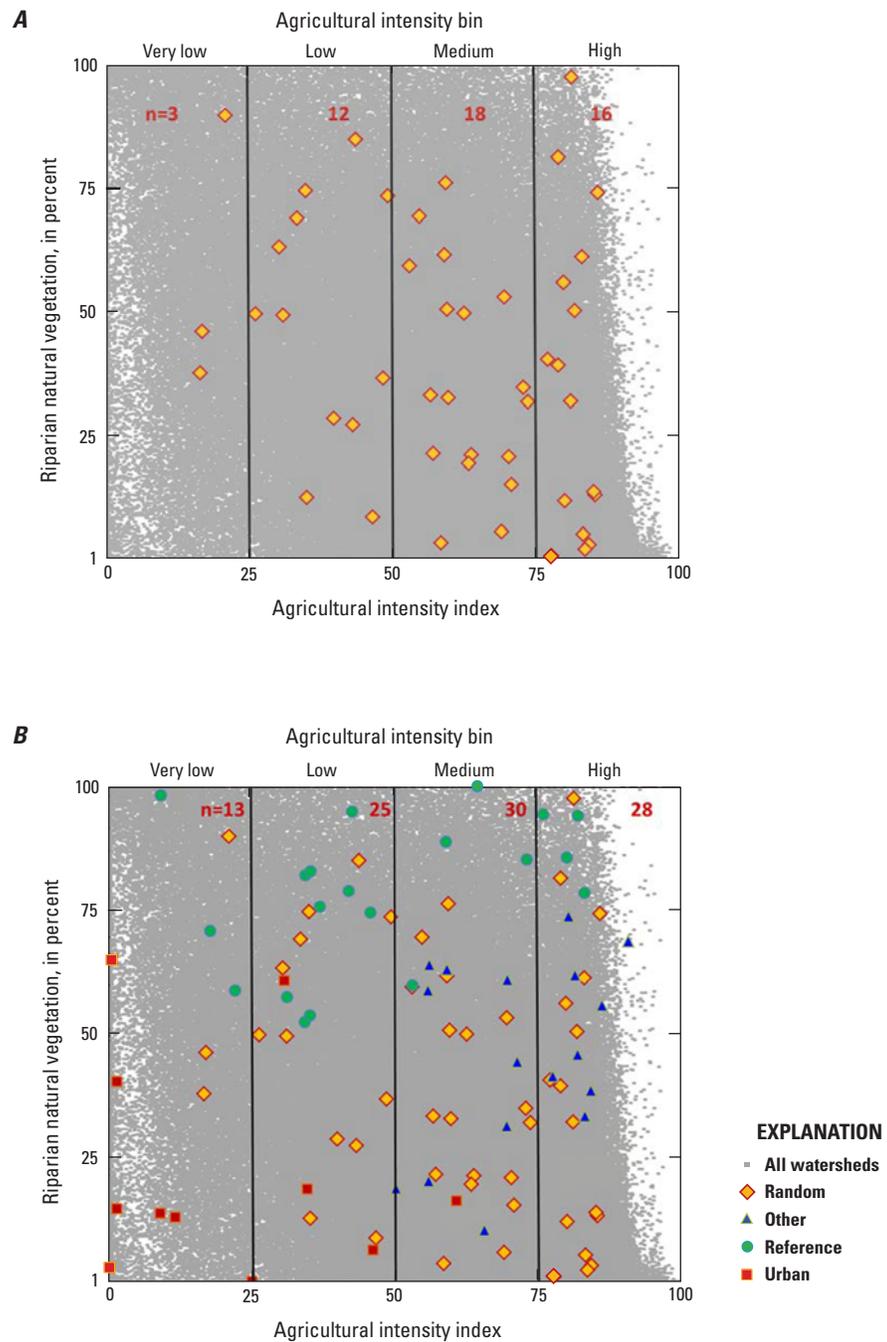


Figure 4. Relation between the agricultural intensity index (Aii) and the percentage of natural vegetation in the riparian buffer of the stream. *A*, watersheds in the Midwest Stream Quality Assessment (MSQA) area (gray dots) and *B*, 50 MSQA random sites (orange symbols) with targeted sites added to achieve a more uniform distribution of sites relative to Aii and riparian natural vegetation.

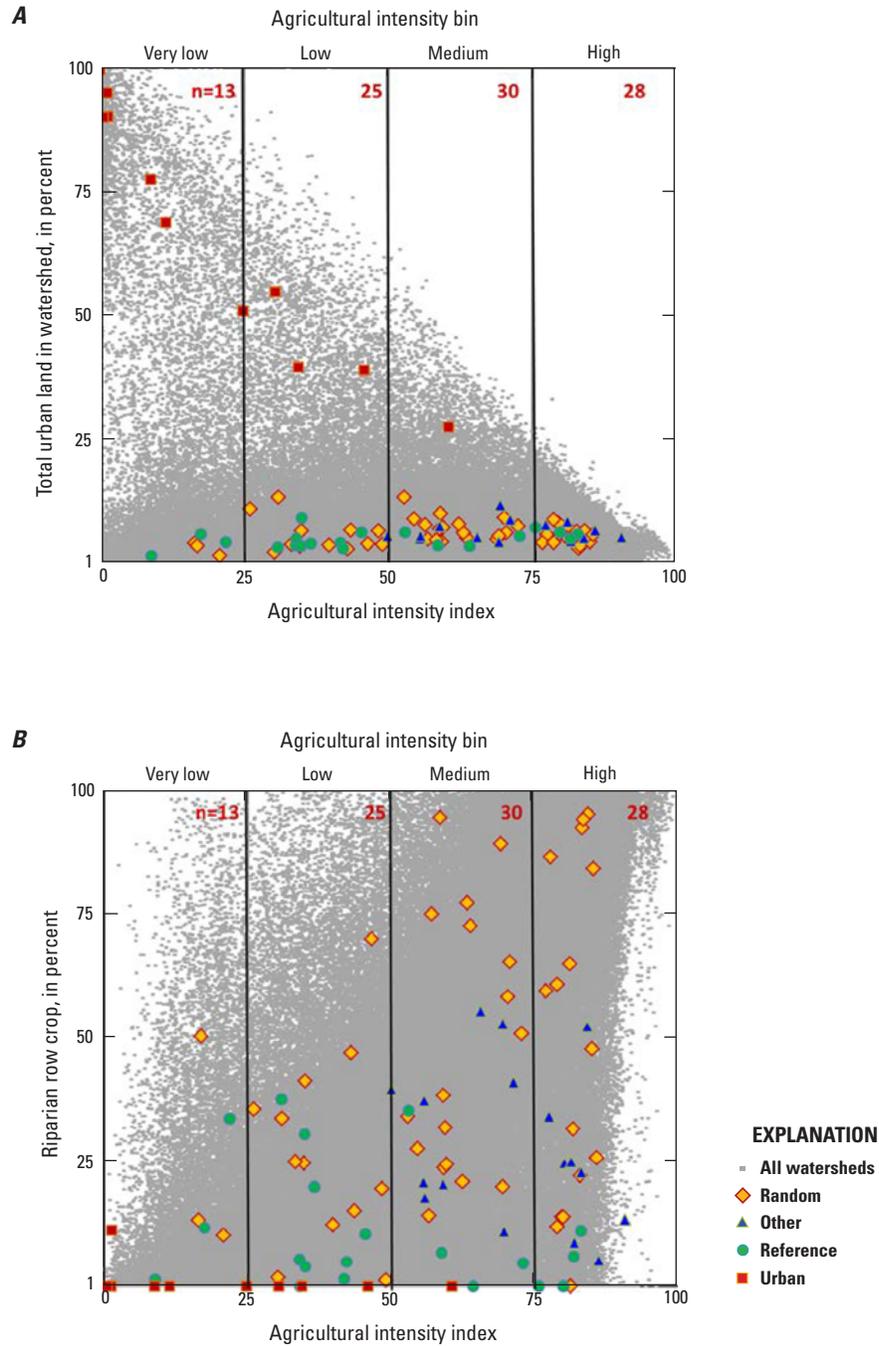


Figure 5. Relation between the agricultural intensity index (Aii) and the percentage of urban land and row crop for Midwest Stream Quality Assessment (MSQA) sites (color coded by site type) and for all watersheds in the study area (gray dots). *A*, urban land in the watershed and *B*, row crop in the riparian buffer of the stream.

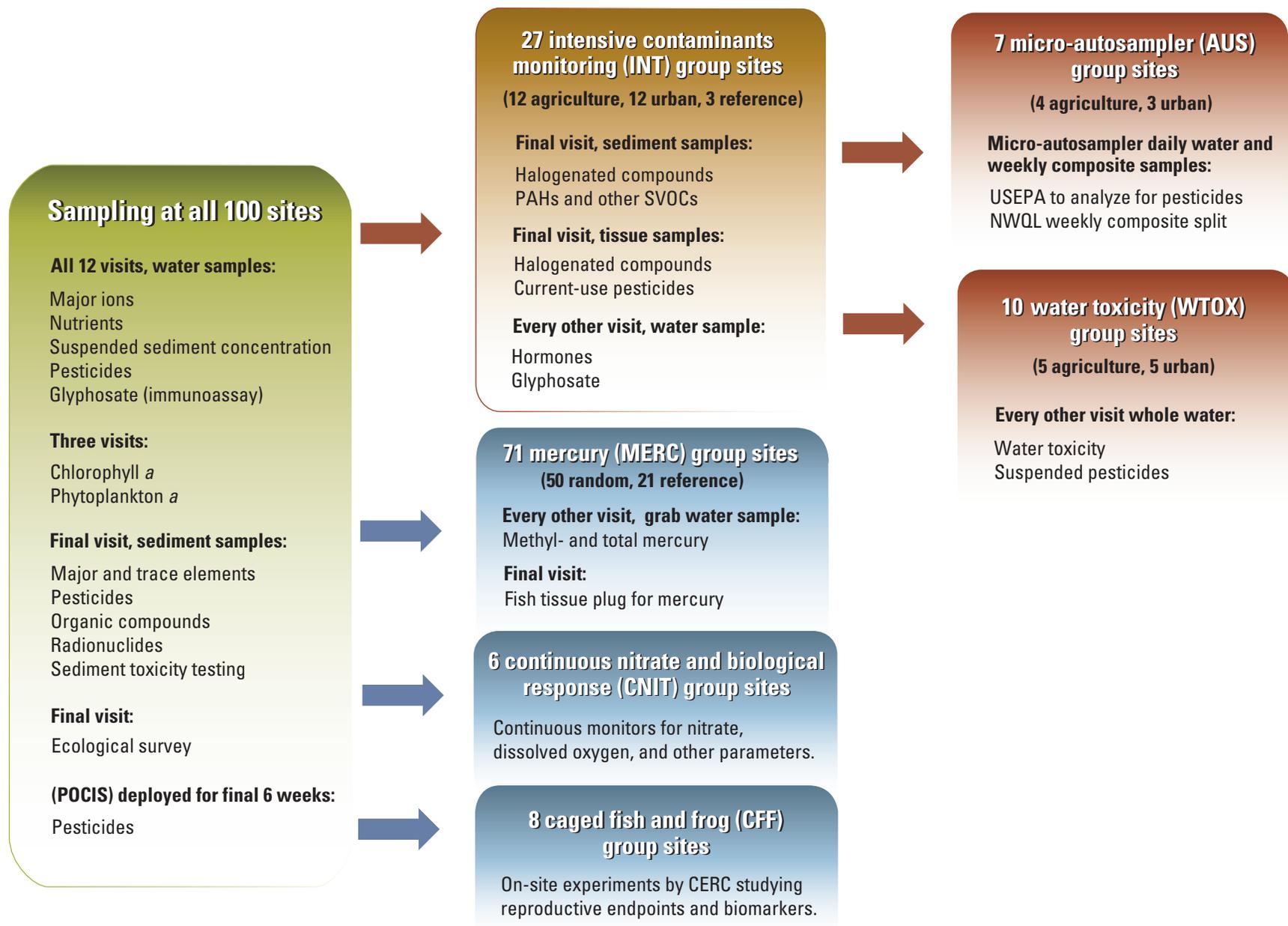


Figure 6. Types and frequency of chemical, physical, and biological sampling and analyses completed as part of the collaborative Midwest Stream Quality Assessment study. [POCIS, polar organic compound integrating samplers; PAHs, polycyclic aromatic hydrocarbons; SVOCs, semivolatle organic compounds; EPA, U.S. Environmental Protection Agency; CERC, Columbia Environmental Research Center; NWQL, National Water Quality Laboratory].

