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Water Quality, Cyanobacteria, and Environmental Factors and Their Relations to Microcystin Concentrations for Use in Predictive Models at Ohio Lake Erie and Inland Lake Recreational Sites, 2013–14

Scientific Investigations Report 2015–5120

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

COVER. View of East Fork Lake State Park Beach (Harsha Lake), Bethel, Ohio. (Photograph by Donna S. Francy, U.S. Geological Survey)

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SALLY JEWELL, Secretary

U.S. Geological Survey
Suzette M. Kimball, Acting Director

U.S. Geological Survey, Reston, Virginia: 2015

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Suggested citation:
Francy, D.S., Graham, J.L., Stelzer, E.A., Ecker, C.D., Brady, A.M.G., Struffolino, Pamela, and Loftin, K.A., 2015, Water quality, cyanobacteria, and environmental factors and their relations to microcystin concentrations for use in predictive models at Ohio Lake Erie and inland lake recreational sites, 2013–14: U.S. Geological Survey Scientific Investigations Report 2015–5120, 58 p., 2 appendixes, http://dx.doi.org/10.3133/sir20155120.

ISSN 2328-0328 (online)

Contents

Figures

Tables

Conversion Factors

Multiply	By	To obtain
	Length	
millimeter (mm)	0.03937	inch (in.)
micrometer (µm)	1,000	millimeter (mm)
inch (in.)	25.4	millimeter (mm)
mile (mi)	1.609	kilometer (km)
	Area	
acre	4,047	square meter (m^2)
square mile (mi^2)	2.590	square kilometer (km^2)
	Volume	
liter (L)	0.2642	gallon (gal)
milliliter (mL)	0.03382	ounce, fluid (fl. oz)
microliter	1,000	milliliter (mL)
cubic foot (ft^3)	0.02832	cubic meter (m^3)
	Flow rate	
cubic foot per second (ft^3/s)	0.02832	cubic meter per second (m^3/s)
cubic foot per second per square mile [$(ft^3/s)/mi^2$]	0.01093	cubic meter per second per square kilometer [$(m^3/s)/km^2$]
	Mass	
gram (g)	0.03527	ounce, avoirdupois (oz)
milligram (mg)	1,000	gram (g)
microgram (µg)	1,000,000	gram (g)

Temperature in degrees Celsius (°C) may be converted to degrees Fahrenheit (°F) as follows:

$°F = (1.8 \times °C) + 32$

Acknowledgments

The authors acknowledge assistance from many individuals and agencies in collecting data and planning and executing this study.

We gratefully acknowledge the following individuals and agencies for help with collecting or compiling data: John McManus, Alex Delvalle, and Hannah Gonzalez with the Clermont County Soil and Water Conservation District, who collected and processed samples at Harsha Lake; Robert England and Craig Ward with the Erie County General Health District, who helped to select sites and collect and process samples at Sandusky Bay; Jean Backs and Jason Wesley with the Ohio Department of Natural Resources, who provided a boat and operator to collect samples at Buckeye Lake Onion Island; Jade Young for providing data on stage/volume relationships at Harsha Lake for calculating residence times; and Joel Allen, U.S. Environmental Protection Agency, Office of Research and Development, who maintained and calibrated the continuous monitor at Harsha Lake and provided these data and calibrations. A special thanks is extended to Chris Nietch and his colleagues at the U.S. Environmental Protection Agency's Environmental Stream Facility in Milford, Ohio, for providing access to their facility for processing and storing samples collected at Harsha Lake.

Finally, we thank Linda Merchant-Masonbrink with the Ohio Environmental Protection Agency, Reed Green with the USGS Lower Mississippi-Gulf Water Science Center, and Chris Kephart with the USGS Ohio Water Science Center for manuscript reviews.

Water Quality, Cyanobacteria, and Environmental Factors and Their Relations to Microcystin Concentrations for Use in Predictive Models at Ohio Lake Erie and Inland Lake Recreational Sites, 2013–14

By Donna S. Francy,[1] Jennifer L. Graham,[1] Erin A. Stelzer,[1] Christopher D. Ecker,[1] Amie M.G. Brady,[1] Pamela Struffolino,[2] and Keith A. Loftin[1]

Abstract

Harmful cyanobacterial "algal" blooms (cyanoHABs) and associated toxins, such as microcystin, are a major water-quality issue for Lake Erie and inland lakes in Ohio. Predicting when and where a bloom may occur is important to protect the public that uses and consumes a water resource; however, predictions are complicated and likely site specific because of the many factors affecting toxin production. Monitoring for a variety of environmental and water-quality factors, for concentrations of cyanobacteria by molecular methods, and for algal pigments such as chlorophyll and phycocyanin by using optical sensors may provide data that can be used to predict the occurrence of cyanoHABs.

To test these monitoring approaches, water-quality samples were collected at Ohio recreational sites during May–November in 2013 and 2014. In 2013, samples were collected monthly at eight sites at eight lakes to facilitate an initial assessment and select sites for more intensive sampling during 2014. In 2014, samples were collected approximately weekly at five sites at three lakes. Physical water-quality parameters were measured at the time of sampling. Composite samples were preserved and analyzed for dissolved and total nutrients, toxins, phytoplankton abundance and biovolume, and cyanobacterial genes by molecular methods. Molecular assays were done to enumerate (1) general cyanobacteria, (2) general *Microcystis* and *Dolichospermum* (*Anabaena*), (3) *mcyE* genes for *Microcystis, Dolichospermum* (*Anabaena*), and *Planktothrix* targeting deoxyribonucleic acid (DNA), and (4) *mcyE* transcripts for *Microcystis, Dolichospermum* (*Anabaena*), and *Planktothrix* targeting ribonucleic acid (RNA). The DNA assays for the *mcyE* gene provide data on cyanobacteria that have the potential to produce microcystin, whereas the RNA assays provide data on cyanobacteria that are actively transcribing the toxin gene. Environmental data were obtained from available online sources. Quality-control (QC) samples were collected and analyzed for all constituents to characterize bias and variability; however, QC data for molecular assays were examined in more detail than for the other constituents. The QC data for molecular assays suggested that sampling variability and qPCR variability were small in comparison with the combined variability associated with sample filtering, extraction and purification, and the matrix itself.