The INT sites include all 12 urban sites and 12 highly agricultural sites (Aii and percentage of RC greater than 50), at which contaminants would be expected to be relatively high, plus 3 reference sites. All 27 are targeted sites, as opposed to random, and most (21 sites) had streamflow gages. Though the nonreference sites were chosen to emphasize high contaminant levels, watershed characteristics related to chemical fate and transport differ. The three reference sites are in three States and have small (28 km²), medium (173 km²) and large (874 km²) watershed areas. The 12 agricultural sites are in 7 States and differ in watershed area (25–1,400 km²), percentage of tile-drained land (0–77 percent), and the extent of soil restrictive layer (0–49 percent) in the basin.

Total Particulate Nitrogen Group Sampling

In addition to routine QC samples (replicates, blanks, and matrix spikes), samples at 18 sites (TPN group; table 3) were analyzed for particulate carbon and nitrogen by high-temperature combustion method (EPA 440.0). Results of analysis of total nitrogen by alkaline persulfate digestion of unfiltered samples, used for all BASIC water samples, can be negatively biased in the presence of suspended sediment. Rus and others (2013) noted this negative bias in synthetic samples and stream water from 77 sites, and the bias was present regardless of suspended-sediment concentration, though more pronounced with suspended-sediment concentrations above 750 milligrams per liter (mg/L). The TPN sites were chosen based on recommendations from WSC personnel and expectations for a wide range of suspended-sediment concentrations or previously documented total nitrogen bias by digestion methods. The TPN sites were sampled throughout the index period to encompass a range of suspended-sediment and total nitrogen concentrations. Comparison data from the alternate analytical methods will be used to quantify analytical bias for the MSQA study.

Data Collection and Processing

Data collected or processed for the MSQA included geospatial datasets; continuous temperature and water-level monitoring; and collection of water, sediment, and ecological samples. In addition to site selection, geospatial variables, which are important in affecting fate and transport of urban and agricultural chemicals and ecological condition, are crucial to further evaluate the distributions of stressor gradients in the region. The types and frequency of data and sample collection differed depending on the site group, as listed in table 3 and shown in figure 6. All sites were monitored for continuous temperature and water level and sampled 12 times for water chemistry during a 14-week index period. A composite bed sediment sample was collected at all sites at the end of the water sampling index period coincident with ecological

sampling, and subsamples were used for contaminant analyses and whole-sediment toxicity testing.

Sampling protocols in most cases required slight differences in site definition between the ecology reach and the water sampling point. Water samples were collected from the nearest bridge to the ecological sampling site or at a shallow-water cross section. The ecological sampling site was defined either as (1) the randomly selected NRSA site for random sites or (2) the reach selected by the USGS sampling crew for targeted sites, typically upstream from the bridge or road crossing. Bridge access for water sampling was necessary because protocols call for collection of depth- and width-integrated samples at higher flows, which are only reasonably collected from a bridge.

Continuous Temperature and Water-Level Monitoring

At sites without existing streamflow gages or temperature monitoring, digital loggers were deployed to record water temperature and water level, as pressure (table 5) (Onset Computer Corporation, 2008; Onset Computer Corporation, 2012). Water-level loggers typically were deployed on temporary structures, such as posts anchored in the stream, with a second logger deployed to measure atmospheric barometric pressure on a nearby permanent structure, such as a bridge or fence. After installation, two reference points were established: one point on a permanently fixed structure such as on a bridge and the second point on the structure that the logger was attached to. The distance to the water surface was measured from both reference points during each site visit to verify the logger readings and that the deployed water-level logger had not moved. During water-quality sampling visits, temperature was periodically measured and recorded near the water-level logger (which includes temperature) or temperature logger. Additional guidelines for deployment and operations of water-temperature loggers are described in Wagner and others (2006).

Water-Quality Data Collection

Sampling is summarized in the diagram presented in figure 6.

Discrete Samples

During 14 weeks, 12 water samples were collected approximately weekly, with 2 “extended weeks” where one sample was collected during the 2-week periods that included the Memorial Day and Fourth of July holidays. The timing of the sampling period was designed to coincide with the high agrochemical use and runoff season preceding the ecological surveys (Gilliom and others, 2006). This 3-month “index period” was selected to characterize the exposure to chemical

Table 5. Deployment details for instrumentation.

[USGS, U.S. Geological Survey; MSQA, Midwest Stream Quality Assessment; USDA–ARS, U.S. Department of Agriculture–Agricultural Research Service]

Type of deployment or instrumentation	Count
Water level and streamflow gaging	
USGS Streamflow gaging station	36
MSQA water level (with temperature)	60
Data provided from USDA–ARS	1
Not deployed	2
Equipment or data lost	1
Total	100
Water temperature	
Existing USGS monitoring	7
MSQA cont nitrate sites	1
MSQA water level with temperature	60
MSQA water temperature (only)	21
Data provided from USDA–ARS	1
Not deployed	2
Equipment or data lost	8
Total	100

stressors for the biological communities leading up to the ecological sampling in late July or early August. The sampling period was selected to capture the runoff after the application of fertilizer and herbicides (typically applied the middle of April to early May), insecticides (which can be applied any time, but generally later in the growing season once infestation has been documented, typically June and July), and fungicides (mostly applied to corn the middle of July to early August). Pesticide application began later than usual in 2013 because cold, wet weather delayed the planting of corn through much of the Midwest (U.S. Department of Agriculture National Agricultural Statistics Service, 2013).

Discrete water samples were collected using USGS methods designed to ensure representative, bias-free samples. Samples were collected and processed using protocols documented in the USGS National Field Manual (U.S. Geological Survey, variously dated). For most constituents, multiple incremental samples from across the channel were collected using DH–81, DH–95, or D–95 samplers and immediately composited. When stream velocities, depth, and safety allowed, multiple vertical sampling followed an isokinetic, equal-width increment or equal-discharge increment approach (U.S. Geological Survey, 2006). Sampling crews that consisted of two people used “clean-hands/dirty-hands” roles and precleaned, acid- and methanol-rinsed Teflon sample collection equipment (isokinetic cap and nozzle on a 1-liter [L] sampling bottle and 14- or 8-L churn for sample compositing). Field properties

of specific conductance, pH, dissolved oxygen, and water temperature were measured at the time of sampling with a calibrated multiparameter sonde (Wilde, variously dated).

Water samples were collected as a grab sample from the centroid of flow for mercury and DOC analysis. Ultra-trace-level clean-sampling procedures and equipment were used to collect surface-water samples at selected sites for low-level total mercury and methyl mercury analysis (U.S. Environmental Protection Agency, 1996; Lewis and Brigham, 2004). The mercury samples were collected in 500-milliliter tetrafluoroethylene bottles, precleaned and acidified immediately with ultra-pure hydrochloric acid (Lewis and Brigham, 2004). The DOC grab samples were collected with methanol-free baked amber glass bottles, filtered through a Pall AquaPrep disc filter, preserved with sulfuric acid, and chilled.

Subsamples of composited water were withdrawn from the Teflon churn splitter for analyses of various constituents, and whole-water and filtered subsamples were preserved, as appropriate (Wilde and others, 2004 with updates through 2009; Hambrook Berkman and Canova, 2007; Sandstrom and Wilde, 2014). Samples were drawn from the churn for most constituents unless equipment cleaning procedures were incompatible (such as with DOC and mercury) or if the volume of water needed exceeded the capacity of the churn (such as with the 14-L water toxicity samples). Whole-water samples were drawn from the churn first while mixing to reduce bias. Protocol for processing, preservation, shipping, and holding times for constituents not described in the National Field Manual (Wilde and others, 2004 with updates through 2009), such as hormones and surfactants, were developed based on similar constituents, such as pesticides, and with guidance from the analytical laboratory. More in-depth details about collection and processing for each constituent are listed in the appendix table 4–1.

Organic Compound Integrated Samplers

Passive samplers (POCIS) collected dissolved chemicals from stream water integrated during an extended deployment period. The POCIS concentrate polar organic chemicals such as water-soluble pesticides, most pharmaceuticals, illicit drugs, polar pesticides, phosphate flame retardants, surfactants, and many metabolite or degradation products (Alvarez, 2010). The POCIS were deployed at all 100 sites for approximately 5–6 weeks (31–45 days) prior to the end of the 14-week sampling period. Care was taken to limit atmospheric exposure. Samplers were installed in a pool section of the targeted sampling reach, typically immediately upstream or in the general vicinity of the weekly water-sampling location. Samplers were transported to field sites on ice, stored frozen (15 °C), and shipped overnight on dry ice. At 10 of the 100 sites, a POCIS field blank was used to characterize contamination of samplers during deployment and retrieval by transporting the POCIS blank to the field and opening it to the air during the time the POCIS environmental sample was being deployed or retrieved.

Sediment Data Collection

At each of the 100 sites, one composite bed sediment sample and one composite sample of eroding banks were collected, typically on the same day as the ecological survey samples. Samples were initially shipped to the CERC and Maryland WSC for consistent processing prior to further laboratory analysis (table 6).

The bed sediment sampling was designed to collect fine-grained sediments deposited within the ecological study reach from hydrologic transport and that are reflective of inputs from the watershed. Sediment collection and processing methods were adapted from those of Shelton and Capel (1994). Bed sediment depositional zones within the ecological stream reach were identified. Depositional zones were avoided near exposed or actively eroding streambanks (where fine-grained bed sediment might represent bank material), point sources such as storm drains, areas indicating evidence of livestock, and areas of obvious stream-side excavation or construction. Each depositional area was sampled in proportion to its presence in the reach, starting at the downstream end of the reach and moving upstream, to minimize sediment disturbance. The top 2 centimeters of sediments were removed using an inverted glass petri dish and Teflon plate and composited to a plastic field bucket. Large particles such as pebbles and sticks were removed by gloved hand or spatula. Samples were maintained on ice or chilled at 4 °C until shipped overnight on ice to the CERC for splitting and further processing.