A total of 46 water-quality samples were collected during 2013 at 8 beach sites—Buck Creek, Buckeye Crystal, Deer Creek, Harsha Main, Maumee Bay State Park (MBSP) Inland (negative control site), MBSP Lake Erie, Port Clinton, and Sandusky Bay. Microcystin was detected in 67–100 percent of samples at all sites except for MBSP Inland, where microcystin was detected in only 20 percent of samples. Microcystin concentrations ranged from <0.10 to 48 micrograms per liter (µg/L), with the widest range found at MBSP Lake Erie and the highest concentrations found at Buckeye Crystal. Saxitoxin was detected in five samples, and cylindrospermopsin was not detected in any samples.

A total of 65 water-quality samples were collected during 2014 at 5 sites on 3 lakes—Buckeye Fairfield and Onion Island, Harsha Main and Campers, and MBSP Lake Erie beach. Four of the sites were bathing beaches and one site, Onion Island, was an offshore boater swim area. Concentrations of microcystin ranged from <0.10 to 240 µg/L and, as in 2013, the widest range was found at MBSP Lake Erie. At Buckeye Lake, microcystin concentrations were consistently high (greater than 20 µg/L), ranging from 23 to 81 µg/L. At Harsha Main and Campers, microcystin concentrations ranged from <0.10 to 15 µg/L. Saxitoxin was detected in four samples collected at MBSP Lake Erie. Throughout the 2014 season, the cyanobacterial community, as determined by molecular and microscopy methods, and the dominance associated with the highest microcystin concentrations were unique to individual lakes. At Buckeye Lake, *Planktothrix* dominated the cyanobacterial community throughout the season and *Planktothrix* DNA and RNA were found in 100 percent of samples; *Microcystis mcyE* DNA was found in low concentrations. At Harsha Lake, *Dolichospermum* and *Microcystis* were a substantial percentage of the community from late May through August,

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and the highest microcystin concentrations occurred in June and July. At MBSP Lake Erie, *Microcystis* generally dominated from mid-July through early November, and the highest microcystin concentrations occurred in August.

Spearman's correlation coefficient (rho) was computed to determine the relations between environmental and water-quality factors and microcystin concentrations at four sites—Buckeye Fairfield, Buckeye Onion Island, Harsha Main, and MBSP Lake Erie. Factors were evaluated for use as potential independent variables in two types of predictive models—daily and long-term models. Easily or continuously measured water-quality factors and available environmental data are used for daily predictions that do not require a site visit. Data from factors used in daily predictions and results from samples collected and analyzed in a laboratory are used for long-term predictions (a few days to several weeks). A few statistically significant correlations ($p \leq 0.05$) between microcystin concentrations and factors for both daily and long-term predictions were found at Buckeye Onion Island, and many were found at Harsha Main and MBSP Lake Erie. There were only a few statistically significant factors for daily predictions at Buckeye Fairfield, likely because of the lack of variability in microcystin concentrations. Among factors for daily predictions, phycocyanin had the highest Spearman's correlation to microcystin concentrations (rho = 0.79 to 0.93) at all sites except for Buckeye Fairfield. Turbidity, pH, algae category, and Secchi depth were significantly correlated to microcystin concentrations at Harsha Main and MBSP Lake Erie. Algae categories were observational categories from 0 (none) to 4 (extreme). Several discharge variables (Maumee River at Waterville, river mouth is approximately 3.5 miles from the beach) at MBSP Lake Erie were promising environmental factors for daily predictions. In addition to discrete water-quality measurements recorded at Harsha Main at the time of sampling, many manipulated measurements (factors derived from mathematical manipulation of time-series data) available from a nearby continuous monitor were strongly correlated to microcystin concentrations; the highest correlation was found for the relation between microcystin concentrations and the antecedent 7-day average phycocyanin (rho = 0.98). For long-term predictions, the most highly correlated molecular assays were *Planktothrix mcyE* DNA at Buckeye Onion Island and *Microcystis mcyE* DNA at Harsha Main and MBSP Lake Erie. Concentrations of several nutrient constituents were significantly correlated to microcystin concentrations including total nitrogen at Buckeye Onion Island, ammonia and nitrate plus nitrite (both negatively correlated) at Harsha Main and MBSP Lake Erie, and total phosphorus at MBSP Lake Erie.

The results of this study showed that water-quality and environmental variables are promising for use in site-specific daily or long-term predictive models. In order to develop more accurate models to predict toxin concentrations at freshwater lake sites, data need to be collected more frequently and for consecutive days in future studies.

Introduction

Harmful cyanobacterial "algal" blooms (cyanoHABs) are quickly becoming a major global water-quality issue (Ho and Michalak, 2015). The cyanoHABs are caused by the release of toxins by a group of photosynthetic bacteria called cyanobacteria. The toxins produced by cyanobacteria are a diverse group of compounds and include neurotoxins (such as anatoxin and saxitoxin) and hepatotoxins (such as cylindrospermopsin and microcystin). Cyanobacteria can produce toxins with different chemical structures; for example, there are over 80 known microcystin variants with a wide range of toxicities (Graham and others, 2010). CyanoHABS have been associated with human and animal death and disease, require additional water treatment measures for water-treatment plants that use affected surface water supplies, and cause economic impacts to communities that rely on revenues from water recreation (Ho and Michalak, 2015).

Over the past 10 years, cyanoHABs have been increasing in Lake Erie and Ohio inland lake waters. Microcystin was detected in 74 percent of the 1,100 samples collected between 2011 and 2013 from Ohio source and recreational surface waters (Ohio Environmental Protection Agency, 2014). Concentrations of microcystin were higher than the Ohio Recreational Public Health Advisory[3] level of 6 micrograms per liter (µg/L) in 44 percent of samples and were higher than 20 µg/L (part of the Recreational No Contact Advisory[4]) in 31 percent of samples; however, it is worth noting that sites with known cyanoHABs were often targeted for repeated sampling. During that same period, saxitoxin was detected in 6 out of 26 samples (23 percent), and cylindrospermopsin was not detected in any of the 23 samples analyzed for that toxin (Ohio Environmental Protection Agency, 2014).