The objective of bank sampling was to collect representative samples of eroding banks throughout the reach. As many as six eroding banks were sampled within the ecological reach from each side of the stream if possible, at least one channel width apart, and avoiding depositional material and structural modifications (for example, riprap). Bank surface (outer 1 centimeter) material was scraped using a precleaned plastic shovel, working from just below the waterline to the top of the bank, and composited into a plastic bag. The bank sediment samples were shipped on ice to the USGS Maryland WSC.

Ecological Data Collection

Ecological assessments followed NRSA protocols and were done by personnel from multiple State agencies, USGS, EPA, and EPA-approved contractors; all team leaders attended training on NRSA protocols (U.S. Environmental Protection Agency, 2013; appendix 1 for ecological crews for each site).

The goal was to sample all 100 sites once for habitat and biological communities (benthic macroinvertebrates, algae, and fish) between July 22 and August 9, 2013, concurrent with the collection of bed and bank sediment samples and the end of the water-chemistry index sampling period. Between July 22 and August 9, 2013, 85 of the sites were sampled. Because of high water, 10 samples were collected between August 12 and September 11. Primarily because of concern that the sites would be dry during the planned sampling period, 4 samples

Table 6. Bed sample analyses in order of priority for limited amount of material.

[mL, milliliter; USGS, U.S. Geological Survey; HDPE, high density polyethylene; PP, polypropylene; °C, degree Celsius; --, not available; L, liter; PAHs, polycyclic aromatic hydrocarbons including other semivolatil organic compounds]

Analysis	Volume required (mL)	Sample container, wide mouth	Preservation and shipping	USGS laboratory
Pesticide	75	125 mL baked clear glass jar	Frozen with headspace	California Pesticide Fate Laboratory, Sacramento, California.
Trace element, carbon	20	125 mL HDPE bottle	Lids left off to dry	Geologic Division Metals Laboratory, Denver, Colorado.
Radionuclides	100	125 mL PP bottle	¹ Refrigerated shipped chilled (4 °C)	National Research Program Laboratory, Menlo Park, California.
Grain size and surface analysis	--	--	--	¹ Maryland Water Science Center, Baltimore, Maryland.
Grain size	400	500 mL PP bottle	Shipped room temperature	Missouri Water Science Center Sediment Laboratory, Rolla, Missouri.
Toxicity testing	1800	14 L cubitainer	Chilled (4 °C) up to 2 months	Columbia Environmental Research Laboratory, Columbia, Missouri.
Hydrophobic organic compounds and PAHs	50	125 mL I-CHEM™ glass jar	Refrigerated shipped chilled (4 °C)	National Water Quality Laboratory, Denver, Colorado.
Hormones	50	125 mL I-CHEM™ glass jar	Refrigerated shipped chilled (4 °C)	National Water Quality Laboratory, Denver, Colorado.

¹Radionuclide samples were shipped initially to Maryland Water Science Center for additional processing, then a subsample returned after the nondestructive analysis.

were collected between June 23 and July 18. The site (LeSeuer River in Minnesota) was not sampled because the water never came down to safely sample the stream before the end of September. On June 23, one site (Sandusky River in Ohio) was sampled by the EPA contractor using a boat and boat methods instead of the wadeable methods that were requested. Lastly, one site sampled on September 11 (Caney Creek in Kentucky) was unwadeable between transects 4 and 11 and, therefore, the fish community data were unusable.

The reach was the fundamental sampling area used in the study and was established following NRSA protocols (U.S. Environmental Protection Agency, 2013). The ecology reach length was determined as 40 times the wetted channel width during ecological sampling (generally base-flow conditions), with a minimum length of 150 m (if the mean width was less

than 4 m), and maximum of 4 kilometers (if the mean width was greater than 100 m). The mean of five wetted width measurements of the stream channel was determined upstream and downstream from middle of the reach. For random sites, the middle of the reach location (called the X-site) was the location on the NHDPlus network randomly selected by the NRSA. Typically, the sampling reach was laid out prior to field sampling using maps, aerial photos, and geographic information system software. For targeted sites the X-site was determined by the field crew at the time of sampling. Eleven equally spaced cross-sectional transects were established in the reach to statistically represent the reach. An example from the LaSeuer River near Rapidan, Minnesota, shows how the site was assessed before sampling to appropriately locate the water-quality site relative to the ecology reach (fig. 7).



EXPLANATION



Water-quality sampling location and identifier

Coordinates for water-quality sampling locations

A, 44.111439 -94.041788	G, 44.112302 -94.045179
B, 44.112067 -94.041746	H, 44.112072 -94.045994
C, 44.112702 -94.041787	I, 44.111807 -94.046757
D, 44.112817 -94.042611	J, 44.111934 -94.047627
E, 44.112603 -94.043451	K, 44.112357 -94.048301
F, 44.112422 -94.044305	

Figure 7. Planning for ecology reach transects and downstream bridge water-quality sampling locations for a wadeable site using available imagery and online tools. (2013 Digital Globe image of the LeSeuer River near Rapidan, Minnesota; water width is 18 meters, reach length is 720 meters, and transect length is 72 meters.)

If necessary, the midpoint of the reach was adjusted upstream or downstream. Conditions for such an adjustment included confluences with higher or lower order streams within the reach, impoundments (pond, lake, or reservoir), physical barriers (for example, a waterfall), or if parts of the reach were inaccessible (including denial of permission to access some portion of the reach). For random sites, which required adjusting the reach upstream or downstream, the X-site from the NHDPlus network was no longer at the midpoint of the reach but still fell within the reach. The reach was not adjusted to avoid manmade obstacles (such as bridges, culverts, riprap, or channelization). Adjusting the reach involved noting the distance of the barrier, confluence, or other restriction from the X-site and flagging the restriction as the endpoint of the reach. The distance to the other end of the reach was added, such that the total reach length remained the same (U.S. Environmental Protection Agency, 2013).

Samples were collected from downstream to upstream. Biological and habitat measures were sampled throughout the reach with measurement points systematically placed in relation to the 11 transects (fig. 8).

Benthic macroinvertebrate samples were collected before periphyton samples at a point 1 m downstream from each transect, at the assigned sampling station using a D-frame net with

500-micron (μm) mesh openings. The 0.09 m^2 (1-square-foot) sampling stations on each transect were at either 25, 50, or 75 percent channel width—left, center, or right (L, C, R, respectively) as looking downstream. Organisms were separated from the substrate—heavy organisms were removed by hand, then the streambed was vigorously kicked for 30 seconds while capturing the material in the net. Material was transferred from the net to a sieve bucket, gravel and detritus were removed, transect samples composited, and samples preserved in 95 percent ethanol with larger predaceous invertebrates preserved immediately to reduce the chances of other specimens being consumed or damaged.

Periphyton samples were collected and composited from a single location at each of the 11 transects after collecting the benthic macroinvertebrate sample. Samples were removed from a 12-square-centimeter area of substrate at each transect by scrubbing or by syringe. The composite sample for the reach was then subsampled and processed for an identification/enumeration sample (PERI), a chlorophyll *a* sample (PCHL), and a biomass sample (PBIO) measured as ash-free dry mass. The PERI samples were preserved with formalin. The PCHL and PBIO samples were filtered using 0.7- and 1.2- μm glass-fiber filters, respectively, and placed on dry ice before being shipped to the appropriate laboratory for analysis.

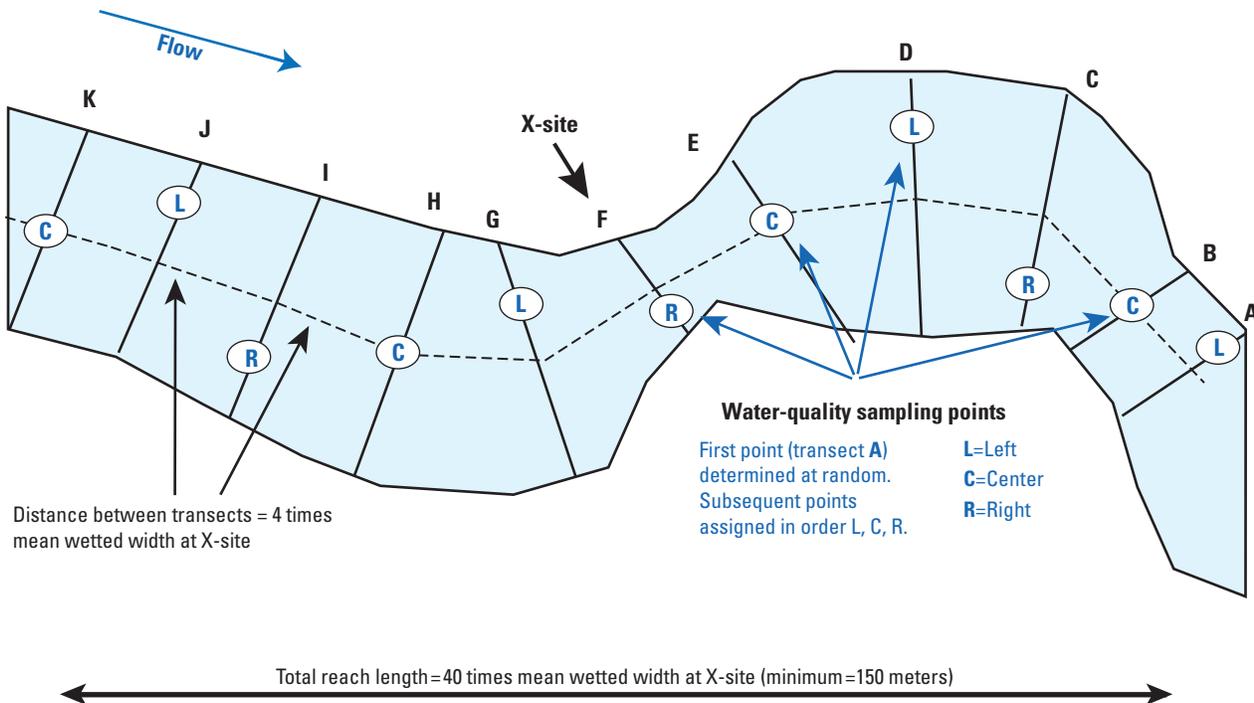
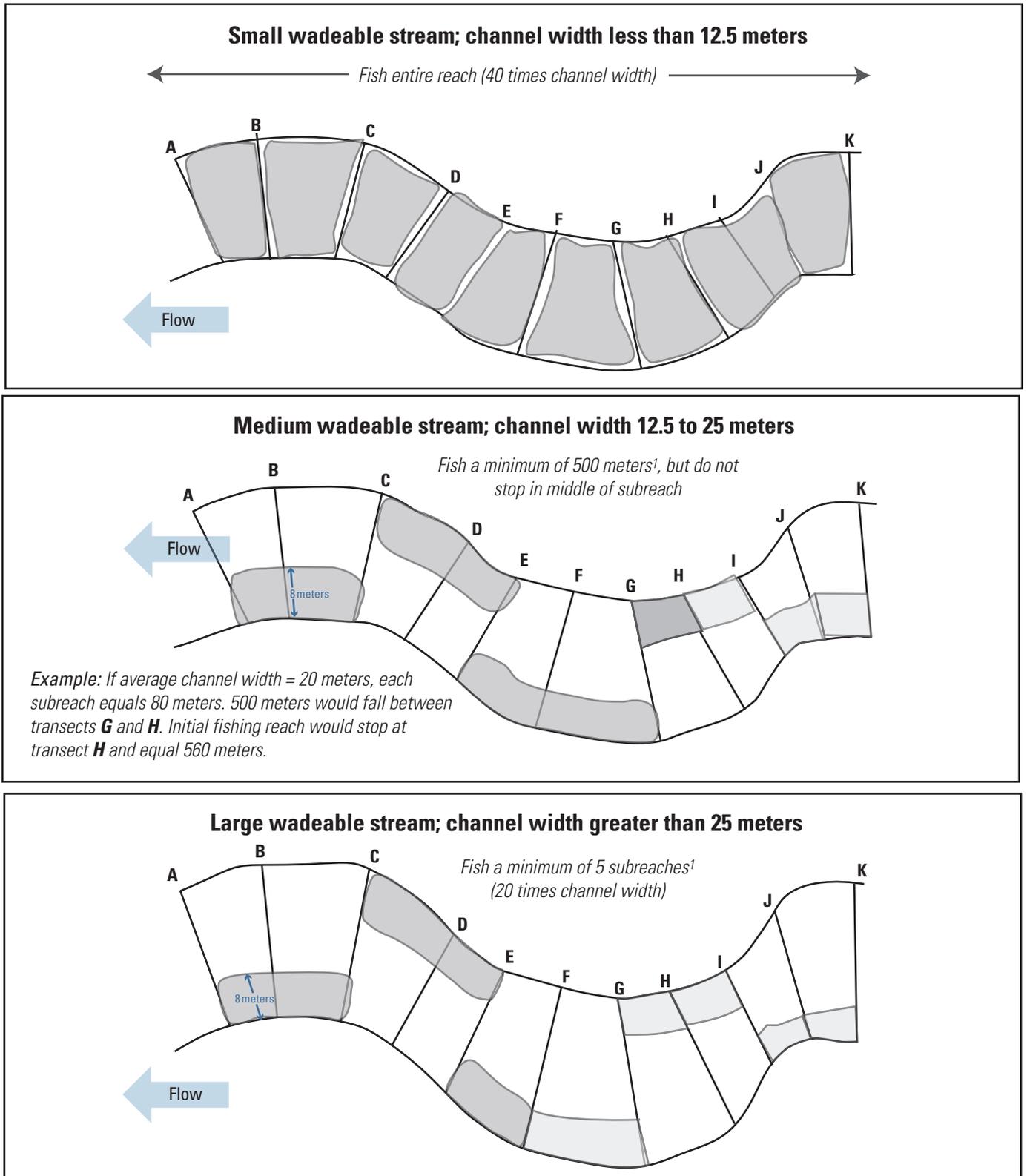