Microcystins are commonly produced by cyanobacteria in the genera *Microcystis, Planktothrix,* and *Dolichospermum* (*Anabaena)* (Rantala and others, 2006). For microcystin production to occur, the microcystin synthetase (*mcy*) gene cluster must be present within the genome of the cell. Known microcystin-producing genera include toxic strains (those with the *mcy* gene cluster) and nontoxic strains (those without the *mcy* gene cluster), which can be differentiated only by molecular detection methods such as quantitative polymerase chain reaction (qPCR). Quantitative PCR methods are used to quantify deoxyribonucleic acid (DNA) gene sequences that are part of the *mcy* gene cluster and indicate the potential for microcystin production, irrespective of whether toxin is being produced. Quantitative RT-PCR methods are used

[3]A Recreational Public Health Advisory for microcystin means that swimming and wading are not recommended, water should not be swallowed, and surface scum should be avoided.

[4]A Recreational No Contact Advisory for microcystin means that the public is recommended to avoid all contact with the water. It is in effect if someone or an animal dies or becomes ill as a direct result of cyanotoxin exposure and microcystin is >20 µg/L or other thresholds are reached.

to quantify ribonucleic acid (RNA) transcripts associated with the toxin gene and indicate that microcystin-producing cyanobacteria are actively transcribing the toxin gene (Sipari and others, 2010). The *mcyE* gene, which is part of the *mcy* gene cluster, is a good choice for detecting microcystin-producing cyanobacteria because it is essential for the synthesis of microcystin (Pearson and Neilan, 2008) and can be used to detect genus-specific *mcyE* genes (DNA) and transcripts (RNA). Molecular methods for cyanobacteria and toxin genes are potentially important tools to help understand and predict toxin production in a water body. For example, in a study of a Canadian bay with frequent cyanoHABs, the potential for microcystin production was detected by use of a qPCR assay for a toxin gene when toxins were still below the limit of detection (Fortin and others, 2010).

A variety of factors such as light, temperature, nutrient concentrations, wind patterns, turbidity, pH, and lake mixing have been shown to influence the proliferation of cyanobacteria and their toxin production. The environmental conditions that support cyanobacterial dominance and trigger toxin production may vary from lake to lake, and even from season to season in the same lake (Jacoby and others, 2000). Predicting when and where a bloom will occur is important to protect the public that uses and consumes a water source; however, predictions are complicated and likely site specific because of the many factors affecting toxin production. Optical sensors that measure algal pigments have been used to provide early warnings of cyanobacterial presence in recreational waters (Marion and others, 2012) and drinking-water sources (Brient and others, 2008; McQuaid and others, 2011).

Optical sensor and molecular methods may provide alternative monitoring approaches to the current method for identifying cyanobacteria by microscopy, which is time consuming, expensive, and unable to identify whether a strain has the ability to produce toxins. These alternative monitoring methods may help to better identify and understand the potential for toxin production in cyanobacterial populations and, at the same time, be used in predictive models to provide an early warning system for toxin production.

The U.S. Geological Survey (USGS), in cooperation with Ohio Water Development Authority and the University of Toledo, collected samples at Ohio recreational sites during two recreational seasons to test alternative monitoring approaches. Most of the sites were at inland lake or Lake Erie beaches known to be affected by cyanoHABs. Water samples were collected and analyzed for toxin and nutrient concentrations and cyanobacteria by microscopy and by molecular methods. Measurements were made at the time of sampling for physical and chemical water-quality characteristics, including phycocyanin, a pigment indicative of cyanobacteria, and chlorophyll, a pigment indicate of all photosynthetic phytoplankton.

Purpose and Scope

The purpose of this report is to present data on concentrations of toxins and total and dissolved nutrients, phytoplankton abundance and community composition, cyanobacterial genes, and associated physical water-quality and environmental factors measured at recreational lake sites in Ohio over two recreational seasons. During 2013, samples were collected approximately monthly at eight sites at eight lakes to facilitate an initial assessment and select sites for more intensive sampling in 2014. During 2014, samples were collected approximately weekly at five sites at three lakes to identify factors affecting the cyanobacterial community composition, toxin concentrations, and cyanobacterial gene concentrations. Correlations between microcystin concentrations and environmental and water-quality factors are presented to identify promising factors that may be used in predictive models for cyanoHABs. Procedures for enumerating cyanobacterial genes by qPCR and qRT-PCR and for measuring bias and variability in quality-control samples also are presented in this report. The goals of the study were to better understand the relations between cyanobacterial community composition, toxin production, and environmental and water-quality factors and to help support predictive capabilities for cyanoHABs.

Methods of Study

Water-quality samples and measurements were collected from Ohio recreational lake sites from May through November 2013–14. Data collection during 2013 constituted an initial assessment of cyanobacterial community composition, toxin concentrations, cyanobacterial genes, and water-quality factors at several sites. Sampling during 2014 involved more intensive data collection at fewer sites than in 2013. Site selection for 2014 was based on 2013 results to include a range of toxin concentrations, different cyanobacterial communities, and promising relations between toxin concentrations and optical sensor measurements or cyanobacterial genes.

Site Descriptions and Sampling Frequency

Samples were collected approximately monthly at eight recreational sites at eight lakes during 2013 and approximately weekly to semiweekly at five recreational sites at three lakes during 2014 (figs. 1 and 2). Official USGS site names, site identification numbers, beach shoreline lengths, and numbers of regular samples (excluding quality-assurance and quality-control samples) collected at each site with collecting agencies are listed in table 1. Samples were collected on predetermined sampling dates and were not initiated to target a bloom.

Table 1. Study sites, agencies collecting and processing samples, and numbers of samples collected at each site, 2013–14.

[USGS, U.S. Geological Survey; CCSWCD, Clermont County Soil and Water Conservation District; UT LEC, University of Toledo, Lake Erie Center; ECGHD, Erie County General Health District; MBSP, Maumee Bay State Park; --, Indicates no samples collected.]