Figure 8. Sampling reach features for a wadeable site. From U.S. Environmental Protection Agency (2013).



¹At medium and large streams, if less than 500 individuals have been collected after minimum sampling reach, continue fishing to next transect (alternating banks) until 500 individuals are collected or transect K is reached (10 subreaches fished).

Figure 9. The reach layouts for fish sampling in small, medium, and large wadeable streams. From U.S. Environmental Protection Agency (2013).

A representative fish community sample was collected at each site using backpack- or barge-mounted electrofishing units. Streams with total reach length less than 500 m (40 times an average stream width of 12.5 m) were fished continuously for all habitats along the sample reach. Streams with wetted widths greater than 12.5 m were sampled in 5–10 sampling zones, on alternating banks, distributed along the reach (fig. 9). Species identifications, tallies, and other information for individuals collected were recorded within four size categories. Federally listed threatened or endangered species or large game fish were processed (identified and measured for length) immediately and released back to the stream. Species not positively identified in the field were separately retained (as many as 20 individuals per species) for laboratory identification or digital photographs were taken. Retained fish were preserved initially in 10 percent formalin with later storage in 45–50 percent isopropyl alcohol or 70 percent ethanol. Tissue plug samples were collected from individual specimens from the fish community sample, targeting commonly consumed species (table 7).

Table 7. Fish target species and minimum size requirements by family.

Common name	Scientific name	Estimated minimum size (millimeters)
Centrarchidae		
Largemouth bass	<i>Micropterus salmoides</i>	280
Smallmouth bass	<i>Micropterus dolomieu</i>	300
Spotted bass	<i>Micropterus punctulatus</i>	280
Black crappie	<i>Pomoxis nigromaculatus</i>	330
White crappie	<i>Pomoxis annularis</i>	330
Ictaluridae		
Channel catfish	<i>Ictalurus punctatus</i>	300
Blue catfish	<i>Ictalurus furcatus</i>	300
Flathead catfish	<i>Pylodictus olivaris</i>	300
Percidae		
Sauger	<i>Sander canadensis</i>	380
Walleye	<i>Sander vitreus</i>	380
Yellow perch	<i>Perca flavescens</i>	330
Moronidae		
White bass	<i>Morone chrysops</i>	330
Esocidae		
Northern pike	<i>Esox lucius</i>	430
Chain pickerel	<i>Esox niger</i>	430
Salmonidae		
Brown trout	<i>Salmo trutta</i>	300
Cutthroat trout	<i>Onchorhynchus clarkii</i>	300
Rainbow trout	<i>Onchorhynchus mykiss</i>	300
Brook trout	<i>Salvelinus fontinalis</i>	330

The reach physical habitat was characterized by measuring substrate, canopy cover, instream fish cover, bankfull condition, riparian vegetation, evidence of human disturbances, discharge, and channel measurements such as depth, width, stream slope, and bank angle. The six components of the physical habitat characterization—(1) thalweg profile; (2) wetted width/bar width; (3) woody debris tally; (4) channel and riparian characterization; (5) assessment of channel constraint, debris torrents, and major floods; and (6) discharge measurement—are detailed in the NRSA field operations manual (U.S. Environmental Protection Agency, 2013, section 8).

Laboratory Analyses

Water samples were analyzed by the USGS NWQL in Denver, Colorado; the USGS Organic Geochemistry Research Laboratory in Lawrence, Kansas; the USGS California Pesticide Fate Research Laboratory (CAPEST) in Sacramento, California; the USGS Sediment Laboratories in Iowa City, Iowa, in Louisville, Kentucky, and in Rolla, Missouri; the USGS Wisconsin Mercury Research Laboratory in Middleton, Wisconsin; the USGS Texas WSC Laboratory; the USGS National Research Program (NRP) Stable Isotope Laboratory in Reston, Virginia; and the EPA OPP Laboratory. Periphyton samples collected during the ecological assessment were analyzed by the NWQL for algal pigments and biomass. Bank-scrape and bed sediment samples collected concurrently with ecological assessments were analyzed by the CAPEST; the NWQL; the USGS Crustal Geophysics and Geochemistry Analytical-Research Laboratory (CGGAR) and the USGS Central Mineral and Environmental Resources Analytical Laboratory (CMERA) in Denver, Colorado; and the USGS Sediment Laboratory in Rolla, Missouri. Fish tissue samples were analyzed for mercury by contract laboratories through arrangements with the EPA NRSA program. Additional analyses are described in the “Special Studies” section.

Field properties, analytical constituents, and field or analytical methods are outlined in appendix 2, tables 2–1 and 2–2 for water samples. Periphyton analysis methods are described in appendix 2, table 2–3. Analytical constituents and methods for bank-scrape and bed sediment samples are listed in appendix 2, table 2–4. Chemical analyses of fish tissue are listed in appendix 2, table 2–5. This report contains Chemical Abstracts Service (CAS) Registry Numbers, which are registered trademarks of the American Chemical Society. The CAS recommends the verification of the CAS Registry Numbers through CAS Client Services.

Index-period water samples at all sites (BASIC group) were analyzed for pesticides (NWQL schedule 2437); nutrients (ammonia, nitrate plus nitrite, nitrite, orthophosphate, phosphorus, and total nitrogen); chloride; sulfate; suspended-sediment concentration and percent smaller than 0.0625 mm; DOC; absorbance at 254 nanometers; glyphosate by immunoassay; and field parameters (water temperature, pH, dissolved

oxygen, and specific conductance). At all 100 sites, samples were analyzed once a month for phytoplankton algal pigments (chlorophyll *a* and pheophytin *a*) and twice a month for stable isotopes in nitrate.

Additional constituents in index-period water samples were analyzed at a subset of sites by group—total and methyl mercury at 71 sites (MERC group) biweekly; glyphosate and related compounds (by LC–MS/MS) and hormones (NWQL schedule 4434) at 27 sites (INT group) biweekly; and additional major ions, laboratory alkalinity, and additional pesticides including pyrethroids in water and suspended sediment at 10 sites (WTOX group) biweekly. Pesticides (NWQL schedule 2437) were analyzed in daily and weekly composite water samples and in reagent-water and matrix (sampled stream water) spikes from the micro-autosamplers (AUS group). Particulate and total dissolved nitrogen were analyzed weekly at selected sites, to assess potential bias in total nitrogen digestion method performance. Glyphosate was determined by LC–MS/MS methods in a subset of samples, as a QC check on the immune-assay method used for glyphosate at all sites and samples. Extracts from POCIS were analyzed for pesticides similar to NWQL schedule 2437. Extraction methods are described by Alvarez (2010), and extracts were analyzed following methods for analysis of water samples (appendix 2).

Bed samples collected during the ecological assessment from all sites (BASIC group) were analyzed for pesticides, carbon, major and trace elements, radionuclides, and grain size. Additional analyses for bed samples at INT group sites were organic waste indicator compounds, hormones, halogenated compounds, and PAHs and other semivolatile organic compounds. Bank samples were analyzed for radioisotopes, major and trace elements, carbon, and grain size from all sites (BASIC group).

Bed sediment samples, analyzed for radionuclides by the USGS NRP Laboratory in Menlo Park, California, and major and trace elements by the CGGAR and CMERA in Denver, Colorado, were first sent to the USGS Maryland WSC for sieving and drying. Samples were wet sieved through a 63- μm polyester sieve; water and fine sediment were collected in 1- or 2-L bottles and refrigerated for a week. If the sediment settled, the sample was then decanted and in each case, the resulting slurry was split. A subsample was sent to the NRP Laboratory where it was freeze dried for analysis of radionuclides. The other subsample was sent to the USGS Texas WSC in Austin, Texas, where it was freeze dried. Some of the subsample sent to the Texas WSC was sent to the Denver laboratories for analyses of major and trace elements, mercury, and forms of carbon.