Site short name	USGS site name	USGS station identification number[a]	Approximate beach shore-line (feet)	Agency collecting/ processing samples	Number of samples in 2013	Number of samples in 2014
Buckeye Fairfield	Buckeye Lake at State Park Fairfield Beach	395520082281500	150	USGS	--	17[b]
Buckeye Crystal	Buckeye Lake at State Park Crystal Beach	395557082283800	75	USGS	3	--
Buckeye Onion Island	Buckeye Lake at Onion Island boat swim area	395431082310000	Open water	USGS	--	10
Buck Creek	C.J. Brown Reservoir at Main Beach North	395705083440100	2,000	USGS	5	--
Deer Creek	Deer Creek Lake at Deer Creek State Park Beach	393707083134100	1,400	USGS	7	--
Harsha Main	Harsha Lake at East Fork State Park Beach	390111084080300	1,000	USGS, 2013 CCSWCD, 2014	7	17
Harsha Campers	Harsha Lake at East Fork State Park Camp Beach	390121084054100	650	USGS, 2013 CCSWCD, 2014	0	4
MBSP Lake Erie (composite)	Maumee Bay at Maumee Bay State Park composite coves 2–4	414111083223201	900	UT LEC	7	--
MBSP Lake Erie (Cove 3)	Maumee Bay at Maumee Bay State Park Cove 3	414111083223200	300	UT LEC	--	17
MBSP Inland	Inland Lake Beach at Maumee Bay State Park	414100083223600	1,500	UT LEC	5	--
Lakeview	Lake Erie at Lakeview Park at Port Clinton	413053082553000	900	UT LEC	6	--
Sandusky Bay	Sandusky Bay at Driftwood Beach at Bay View	412810082491000	170	UT LEC and ECGHD	6	--

[a]Corresponds to the latitude and longitude of the site (concatenated in degree-minute-second format) plus two additional numbers (usually 00 unless there are multiple locations at the same site).

[b]Includes samples on 3 days, collected in the morning and afternoon.

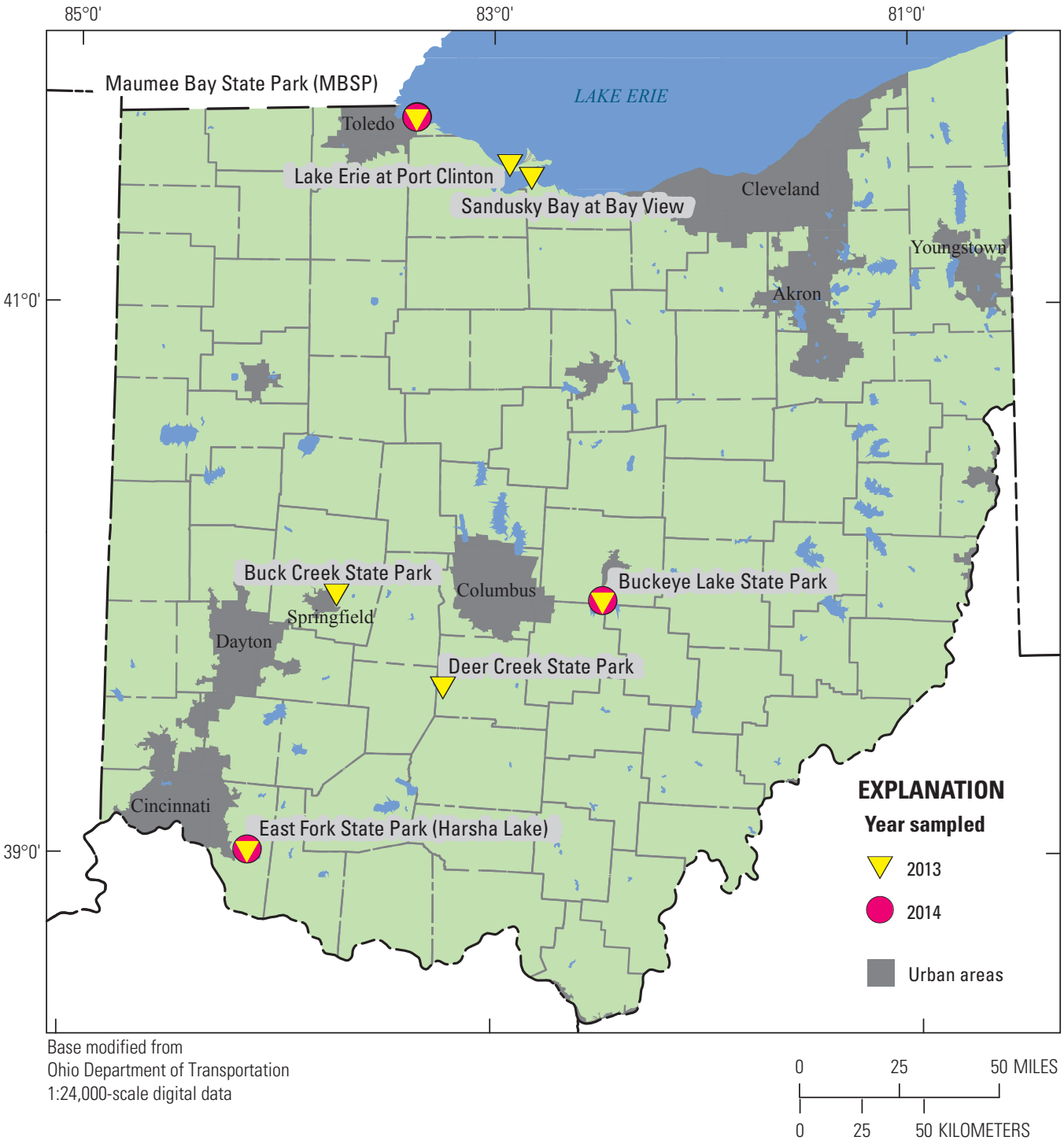


Figure 1. Locations of Ohio recreational lake sites and year(s) sampled.

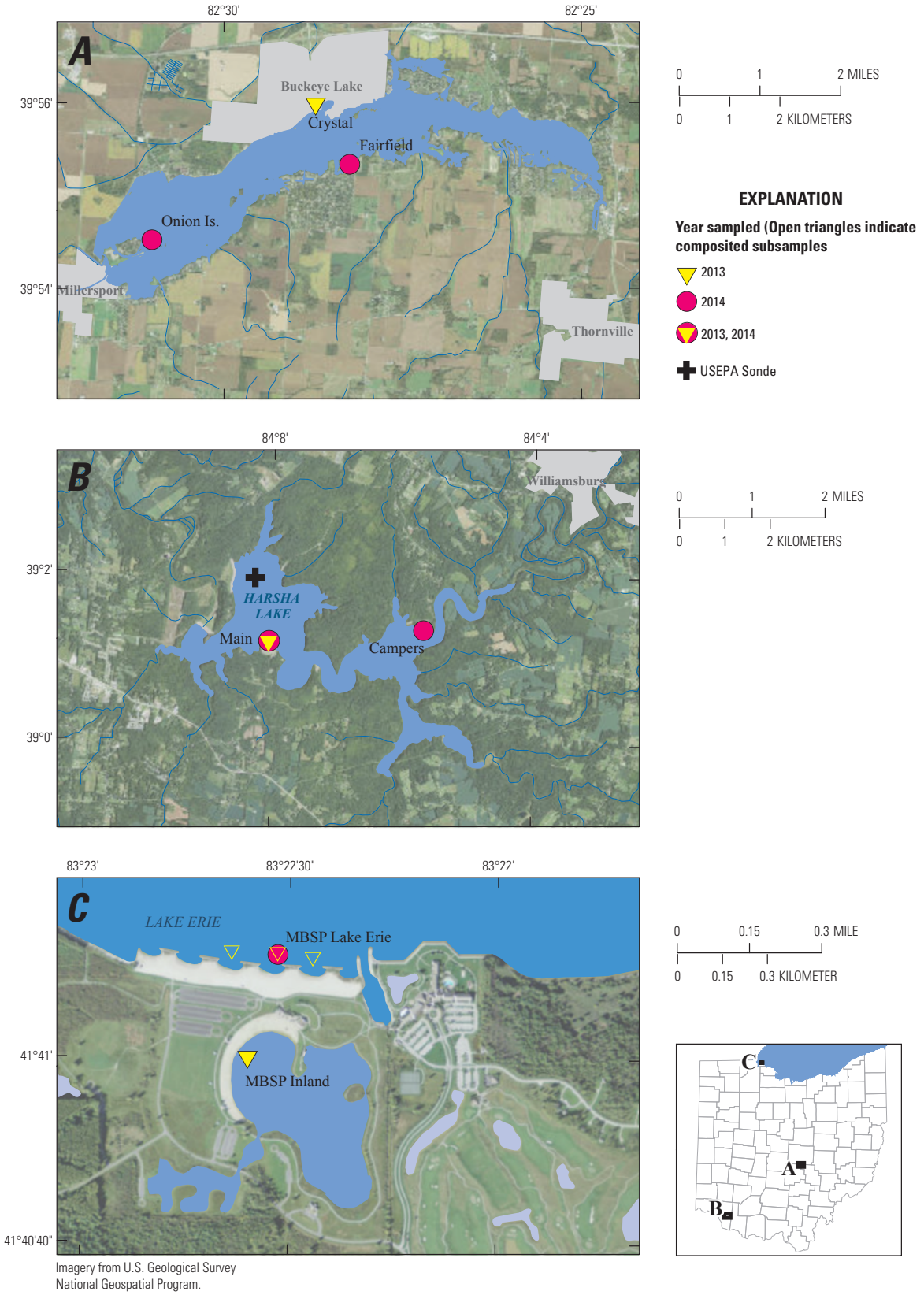


Figure 2. Sampling locations and year(s) sampled: *A*, Buckeye Lake State Park. *B*, East Fork State Park (Harsha Lake). *C*, Maumee Bay State Park (MBSP). *D*, C.J. Brown Reservoir at Buck Creek State Park. *E*, Deer Creek State Park. *F*, Lake Erie at Lakeview Park at Port Clinton and Sandusky Bay at Bay View.