Bank samples were maintained chilled (4 °C), wet sieved through a 63- μm polyester sieve, and decanted after settling 1 week. The resulting slurry samples were shipped on ice to the NRP Laboratory in Menlo Park, California, where they were freeze dried for radioisotope analysis. The freeze-dried sample was then shipped on ice to the Texas WSC where the sample

was split, and subsamples were shipped on ice to the CGGAR and CMERA in Denver, Colorado, for analysis of major and trace elements and carbon.

Water and periphyton samples collected during the ecological assessment at all sites (BASIC group) were analyzed through arrangements with the EPA NRSA for algae identification and algal biomass. Fish tissue samples collected during the ecological assessment at sites in the MERC group were analyzed for mercury in tissue plugs through arrangements with EPA NRSA.

Quality Assurance and Quality Control

The QA procedures and data from QC samples maintain the integrity, accuracy, and legal defensibility of results from data collection and assessment. Documented QA policies and procedures were implemented in the MSQA study to ensure that the data can be interpreted properly and be scientifically defensible (Mueller and others, 1997; U.S. Geological Survey, 2006). The QC samples were collected to identify, quantify, and document bias and variability in data that result from the sampling procedure, including collection, processing, shipping, and handling of samples. Training was held for all field personnel prior to the sampling period to ensure that appropriate and consistent methods were used. Data management QA procedures included steps for planning, data collection, sample status tracking, data transfer, database management, and data review for completeness, precision, bias, and transcription errors.

Quality Control

The QC samples included field blanks, matrix spikes, and replicates (table 8, appendix 3). Field blanks were used to demonstrate that cleaning procedures were adequate to remove any sampling equipment contamination introduced by samples collected at previous sites and to ensure that sample collection, processing, handling, and shipping did not result in contamination (Mueller and others, 1997; U.S. Geological Survey, 2006). Field split replicates were prepared by dividing a single volume of water into two samples. These replicates provided a measure of the variability introduced during sample processing and analysis (Mueller and others, 1997; U.S. Geological Survey, 2006). Field and laboratory matrix spikes were used to assess the potential bias for analytes in a particular sample matrix. Bias is estimated from spiked samples by calculating the percentage of the added analyte (spike material) measured (recovered) in the sample (Mueller and others, 1997; U.S. Geological Survey, 2006). Recovery either can be greater than or less than 100 percent, so the bias either can be positive or negative; however, matrix interference and analyte degradation generally result in a negative bias.

Table 8. Summary counts of environmental, field blank, replicate, and spike samples of streamwater from 100 stream sites sampled in the U.S. Geological Survey (USGS) Midwest Stream Quality Assessment (MSQA) study in 2013.

[Recommended percentage from Mueller and others (1997); QC, quality control; --, not applicable; NA, not applicable because no recommendation given; TPN, total particulate nitrogen; MERC, mercury; INT, intensive contaminants monitoring; KS OGRL, Kansas organic geochemistry research laboratory; WTOX, water toxicity; QC samples in bold do not meet recommended guidelines within the project, but samples were collected and analyzed with protocols consistent with other national USGS programs to allow for pooled assessment (Mueller and others, 2015)]

Laboratory analyses	Type of sample	Sample counts	Ratio of QC to environmental samples (percent)	
			Project	Recommended
BASIC group				
Chloride and sulfate	Environmental	1,200	--	--
	Replicate	55	4.6	¹ 1.4
	Blank	44	3.7	¹ 1.4
Nutrients	Environmental	1,200	--	--
	Replicate	59	4.9	¹ 1.4
	Blank	60	5.0	¹ 1.4
Dissolved organic carbon	Environmental	600	--	--
	Replicate	50	8.3	¹ 2.8
	Blank	50	8.3	¹ 2.8
Pesticides	Environmental	1,200	--	--
	Replicate	50	4.2	10.0
	Blank	50	4.2	¹ 1.4
	Spike	100	8.3	8.3
Glyphosate (immunoassay)	Environmental	1,200	--	--
	Replicate	50	4.2	10.0
	Blank	50	4.2	¹ 1.4
Chlorophyll <i>a</i>	Environmental	300	--	--
	Replicate	30	10.0	NA
	Blank	30	10.0	NA
Nitrate isotopes	Environmental	200	--	--
	Replicate	30	15.0	NA
TPN group				
Particulate and total filtered nitrogen	Environmental	216	--	--
	Replicate	11	5.1	5.0
	Blank	10	4.6	5.0
MERC group				
Total and methyl mercury	Environmental	426	--	--
	Replicate	25	5.9	5.0
	Blank	25	5.9	5.0

Table 8. Summary counts of environmental, field blank, replicate, and spike samples of streamwater from 100 stream sites sampled in the U.S. Geological Survey (USGS) Midwest Stream Quality Assessment (MSQA) study in 2013.—Continued

[Recommended percentage from Mueller and others (1997); QC, quality control; --, not applicable; NA, not applicable because no recommendation given; TPN, total particulate nitrogen; MERC, mercury; INT, intensive contaminants monitoring; KS OGRL, Kansas organic geochemistry research laboratory; WTOX, water toxicity; QC samples in bold do not meet recommended guidelines within the project, but samples were collected and analyzed with protocols consistent with other national USGS programs to allow for pooled assessment (Mueller and others, 2015)]

Laboratory analyses	Type of sample	Sample counts	Ratio of QC to environmental samples (percent)	
			Project	Recommended
INT group				
Glyphosate (KS OGRL)	Environmental	162	--	--
	Replicate	7	4.3	10.0
	Blank	7	4.3	5.0
	Spike	14	8.6	8.3
Hormones	Environmental	162	--	--
	Replicate	7	4.3	² 10.0
	Blank	7	4.3	² 5.0
	Spike	14	8.6	8.3
WTOX group				
Major ions	Environmental	60	--	--
	Replicate	5	8.3	5.0
	Blank	4	6.7	3.3
Pyrethroids	Environmental	60	--	--
	Replicate	5	8.3	10.0
	Blank	4	6.7	5.0

¹Mueller and others (1997) recommends a reduced alternate percentage of once per month if a large number of environmental samples are collected in a short period, with a minimum of one blank and one replicate prepared by each crew. For the MSQA study, the alternate percentage is, therefore, based on 17 crews.

²Recommendation from Mueller and others (1997) for pesticides given for comparison because none given for hormones.

Field blanks were collected once or twice at 44–59 sites, depending on analyte, and replicates were collected at 48–51 sites, for the basic laboratory schedules (chloride, sulfate, nutrients, pesticides, and glyphosate by immunoassay) collected weekly plus DOC collected biweekly (table 8, appendix 3). For QC samples collected as part of NAWQA, Mueller and others (1997) recommends 1 field blank per 30 samples (3.3 percent) for major ions, 1 replicate per 10 samples (10 percent) for pesticides, 1 matrix spike per site (8.3 percent for MSQA) for pesticides, and a ratio of 1 per 20 samples (5 percent) for field blanks and replicates for most other constituent groups, including major ions, nutrients, DOC, pesticides, and trace elements. If a large number of environmental samples are collected in a short period of time for field blanks and replicates for major ions, nutrients, DOC, and trace elements and for field blanks for pesticides lowering the QC sample frequency to one per month is recommended, but not less than one for each field crew. For BASIC group constituents, therefore, the expected QC was 1.4 percent, based on 17 field crews. Actual field blanks represented 3.7–8.3 percent of the weekly environmental samples and split replicates for the same analyses represented 4.2–8.3

percent of the environmental samples, which met the recommended frequency for most BASIC group constituents (table 8; Mueller and others, 1997).

No recommendation was provided for the organic compounds of emerging concern (pharmaceuticals, organic wastewater indicators, and hormones) in Mueller and others (1997). Field blanks and split replicates for pharmaceutical and organic wastewater indicator analyses represented 4.7 percent of the environmental samples and increased to 5 and 25 percent, respectively, for hormone analysis (table 7). Matrix spikes were analyzed for organic compounds, with the exception of glyphosate analysis by immunoassay. The frequency of these spikes was about one spike per every 20 environmental samples (table 8).

Additional guidelines for assessing QC sample data described by Mueller and others (2015) emphasize collecting a sufficient total number of QC samples for a robust analysis. Because sample collection protocols and analytical procedures are consistent within each RSQA, and in most cases with the overall NAWQA Project, an assessment of pooled QC samples will be possible. In some cases, constituent groups did not meet the NAWQA-recommended QC ratios (Mueller and others,

1997), but a sufficient total QC sample size was collected for a robust analysis. For example, the recommended ratio for pesticides replicates is 10 percent; however, 50 MSQA replicates will allow an estimate of standard deviation within 20 percent of the true standard deviation with 95 percent confidence (Mueller and others, 2015). For analyte groups with small sample counts, the recommended QC ratios yield an insufficient number of replicates for a statistically robust assessment of variability. For example, although the recommended replicate ratios for major ions (5 percent) was met (8.3 percent for the project) for the 60 samples in the WTOX group, the 5 replicates will be pooled with other RSQAs and NAWQA QC data.

Quality Assurance

The QA of field data collection included maintaining standardized sample collection and handling protocols among all field personnel as described in the National Field Manual (U.S. Geological Survey, variously dated) for water and sediment sampling and by the EPA NRSA Field Operations Manual (U.S. Environmental Protection Agency, 2013) for ecological sampling. Additional guidance on specific types of sample or data collection are obtained from Wagner and others (2006) and Sauer and Turnipseed (2010) for continuous data, from Alvarez (2010) for passive samplers, from Shelton and Capel (1994) for bed sediment, and from Moulton and others (2002) for ecological sampling. Field personnel involved in the MSQA complete annual performance assessments to verify proficiency in collecting field data, including temperature, pH, dissolved oxygen, alkalinity, and specific conductance. Applicable sampling and handling protocols were reviewed by field personnel involved in the MSQA and NRSA studies during training courses prior to field work.

Water-quality data from each sample event were reviewed for completeness, precision, bias, and transcription errors when received from the laboratory as part of the QA/QC procedures. Water-quality and sediment-quality data were stored in the National Water Information System (NWIS) database. Quality-assured water-quality and sediment-quality data are available for retrieval on the internet at <https://water-data.usgs.gov/nwis/sw> and most MSQA data are publicly available on the RSQA Web site at <https://txpub.usgs.gov/RSQA/>. The NWQL provides all QA/QC documentation for their analytical services on the internet at <http://nwql.usgs.gov/Public/quality.shtml>.

The final goal of the data management process for the RSQA, including MSQA, is to have all appropriate data reviewed, approved, and stored with the appropriate data quality indicator (DQI) code in the NWIS database or RSQA team database. The sampling locations and teams for the MSQA study were in multiple States. Each State has a USGS WSC NWIS database host for data entry and retrieval. Additionally, a central team of national RSQA and regional MSQA members play a role in the data management process. The centralization of the data management process was essential to ensure

consistency among the WSCs and among RSQA study areas. The nine main steps implemented for the data management process were as follows:

- Sampling matrix and sample coding design
- Electronic field form utilization
- Sample status checks at all laboratories
- NWIS sample record checks
- Data transfer from laboratory to NWIS
- Establishment of project networks
- Sample coding and field parameter checks
- Data quality checks (water, sediment)
- Approval of data in NWIS

Prior to the start of sampling, the MSQA team prepared the sample matrix design and sample coding plan for all aspects of the field process. The sampling matrix distributed replicate, blank, and matrix spike QC samples equally across sites, sample teams, and time periods for optimum coverage. The matrix also served as a summary diagram for the type, frequency, and location of environmental and QC samples to be collected and is summarized in table 8 and appendix 3. Field data and field supply managers of the central team provided bottle sets along with the corresponding preprinted analytical services request forms (ASRs) each week. The field data and field supply managers of the central team used a consistent sample coding scheme among the MSQA sampling teams to ensure a well-structured and manageable dataset. Additionally, training and written guidelines for sampling coding were made available to sampling teams prior to the start of sampling.

The MSQA sampling teams from all the WSCs used the Personal Computer Field Form (PCFF) version 6.1F or 7.0 software created by the USGS, which provides electronic field forms for data collection at sampling sites. The PCFF software streamlines the process of uploading (logging in) field data and sample codes to NWIS by automatically generating the batch load files required by NWIS (qwsample and qwresult), thereby resulting in a more efficient process of data flow from field and laboratory to database. For each sample, the information uploaded to NWIS and later the results received from the laboratory are identified based on station identification number, date, time, and medium code. In addition to the unique number assigned in NWIS, each MSQA sample was labeled with a unique barcode as a backup sample tracking identifier. The automation of data upload to NWIS limits the incidence of transcription errors associated with the manual entry of data into NWIS.

The field data manager of the central team continuously tracked the shipments to verify that the shipped samples were received at all laboratories (1) within the correct holding times, (2) in the proper condition (for example, chilled samples received at the appropriate temperature of 4 °C or

below), and (3) with proper documentation. Sample shipment schedules were established prior to the start of sampling for MSQA, which ranged from once per day to once per week depending on the sample type (appendix 4). Sampling teams and other WSC personnel were responsible for the shipment process. The field data manager worked with the laboratories to correct problems with mislabeled samples or ASRs in a timely manner and to communicate problem-resolution approaches to WSC personnel. During this process, the field data manager also established the connection between the USGS Laboratory Information Management System (LIMS) used to transfer sample results and the NWIS database used to receive and store sample results.

During sampling and the corresponding establishment of sample records in NWIS, the field data manager of the central team inspected sample coding and procedures of sample records in NWIS to ensure that samples were established properly and in a consistent manner. Sample coding or procedures were modified if determined to be inaccurate or inconsistent. These modifications involved changes or corrections to sample time offsets, sample type coding, or other documentation at the laboratory or in NWIS. Modifications in sample coding or procedures related to data management or sample submittal were communicated immediately to sampling teams to ensure that appropriate adjustments were made before the next sampling.

Most of the laboratories used for sample analysis by the MSQA transmitted sample results through the Water Quality Data Exchange (QWDX) for automatic upload into the NWIS database (appendix 1, table 1–2). For laboratories without the ability to use QWDX, sample results were loaded into NWIS using manually created batch files. Batch files were created by the field data manager upon receipt of electronic data from the laboratory. Batch files were loaded into the respective WSC NWIS host by the field data manager, WSC personnel, or the local data manager. The field data manager verified that the manually loaded data were properly loaded into NWIS. Data files provided by laboratories through email and data not applicable to NWIS (for example, CERC toxicity data) were stored electronically in a centrally located project database rather than NWIS.

Once sampling sites were selected for MSQA, the field data manager, with input from the central team, identified the appropriate network designations in NWIS ProjectNetworks to allow integration of similar sites across many regions and to designate the site type in the NAWQA Data Warehouse. These network designations were obtained from the project planning documents and, where possible, kept consistent with other network designations from previous regional studies. The ProjectNetworks documentation was provided to local WSC personnel so they could establish the sites in NWIS ProjectNetworks.

After sampling was completed, the field data manager inspected the NWIS sample records for completeness regarding field data collection, including stream measurements (streamflow, stage, sampling points, stream width, and so forth), field parameters (pH, air and water temperature, specific conductance, and dissolved oxygen), and proper sample coding (sample purpose, purpose of site visit, sampling method, sampler type, and multiple QC-related sample codes). Manual checks were made for each sample and any corrections were communicated to WSC personnel. The field data manager, WSC personnel, or local data manager made any needed changes in NWIS.

National RSQA team members provided final reviews of the water-quality and sediment-quality results received from the laboratories. The water-quality data reviews included identification of extremes in the data (outliers), inconsistencies or unexpected results in the data, major differences between environmental samples and replicates, detected values in blanks, and low analyte recoveries in spike samples. These team members communicated requests for reruns, reloads, and verification of results from the laboratory as necessary. The national RSQA team members involved in the review process worked closely with the field data manager to verify the completeness of sample results and to verify that a final dataset was established in NWIS and the RSQA team database.

Upon completion of the data review process by the national RSQA team members, the field data manager provided the appropriate WSC personnel with a table of the data review results from the RSQA team database for their own internal review. Subsequently, WSC personnel changed the DQI codes for each individual water-quality parameter, based on the results of the review, to reviewed and accepted (R) or reviewed and rejected (Q). Data that were considered reviewed and rejected were neither used in the data analysis nor in the interpretation process of the study.

Special Studies

Several special studies were completed as components within the MSQA. These studies provide finer-scale temporal data or more extensive analysis at a logistically manageable number of sites by using alternate sampling regimes or experimental designs, or both. Special study groups were continuous nitrate and biological response (CNIT), micro-autosampler for pesticides (AUS), water toxicity (WTOX), caged fish and frog (CFF), and sediment source (SED). Detailed procedures for experiments in these groups are beyond the scope of this report, but overall objectives, approach, and sampling for each component are described in the following section.

Continuous Nitrate and Biological Response

Nutrients and their effects on biological communities remain a major concern for State and Federal agencies. An investigation of nutrients and associated biological response was completed at six sites in the MSQA region to provide high-resolution information on seasonal nutrient dynamics (table 9). The objective of this study was to determine how short-term variability in physical, chemical, and biological components of the ecosystem might affect trophic classification of a stream. Continuous nitrate records provide a temporal context for discrete measurements and can be used to elucidate the effects of various stream processes on nitrate levels. Dissolved oxygen levels can change rapidly within the stream, typically in response to changes in nutrient and algal dynamics, and continuous monitoring can be used to track low-oxygen events that may adversely affect aquatic life.

The CNIT group included six sites in Illinois, Indiana, and Iowa, with sites split evenly between high or low nutrient loading, based on fertilizer, manure, and wastewater input of nitrogen and phosphorus to the basin (table 9, appendix 1). Of the six sites, four sites had existing streamflow gages. The sites were instrumented with continuous water-quality monitors from May to September, measuring temperature, dissolved oxygen, pH, specific conductance, turbidity, chlorophyll *a*, fluorescent dissolved organic matter (a proxy for carbon concentrations), and nitrate using YSI EXO meters and Satlantic SUNA nitrate sensors (YSI Incorporated, 2012; Satlantic LP, 2013). Data collection and computations followed

guidelines for deployment and operations for water-quality meters described by Wagner and others (2006) and for nitrate sensors described by Pellerin and others (2013).

In addition to sampling during the MSQA 14-week index period and the ecological surveys done at all MSQA sites, periodic sampling and data collection were completed six times from May to September at the six CNIT sites to assess changes in the trophic state of the stream. Artificial habitat (tiles) placed at the start of the water-quality index period were used to assess the accrual rates of benthic algae at the six sites. The following were completed during each of the six trophic state assessments:

1. Reach-scale benthic chlorophyll *a* samples were collected.
2. Aquatic macrophyte cover was determined using established transect methods, along with data for center channel densitometer readings, sample depth, and substrate type.
3. Three tiles were removed and analyzed for chlorophyll *a* and biomass.
4. Subsurface (hyporheic) water samples were collected and analyzed for chloride and nutrients.

Periphyton from natural and artificial substrate (tiles) samples were analyzed by the USGS NWQL in Denver, Colorado, for algal pigments (chlorophyll *a* and pheophytin *a*) and biomass (appendix 2, table 2–3).

Table 9. Midwest streams selected for continuous nitrate and biological response (CNIT) special study.

[NWIS, U.S. Geological Survey National Water Information System database; mi², square mile; kg/km², kilograms per square kilometer; Ill., Illinois; Ind., Indiana]

Station name	NWIS station number	Gaged	Drainage area (mi ²)	Watershed base-flow index	Riparian canopy (percent)	Nitrogen (kg/km ²)	Phosphorus (kg/km ²)
Low nutrient							
Lusk Creek near Eddyville, Ill.	03384450	Yes	43	14	81	502	114
Otter Creek at N County Road 560 E near Butlerville, Ind.	390033085300301	No	62	18	63	3,502	747
Nineveh Creek at Stone Arch Road near Nineveh, Ind.	392158086035901	No	6	28	54	5,755	1,278
High nutrient							
North Fork Maquoketa River near Fulton, Iowa	05418400	Yes	505	52	10	9,874	2,139
South Fork Iowa River NE of New Providence, Iowa	05451210	Yes	224	41	40	11,578	3,289
Eagle Creek at Zionsville, Ind.	03353200	Yes	106	28	46	6,288	1,233

Micro-Autosampler

Weekly discrete water chemistry might be insufficient to describe stressor effects on biota for pesticides, which can have acute toxic and cumulative sublethal effects. Though discrete samples used with continuous streamflow can be used to calculate chemical transport appropriate for monthly or longer average time steps (fig. 10), increasing sample frequency might better describe conditions experienced by the biota, such as brief, acutely toxic events. An example of continuous atrazine concentration estimated by rating-curve method from discrete samples and continuous streamflow is shown in figure 10. The example shows that atrazine concentrations modeled in this way are a poor match to sampled peak atrazine concentrations, such as samples in May and July in the example. Logistical and practical constraints, however, typically limit sample collection intervals. Data from daily and weekly composite samples better describe the occurrence and short-term variability in constituent concentrations than periodic (weekly) samples, providing a better understanding of potential chronic and acute exposures of biota to contaminants. The objectives of this special study were to evaluate the feasibility and effectiveness of micro-autosamplers in a field test and to compare pesticide concentrations in daily and weekly composite samples from the automated samplers to other sampling

methods to evaluate the benefits of various compositing and sampling strategies.

Micro-autosamplers collected daily and weekly composite samples (6-hour aliquot interval for daily samples and 12-hour interval for weekly samples) for analysis of pesticides (fig. 11). A ninth vial contained a solution of known pesticide concentrations (a spike) to assess possible degradation of compounds during deployment. During the first few weeks of sampling, the spike was added to laboratory reagent (blank) water. During the rest of the sampling period, the spike was added to a split of the water collected from the stream for the weekly water sample (an environmental matrix spike). All vials in the sampler had a buffer solution added as a preservative prior to deployment in an attempt to limit compound degradation. Samplers were swapped weekly to collect sample vials, charge batteries, clean tubing, and replace consumable components such as filters. The seven MSQA sites—two urban sites and five agricultural sites—for this special study were selected to target watersheds with expected high occurrence of pesticides, considering logistical limitations for the sampler installations, and to maximize additional data available to provide context for results (table 10).