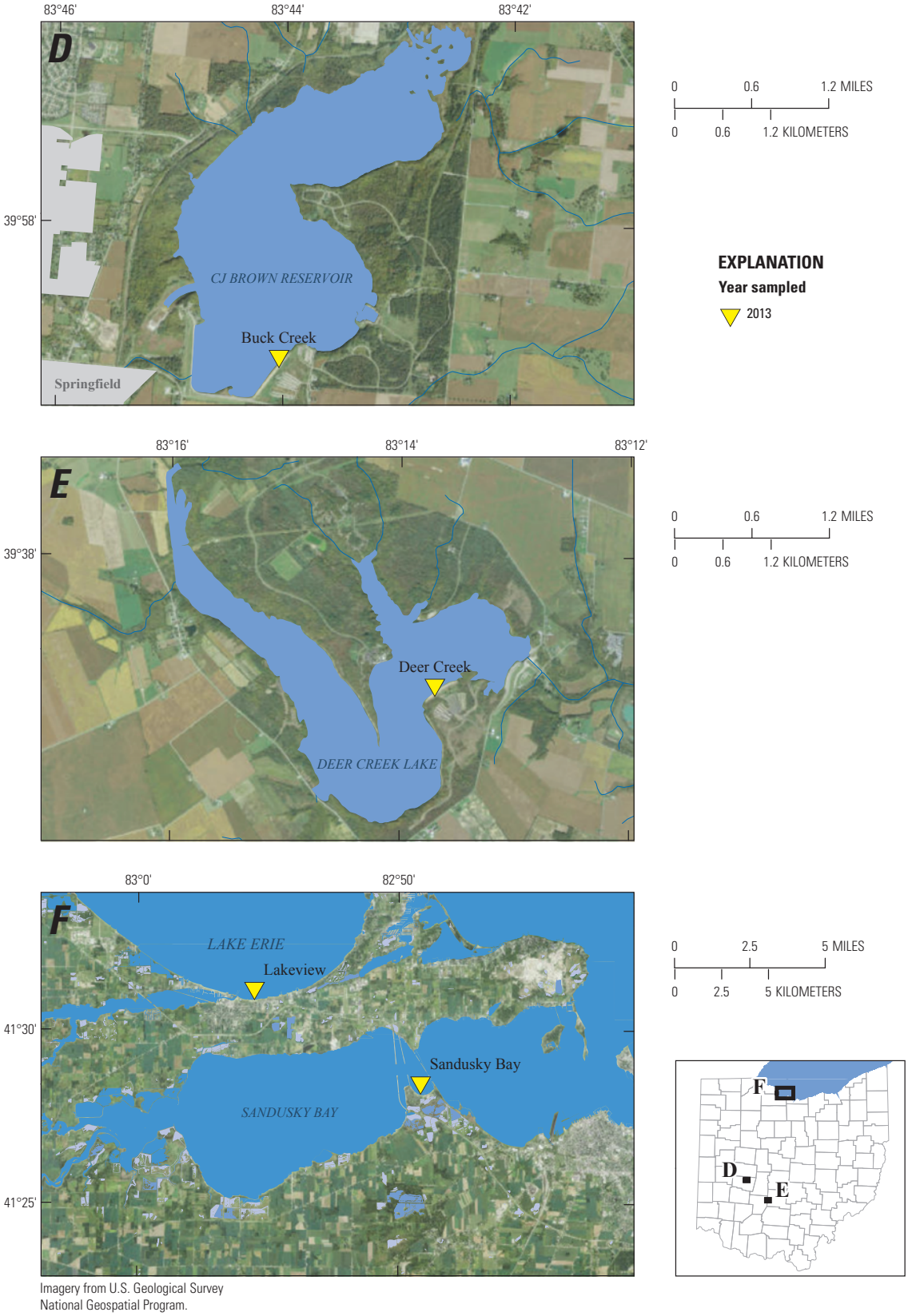


Figure 2. Sampling locations and year(s) sampled: *A*, Buckeye Lake State Park. *B*, East Fork State Park (Harsha Lake). *C*, Maumee Bay State Park (MBSP). *D*, C.J. Brown Reservoir at Buck Creek State Park. *E*, Deer Creek State Park. *F*, Lake Erie at Lakeview Park at Port Clinton and Sandusky Bay at Bay View.—Continued

Buckeye Lake is a shallow manmade lake 30 miles (mi) east of Columbus, Ohio. It was constructed in the early 19th century for the Miami and Erie Canal, which connected the Ohio River with Lake Erie. Sampling at the beach at Buckeye Crystal was added late in the 2013 season (August–September) because of high microcystin concentrations measured at that location by the Ohio Environmental Protection Agency (Ohio EPA; 2014). Buckeye Crystal is on the north side of Buckeye Lake in a protected bay where circulation is obviously limited (fig. 2*A*). During 2014, sampling was moved to Buckeye Fairfield, a beach on the south side of Buckeye Lake that is open to the lake and more representative of general lake conditions than Buckeye Crystal. During 2014, a third sampling location, accessible by boat and frequently visited by the public as an offshore boater swim area, was established at Buckeye Onion Island.

William H. Harsha Lake (Harsha Lake, also known as East Fork Lake) is a reservoir located 25 mi east of Cincinnati, Ohio. East Fork State Park has two official swimming beaches on Harsha Lake—Harsha Main on the southwest side of the reservoir and Harsha Campers on the northeast side of the reservoir (fig. 2*B*). In 2014, Harsha Campers was added as a second sampling site because of high microcystin concentrations reported by Ohio EPA (2014).

Maumee Bay is in the southwest corner of Lake Erie, east of Toledo, Ohio. Maumee Bay State Park (MBSP) is a popular Ohio recreational attraction and has two swimming beaches—one along the Lake Erie shoreline composed of five coves and one small inland lake (fig. 2*C*). The MBSP Lake Erie beach is often impaired by high *Escherichia coli* (*E. coli*) concentrations and more recently, by cyanoHABs. The western basin of Lake Erie, including Maumee Bay, experienced record-setting cyanobacterial blooms in 2011; this trend is expected to continue because of phosphorus loading from the Maumee River and other sources (Michalak and others, 2013). The MBSP inland beach, which is not connected to Maumee Bay, has not been affected by cyanoHABs and served as the negative control for this study (to verify that gene and toxin concentrations were indeed low at a negative control site). During 2013, samples were collected from the inland lake beach and from the Lake Erie beach (as a composite from three coves). During 2014, sampling was discontinued at the inland lake beach because of low microcystin concentrations and, to simplify sampling efforts, samples were collected from the Lake Erie beach from the most popular swimming cove (cove 3).

At four sites, samples were collected in 2013, but not in 2014. The Buck Creek State Park beach is on the southeast side C.J. Brown Reservoir (also known as Buck Creek Lake) near Springfield, Ohio (fig. 2*D*). The Deer Creek State Park beach is on the southeast side of Deer Creek Reservoir (fig. 2*E*) approximately 30 mi southwest of Columbus, Ohio. Low microcystin concentrations have been reported for both of these sites by the Ohio Environmental Protection Agency (2014). Lakeview Park includes a Lake Erie beach in the City of Port Clinton, Ohio (fig. 2*F*). Lakeview is in the western basin of Lake Erie, where cyanoHABs are common; however, samples for toxins are not routinely collected at Lakeview. Sandusky Bay at Bay View is a private beach in the small community of Bay View, Ohio (fig. 2*F*), and was selected for sampling to represent a site on Sandusky Bay, a 41,000-acre shallow arm of the western basin of Lake Erie.

Sampling Procedures

At each site, subsamples were collected from several locations within the designated swimming area and composited into one sample (Graham and others, 2008). Depending on the length of the swimming area, from 3 to 6 subsamples were collected from the same locations and depths (2–3 feet [ft]) throughout the season. The 1-liter (L) glass sample bottle and 5-L glass amber composite bottle were first triple rinsed with native water. The 1-L bottle was lowered to about 1 ft below the water's surface, the lid was removed, and the bottle was filled while bringing it up to the water's surface. The water was composited into the 5-L bottle, which was contained in an ice-filled cooler placed in an inflatable raft. The sample bottles were previously washed with nonphosphate detergent and tapwater, submerged in10 percent reagent grade hydrochloric acid (HCl), rinsed with deionized water, and autoclaved.

Water temperature, pH, dissolved oxygen, specific conductance, chlorophyll, and phycocyanin were measured at each subsample location by using a hand-held sensor calibrated and operated by use of standard USGS methods (Wilde, variously dated) or per the manufacturer's instructions (YSI Incorporated, Yellow Springs, Ohio). The optical sensors for algal pigments were different in the two types of sensors used in this study. For the YSI EXO, the total algae sensor emitted excitation beams to irradiate chlorophyll (470 nanometers [nm]), indicative of all photosynthetic cells, and phycocyanin (590 nm), indicative of cyanobacteria. For the YSI 6-series, separate probes measured chlorophyll (6025 sensor, 650–700 nm) and phycocyanin (6131 sensor, 590–595 nm). Secchi depth was also measured, and general field observations, such as wave heights and algae category, were recorded. Algae categories were 0, none; 1, mild; 2, moderate; 3, serious; and 4, extreme.

Sample Processing

Composited samples were processed in the field or in a local laboratory within 1 hour of collection. The composited sample was evenly distributed by shaking the 5-L bottle before removing any aliquot for processing.