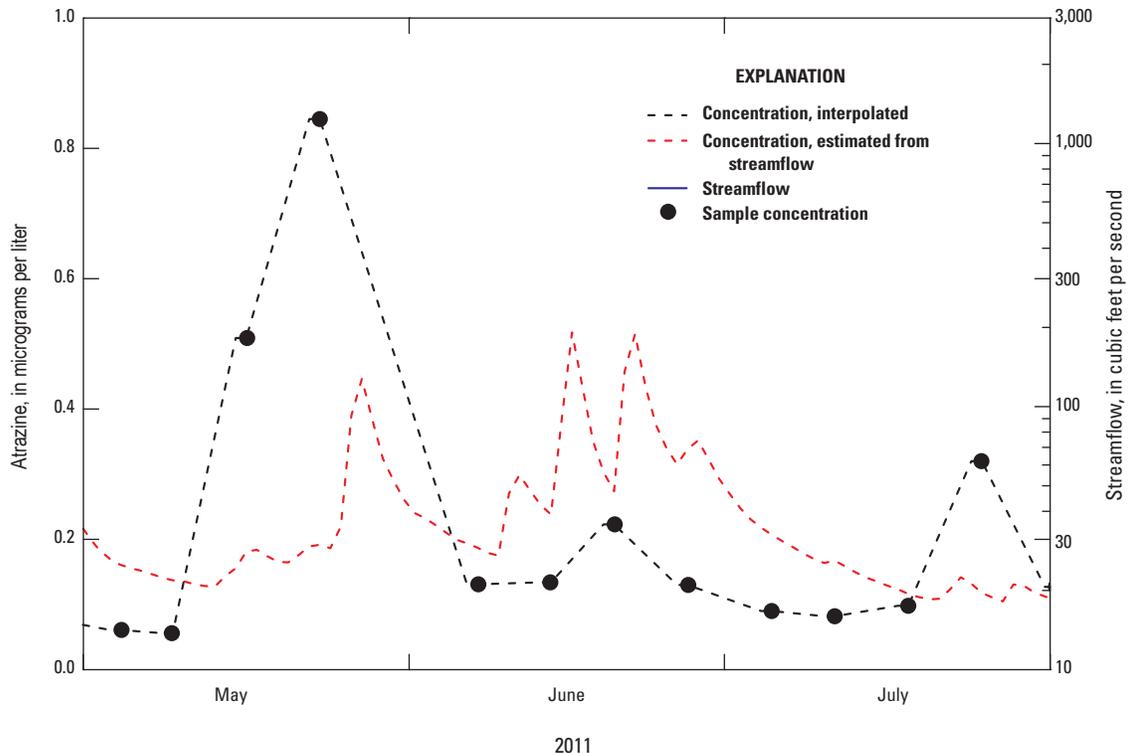
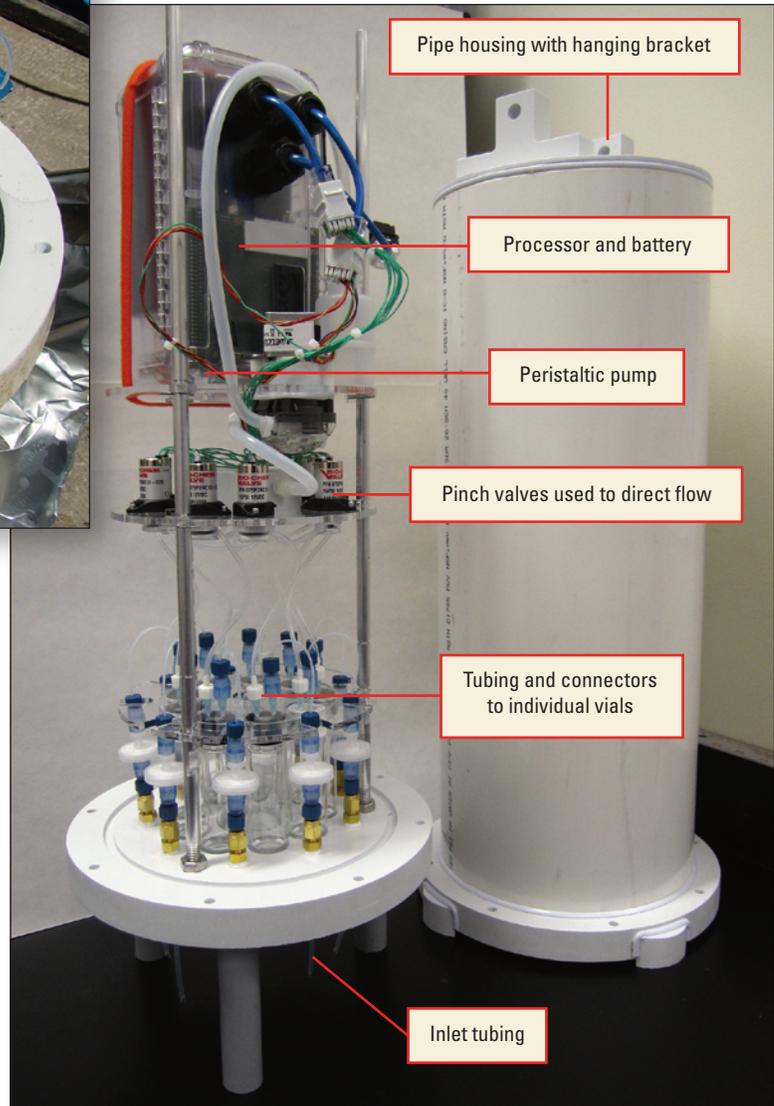


Figure 10. Example of periodic atrazine samples and continuous streamflow used to determine chemical transport, based on the assumption that streamflow and concentrations are empirically related; poor model fit during episodic high pesticide concentrations make these estimates poor for describing acutely toxic events.



Figure 11. Micro-autosamplers designed and built at Portland State University were used to collect filtered water at subdaily intervals for pesticide analysis.



The low-volume micro-autosamplers take advantage of direct aqueous-injection liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods for pesticide analysis with nanograms per liter detection limits that require only a few milliliters of water. Splits from the weekly composite water sample and spike samples from the micro-autosamplers were analyzed by the NWQL for pesticides by direct aqueous-injection LC-MS/MS (appendix 2, table 2-2). Splits from those samples and each of the 7-daily composite samples

were analyzed at the EPA OPP Laboratory in Fort Meade, Maryland, for pesticides. The OPP Laboratory used the NWQL direct aqueous-injection LC-MS/MS method with the exception that the OPP Laboratory used a different LC-MS/MS instrument than the NWQL. The OPP Laboratory used a Waters Model Xevo TQ, and the NWQL used an Agilent Model 6460. The result of this difference is that reporting and detection levels for the OPP Laboratory results are higher than for the NWQL results, generally by a factor of about 10.

Table 10. Midwest streams selected for micro-autosampler (AUS) special study.

[NWIS, U.S. Geological Survey National Water Information System database; mi², square mile; Aii, agricultural intensity index; TWUI_13bs_kgkm², toxicity weighted pesticide use in 2013 relative to benthic invertebrate species; TWUI_13cs_kgkm², toxicity weighted pesticide use in 2013 relative to cladocerans; TWUI_13fs_kgkm², toxicity weighted pesticide use in 2013 relative to fish species; Wis., Wisconsin; Ind., Indiana; Mo., Missouri; Nebr., Nebraska]

Station name	NWIS station number	Drainage area (mi ²)	Urban	Cultivated crops	Aii	Gaged	TWUI_13bs_kgkm ²	TWUI_13cs_kgkm ²	TWUI_13fs_kgkm ²
Urban									
Honey Creek at Wauwatosa, Wis.	04087119	10.3	99.6	0.0	0.0	Yes	0.00	0.00	0.00
Lincoln Creek at Sherman Boulevard at Milwaukee, Wis.	040869416	13.5	93.8	0.0	1.3	Yes	0.00	0.00	0.00
High agricultural intensity									
Eagle Creek at Zionsville, Ind.	03353200	106	14	70	69	Yes	6,913	2,947	433
South Fork Iowa River NE of New Providence, Iowa	05451210	224	6.7	86	80	Yes	30,635	22,551	1,826
Goodwater Creek near Centralia, Mo.	391815009203901	28	7.9	67	59	No	2,785	1,453	152
Sugar Creek at County Road 400 S at New Palestine, Ind.	394340085524601	93	8.0	82	77	Yes	8,305	3,938	503
Bell Creek near Arlington, Nebr.	413012096210001	167	3.8	88	83	No	63,100	17,353	3,856

Water Toxicity

The water toxicity special study investigated stream-water toxicity under controlled laboratory conditions. Specific objectives pertaining to the WTOX group sites were to assess the toxicity of water to daphnids and fish during 7-day exposure and to evaluate the extent to which toxicity is related to concentrations of pesticides in water and suspended sediment. Though details of the laboratory experiments of this special study are beyond the scope of this report, the overall design is summarized. The number of sites and samples were limited by laboratory capacity and method requirements for minimal holding times. The 10 sites selected for water toxicity testing consisted of 3 urban and 7 intensive agricultural sites and were selected assuming relatively high occurrence of pesticides based on variables important in affecting the occurrence and transport of agrochemicals (tables 1 and 11). All 10 sites also are in the INT group, 8 sites are gaged, and the 2 sites without streamflow gages are in the caged fish and frog group.

Every other week at the 10 sites in the WTOX group, additional water was collected in a 14-L container for use in ambient water toxicity testing. Water samples were collected, chilled at 4 °C, and shipped overnight to the CERC Yankton Field Research Station (Yankton, South Dakota).

Additional chemical water analyses were included (drawn from the churn splitter) when ambient water toxicity samples were collected to potentially link water toxicity findings from the experiments with specific chemical stressors. Samples were analyzed for additional major ions, metals, and alkalinity by the NWQL (appendix 2, table 2–1). Pyrethroids and other moderately to strongly hydrophobic pesticides in filtered water samples and in suspended sediment (trapped on the filters) were analyzed by the CAPEST (appendix 2, table 2–2).

Table 11. Midwest streams selected for water toxicity (WTOX) special study.

[NWIS, U.S. Geological Survey National Water Information System database; mi², square mile; LMH, sum of low, medium, and high categories; Aii, agricultural intensity index; Wis., Wisconsin; Nebr., Nebraska; Ind., Indiana; Mo., Missouri]

Station name	NWIS station number	Gaged	Drainage area (mi ²)	Watershed base-flow index	Stream slope (percent)	Watershed tile-drained land use (percent)	Urban LMH	Cultivated crops	Aii
Urban									
Honey Creek at Wauwatosa, Wis.	04087119	Yes	10	38	2.1	0.0	88	0.0	0.0
Little Papillion Cr at Ak-Sar-Ben at Omaha, Nebr.	06610765	Yes	50	41	7.3	0.0	37	31	25
Lincoln Creek at Sherman Boulevard at Milwaukee, Wis.	040869416	Yes	13	40	1.6	0.0	81	0.0	1.3
High agricultural intensity									
Eagle Creek at Zionsville, Ind.	03353200	Yes	106	28	1.3	46	5.5	70	69
North Fork Maquoketa River near Fulton, Iowa	05418400	Yes	505	52	6.9	4.7	1.9	59	56
South Fork Iowa River NE of New Providence, Iowa	05451210	Yes	224	41	1.8	50	1.2	86	80
Maple Creek near Nickerson, Nebr.	06800000	Yes	368	41	5.1	1.3	0.6	81	69
Fish Branch near Mexico, Mo.	391443091534001	No	17	13	2.9	0.0	0.2	77	56
Goodwater Creek nr Centralia, Mo	391815009203901	No	28	9.0	1.4	0.0	3.4	67	59
Sugar Creek at County Road 400 S at New Palestine, Ind.	394340085524601	Yes	93	36	1.1	68	2.2	82	11

Caged Fish and Frog

The overall objective of this component study was to test the hypothesis that fish reproduction is reduced and amphibian development is impaired in streams with high pesticide loads from agricultural runoff. Though details of the field experiments of this special study are beyond the scope of this report, the overall design is summarized.