Aliquots to be analyzed for cyanobacterial genes were filtered onto three to five replicate Nucleopore polycarbonate filters (Whatman/GE Healthcare, Piscataway, New Jersey) with 0.4-micrometer (μm) pore size, using filter funnels that were sterilized as described above for sample bottles. The volume of water filtered for each replicate ranged from 10 to 70 milliliters (mL), depending on the clarity of the water and how quickly the filter clogged. One filter blank was prepared with sterile buffered water each day that samples were filtered. After rinsing the filter funnel with sterile buffered water, each filter was placed into a 2-mL screw-capped vial with 0.3 gram (g) of acid-washed glass

beads (Sigma-Aldrich, St. Louis, Missouri). Because the RNA targets are unstable, the vials were immediately flash frozen in liquid nitrogen and subsequently transferred to a freezer for storage.

After samples were processed for the analysis of cyanobacterial genes, samples were processed for water-quality constituents. An aliquot from the composited sample bottle was removed to measure turbidity by use of a turbidimeter (Hach Company, Loveland, Colorado). For toxin analysis, two 125-mL high-density polyethylene (HDPE) bottles were triple rinsed with native water and filled with the composited sample; samples were kept on ice and transferred to a freezer. Two 250-mL aliquots were removed for analysis of phytoplankton abundance and community composition and preserved with 3 percent Lugol's iodine, kept on ice, and refrigerated for storage. Samples for subsequent analysis of total and dissolved nitrogen and phosphorus and for sulfate were processed and preserved by use of standard USGS methods (Wilde and others, 2004–9).

Laboratory Analyses and Associated Laboratory Quality Control

Nutrient and Sulfate Analyses

Samples for nutrient analysis were shipped on ice within three days of collection to the USGS National Water Quality Laboratory (NWQL) in Denver, Colo. In the NWQL, samples were analyzed for dissolved nitrite, dissolved nitrite plus nitrate, dissolved ammonia, dissolved orthophosphorus, total nitrogen, and total phosphorus (table 2). Nitrogen to phosphorus ratios for each sample were calculated by dividing the total nitrogen by

Table 2. Laboratory method information for constituents analyzed in water samples collected at recreational sites, 2013–14.

[mg/L, milligram per liter; µg/L, microgram per liter; mL, milliliter; NWQL, U.S. Geological Survey National Water Quality Laboratory; OSU, The Ohio State University; OGRL, U.S. Geological Survey Organic Geochemistry Research Laboratory; BSA, BSA Environmental Services; OWML, U.S. Geological Survey Ohio Water Microbiology Laboratory; qPCR, quantiative polymerase chain reaction; qRT-PCR, quantiative reverse-transcriptase polymerase chain reaction; DNA, deoxyribonucleic acid; RNA, ribonuceic acid]

Constituent	Method	Reporting limit	Laboratory
Dissolved nitrite nitrogen	Colorimetry (Fishman, 1993)	0.001 mg/L	NWQL
Dissolved nitrite plus nitrate nitrogen	Colorimetry with enzyme reduction-diazolization (Patton and Kryskalla, 2011)	0.04 mg/L	NWQL
Dissolved ammonia, as nitrogen	Colorimetry and salicylate-hypochlorate (Fishman, 1993)	0.01 mg/L	NWQL
Dissolved orthophosphorus	Colorimetry with phosphomolybdate (Fishman, 1993)	0.004 mg/L	NWQL
Total nitrogen	Alkaline persulfate digestion (Patton and Kryskalla, 2003; U.S. Environmental Protection Agency, 1993)	0.05 mg/L	NWQL
Total phosphorus	Alkaline persulfate digestion (Patton and Kryskalla, 2003; U.S. Environmental Protection Agency, 1993)	0.004 mg/L	NWQL
Sulfate	Ion chromatography (American Public Health Association and others, 2011)	2.5 mg/L	OSU
Cylindrospermopsins	Enzyme-linked immumosorbent assay (Abraxis microtiter plate kit; Graham and others, 2010)	0.05 µg/L	OGRL
Microcystins/nodularins	Enzyme-linked immumosorbent assay (Abraxis ADDA kit; Graham and others, 2010)	0.10 µg/L	OGRL
Saxitoxins	Enzyme-linked immumosorbent assay (Abraxis microtiter plate kit; Graham and others, 2010)	0.02 µg/L	OGRL
Phytoplankton abundance and community structure	Microscopy (Beaver and others, 2013)	1 cell/mL	BSA
Cyanobacteria, general	qPCR (Rinta-Kanto and others, 2005)	4.16 log copies/100 mL	OWML
Microcystis, general	qPCR (Rinta-Kanto and others, 2005)	3.57 log copies/100 mL	OWML
Dolichospermum (*Anabaena*), general	qPCR (Doblin and others, 2007)	4.36 log copies/100 mL	OWML
Microcystis mcyE toxin gene (DNA) or transcript (RNA)	qPCR (DNA) or qRT-PCR (RNA) (Sipari and others, 2010)	2.94 – 3.35 log copies/100 mL (DNA) 2.18 – 4.90 log copies/100 mL (RNA)	OWML
Dolichospermum (*Anabaena*) *mcyE* toxin gene (DNA) or transcript (RNA)[a]	qPCR (DNA) or qRT-PCR (RNA) (Sipari and others, 2010)	3.36 – 4.14 log copies/100 mL (DNA)	OWML
Planktothrix mcyE toxin gene (DNA) or transcript (RNA)	qPCR (DNA) or qRT-PCR (RNA) (Vaitomaa and others, 2003; Rantala and others, 2006)	3.58 – 3.71 log copies/100 mL (DNA) 2.63 – 4.88 log copies/100 mL (RNA)	OWML

[a] *Dolichospermum mcyE* DNA was not detected in any samples in 2013–14; as a result, samples were not analyzed for *Dolichospermum mcyE* RNA.

the total phosphorus concentration. Samples were analyzed for sulfate at The Ohio State University by using Standard Method 4110C (American Public Health Association and others, 2011) with modifications. Samples were prepared by centrifuging 1.5 mL of sample at 12,000 × *g* for 60 seconds and filtering the supernatant through a 0.45-µm PES syringe filter. Samples were analyzed for sulfate by single-column ion chromatography using the Dionex ICS-2100 and a Dionex AS18 column. Modifications included the substitution of an Eluent Generator Module (Dionex, Cat. No. 074218, Sunnyvale, California) for the eluent solution described in Standard Method 4110C and elution with sodium hydroxide instead of a borate, gluconate, acetonitrile solution. A standard curve was prepared by plotting the concentration of sulfate from known standards against the peak area. Calculated concentrations of sulfate in the sample were based on the linear equation of the standard curve.