Laboratory studies have determined that atrazine causes endocrine disruption in fish and amphibians (Tillitt and others, 2010; Hayes and others, 2010), and field or mesocosm studies have indicated a relation between atrazine and fewer offspring, immunosuppression, testicular oocytes, and altered sex ratios (Kettle and others, 1987; Hayes and others, 2002; Rohr and others, 2008; Langlois and others, 2010). These effects are present at low atrazine concentrations (for example, fish egg production was reduced by concentrations of atrazine as low as 0.5 µg/L under laboratory exposure conditions; Tillitt and others, 2010)—well within or below the range of atrazine concentrations (1–25 µg/L) commonly detected in Midwest streams (Gilliom and others, 2006).

For fish, the specific objectives were to (1) measure realized fecundity of fathead minnow exposed in place during the spawning season to streams receiving varying amounts of agricultural runoff; (2) measure a suite of molecular, biochemical, and physiological endpoints indicative of exposure to endocrine disrupting chemicals and of effects along the

brain-pituitary-gonad axis; (3) evaluate the phenotypic and genotypic sex ratios of offspring exposed to the same stream water as adults from fertilization through the period of sexual differentiation; and (4) relate water quality and pesticide chemical exposures to biological effects on fathead minnow reproduction.

For frogs, the specific objectives were to (1) measure survival, growth, and somatic and sexual development of leopard frog (*Lithobates blairi*) exposed in place during the larval period to streams receiving varying amounts of agricultural runoff; (2) measure a suite of physiological endpoints indicative of exposure to endocrine disrupting chemicals; and (3) relate water quality and pesticide chemical exposures to biological effects on leopard frog development.

To meet these objectives, field experiments were done in which fish (fathead minnow) or frogs (leopard frog) were placed into in-place chambers and exposed to ambient water at sites expected to have high or low atrazine concentrations (fig. 12). Morphological, physiological, biochemical, and molecular endpoints were monitored that previously have been determined to be associated with exposure to pesticides or endocrine disrupting compounds.

Because the caged fish and frog studies are labor intensive, these studies were done at a subset of only eight sites (table 12). To minimize travel and the effects of confounding environmental variables (physical and physicochemical habitat characteristics), the sites were located within a limited



Figure 12. Fish and frogs in chambers were exposed in place to ambient water conditions at sites with a range of chemical stressor concentrations. (Photograph by Peter Van Metre, U.S. Geological Survey)

Table 12. Midwest streams selected for caged fish and frog (CFF) special study.

[NWIS, U.S. Geological Survey National Water Information System database; mi², square mile; Aii, agricultural intensity index; ePestHigh, estimated pesticide use; kg/km², kilograms per square kilometer; Mo., Missouri; ag, agriculture]

Station name	NWIS station number	Drainage area (mi ²)	Site selection type	Final nutrient site type	Cultivated crops	Aii	Aii bin	Watershed base-flow index	Atrazine 2012 ePestHigh (kg/km ²)
High estimated atrazine concentrations									
Skull Lick Creek near Mexico, Mo.	391308091550901	29	Random	Low ag	49.1	49.01	2	7.00	34.64
Bear Creek near Gilliam, Mo.	391504093003301	8	Random	High ag	57.0	52.82	3	13.68	29.49
Goodwater Creek near Centralia, Mo.	391815009203901	28	Other	High ag	66.9	58.90	3	8.96	41.16
Fish Branch near Mexico, Mo.	391443091534001	17	Other	High ag	77.1	55.77	3	13.08	82.90
Low estimated atrazine concentrations									
Perche Creek near Columbia, Mo.	390227092234101	178	Reference—geospatial	Least developed	11.7	33.92	2	10.10	5.64
North Moreau Creek near Jefferson City, Mo.	383158092192001	336	Reference—geospatial	Least developed	12.4	17.53	1	14.63	5.66
Moniteau Creek near Rocheport, Mo.	390200092341701	126	Reference—geospatial	Least developed	13.2	21.82	1	10.61	5.27
Loutre River near Montgomery City, Mo.	385638091364601	117	Random	Low ag	27.0	33.27	2	8.06	15.20

geographic area in Missouri. Sites were selected carefully such that the water-quality conditions could support survival and growth of young fathead minnows; the physical conditions were conducive to deploying chambers with small, fragile larval fish, as ascertained during the site reconnaissance; and contaminants of concern were predicted to be in the range of concentrations of interest. The watershed regressions for

pesticides model for the Corn Belt region (Stone and Gilliom, 2012) was used to estimate atrazine concentrations in Missouri streams, and four sites were selected that had high estimated atrazine concentrations (greater than or equal to 10 µg/L) and four sites with low estimated atrazine concentrations (less than or equal to 1 µg/L).

Sediment Source

The overall objective of this sediment source study was to determine the sources of fine-grained sediment to streams in the MSQA region. Increased sediment loading is one of the most common causes for habitat degradation and the subsequent loss of stream biological integrity in the United States. In 2014, sediment and turbidity together were the second (pathogens were the first) leading cause of impairment of U.S. waterways (U.S. Environmental Protection Agency, 2014). Excess sediment can alter benthic environments, bury spawning grounds, promote gill damage, and attenuate light. Sediment also is a vector for pollutants. Commonly, the fine-grained fraction of the sediment is the most responsible for degrading aquatic habitats and is the vector for sorbed pollutants (Owens and others, 2005; Larsen and others, 2010). Understanding the sources of this sediment is a necessary component of effective management actions and policies aimed at reducing sediment inputs (Owens, 2005).

Sediment-source characterization (fingerprinting) compares fine-grained depositional or suspended-sediment samples with samples from potential source areas within the watershed (Williamson and others, 2014). Potential sources investigated by this study are watershed soils, stream channel material, and bank material. Sediment-source characterization relied on bed sediment and bank sediment sampling at all 100 MSQA sites. Radionuclides and major and trace elements were analyzed in fine-grained bed and bank sediment (sieved at 63 mm) from 99 of the 100 MSQA sites (sediment was not sampled at 1 site). The approach primarily relied on differences in the fallout radionuclides lead-210 and beryllium-7 in surface soils and streambanks.

At three sites, passive-sampler tubes (Phillips and others, 2000) were placed in the stream to collect suspended sediment (fig. 13). The sites—Walnut Creek near Vandalia, Iowa; Mill Creek near Choctaw, Illinois; and Sugar Creek at Co Rd 400 S at New Palestine, Indiana—were in largely agricultural basins (cultivated crops 54–82 percent), had evidence of bank erosion, and had some historical data available (table 1). At each site, four tubes were mounted on steel struts in pairs in the center of the channel with the bottom tubes submerged during low flow. The samplers were made from commercially available PVC pipe and threaded end caps (dimensions of 98-mm inner-diameter width by 1-m length). A 4-mm plastic tube inserted through holes drilled into the center of each end cap allowed water to flow through the sampler, with a funnel-shaped tube end facing into the flow. Sediment accumulated in the sampler because flow through the small inflow and outflow tubes was slow enough to allow for settling in the larger PVC pipe. Sediment was retrieved from the passive samplers approximately weekly or shortly after a high-flow event. Sample material was composited (all four samplers at the site), maintained chilled, settled, decanted, and sieved (fig. 14). Analyses of sediments from passive samplers included radionuclide and elemental analyses for source identification following similar approaches as bed sediment.

Sediment fingerprinting samples from passive suspended-sediment samplers, bed material, and bank-scrap samples were analyzed by the USGS NRP Laboratory in Menlo Park, California, for radionuclide tracers (appendix 2, table 2–4) and by the CCGAR and CMERA in Denver, Colorado, for trace elements.



Figure 13. Passive suspended-sediment samplers deployed in Sugar Creek, Indiana



Figure 14. Suspended-sediment samples from four passive-sampler tubes for each site. *A*, composited; *B*, settled; *C*, decanted; and *D*, transferred to a smaller container before shipping to the laboratory for analysis.

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Appendixes 1–4

Appendixes are available for downloading from <https://doi.org/ofr20171073>.

Appendix 1. Additional Site, Reach, and Watershed Characteristics of Selected Sites Assessed as Part of the U.S. Geological Survey (USGS) National Water-Quality Assessment (NAWQA) Midwest Stream Quality Assessment (MSQA) in 2013.

Table 1–1. Definition of site, reach, and watershed characteristics of selected sites assessed as part of the U.S. Geological Survey (USGS) National Water-Quality Assessment (NAWQA) Midwest Stream Quality Assessment (MSQA) in 2013.

Table 1–2. Description of site, reach, and watershed characteristics of selected sites assessed as part of the U.S. Geological Survey (USGS) National Water-Quality Assessment (NAWQA) Midwest Stream Quality Assessment (MSQA) in 2013.

Appendix 2. Description of the Laboratory Analyses Used for Water, Periphyton, Sediment, Fish Tissue, and Passive Integrated Samples.

Table 2–1. Physical properties, suspended sediment, ions, carbon, nutrients, algal pigments, and stable isotopes analyzed in water samples from selected Midwest streams, 2013.

Table 2–2. Pesticides and other organic compounds analyzed in water samples from selected Midwest streams with method surrogates, 2013.

Table 2–3. Algal pigments and biomass in periphyton samples from selected Midwest streams, 2013.

Table 2–4. Compounds analyzed from bed sediment samples from selected Midwest streams with method surrogates, 2013.

Table 2–5. Compounds analyzed from fish tissue samples from selected Midwest streams, 2013.

Appendix 3. Description of Quality Control Samples.

Table 3–1. Counts of planned environmental (env), field blank, replicate (rep), and spike samples of stream water by site and laboratory analysis for the 100 stream sites sampled in the U.S. Geological Survey (USGS) Midwest Stream Quality Assessment (MSQA) study in 2013.

Appendix 4. Description of the Sampling Timelines, Matrix, Collection, and Processing for Water, Sediment, and Ecological Samples.

Table 4–1. Summary of the collection and processing of water samples for chemistry and toxicity.

Table 4–2. Major data-collection elements of the Midwest Stream Quality Assessment.

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For additional information contact:
Director, [National Water-Quality Program](#)
U.S. Geological Survey
413 National Center
12201 Sunrise Valley Drive
Reston, VA 20192