Toxin Analyses

Frozen whole-water samples for toxin analysis were shipped approximately monthly by overnight mail on ice to the USGS Organic Geochemistry Research Laboratory in Lawrence, Kansas. The samples were processed and analyzed as described in Graham and others (2010). Briefly, samples were lysed by three sequential-freeze/thaw cycles followed by syringe filtration with a 0.7-µm glass fiber syringe filter. Analysis of filtered samples was done by using three separate enzyme-linked immunosorbent assay (ELISA) kits (Abraxis LLC, Warminster, Pennsylvania) for measurement of cylindrospermopsins, microcystins/nodularins, and saxitoxins (table 2). The ELISA for microcystin measures multiple congeners and is referred to in the report as "microcystin concentrations." Nodularins are rarely detected in inland freshwater lakes, and their known producers, *Nodularia* spp., typically require brackish waters to survive (Graham and others, 2010). Four-parameter calibration curve fits were used for quantitation and minimum reporting levels for each assay. Samples with concentrations exceeding those of the highest calibration standard were diluted with reagent water until the measured concentration was within the calibration curve. Final concentrations were reported with corrections for dilution.

Laboratory quality-control checks on ELISA measurements included assessment of interassay variability, laboratory duplicates, and blind spiked samples (microcystins only). Repeated analyses of a field sample from this study were used to assess interassay variability across the entire study. All quality-control (QC) data were considered acceptable if within 28.3 percent relative standard deviation (RSD) of mean or expected values.

Phytoplankton Abundance and Community Composition

Samples preserved in Lugol's iodine were shipped approximately monthly by overnight mail to BSA Environmental Services, Inc., in Beachwood, Ohio. Phytoplankton slides were prepared by using standard membrane-filtration techniques (McNabb, 1960). A minimum of 400 natural units (colonies, filaments, and unicells) were counted from each sample; in accordance with Lund and others (1958), counting 400 natural units provides accuracy within 90-percent confidence limits. In addition, an entire strip of the filter was counted at high magnification (usually 630×) along with one-half of the filter at a lower magnification (usually 400×) to ensure complete species reporting. Phytoplankton identifications were confirmed by at least two phycologists. Biovolume was calculated by using mean measured cell dimensions as described in Hillebrand and others (1999).

Molecular Analyses for Cyanobacteria

Filters were transported to the USGS Ohio Water Microbiology Laboratory (OWML) on dry ice for batch analysis. In the OWML, samples for toxin genes and transcripts were extracted and analyzed by use of qPCR (DNA) and qRT-PCR (RNA) according to procedures in Stelzer and others (2013) that are summarized below. Molecular assays for cyanobacteria were done to enumerate the following (table 2):

- general cyanobacteria,
- general *Microcystis* and *Dolichospermum* (*Anabaena*) (an assay for *Planktothrix* was not available at the time of this study),
- *mcyE* toxin genes for *Microcystis*, *Dolichospermum* (*Anabaena*), and *Planktothrix* targeting DNA, and
- *mcyE* transcripts for *Microcystis*, *Dolichospermum* (*Anabaena*), and *Planktothrix* targeting RNA.

DNA Extraction and qPCR Analyses

For DNA assays, one frozen filter from each sample was extracted according to procedures in Stelzer and others (2013), and the final elution volume was 100 and 150 microliters (µL) for samples collected in 2013 and 2014, respectively. The larger elution volume in 2014 was needed for the addition of a general *Dolichospermum* qPCR assay. An extraction blank was included with each batch of sample extractions.

From each sample extract, 5 µL was analyzed by qPCR in duplicate for each DNA assay described above (assays 1–3) by using primer and probe sets as well as qPCR run conditions listed in Stelzer and others (2013) and described elsewhere (Vaitomaa and others, 2003; Rinta-Kanto and others, 2005; Rantala and others, 2006; Doblin and others, 2007; and Sipari and others, 2010). A general *Dolichospermum* qPCR assay was added in 2014; primers, probes, and run conditions for this assay are described in Doblin and others (2007). All assays were run on either an Applied Biosystems 7500 or a StepOne Plus (Life Technologies, Carlsbad, Calif.) thermal cycler. TaqMan Universal PCR Master Mix (Life

Technologies, Carlsbad, Calif.) was used if a probe for the assay was available, whereas SYBR Green PCR Master Mix (Life Technologies, Carlsbad, Calif.) was used if a probe was not available. Sample inhibition was determined according to procedures in Stelzer and others (2013). If the sample results were considered inhibited, the extracts were diluted and rerun.

RNA Extraction and qRT-PCR Analyses

For RNA assays, one frozen filter from each sample was extracted according to procedures in Stelzer and others (2013). An extraction blank was included with each batch of sample extractions. A DNase treatment was included during extraction, and a DNA *Microcystis mcyE* toxin gene qPCR assay was run to verify that the RNA extracts were free of DNA.

RNA was reverse transcribed into DNA according to procedures in Stelzer and others (2013). There is a higher potential for contamination during the two-step RT method as opposed to a traditional qPCR because plates have to be opened and resealed multiple times; therefore, a no-template control (or qRT-PCR negative control) was added after every four samples.

After the RT reaction, each reverse transcribed sample was analyzed in duplicate by qPCR for *Microcystis*, *Dolichospermum*, and *Planktothrix mcyE* RNA transcripts under the same conditions as the qPCR DNA toxin gene assays described above. Inhibition of the RT reaction was measured according to procedures in Dumouchelle and Stelzer (2014). If RNA extracts were considered inhibited, the extracts were diluted and rerun.

Standard Curves and Quantifying Cyanobacteria by qPCR and qRT-PCR

Standards were included in duplicate with each qPCR and qRT-PCR run to construct a six-point standard curve. Plasmid standards for each assay were used to establish standard curves for quantification. For all assays except general *Dolichospermum* and the *Dolichospermum mcyE* toxin gene, plasmid standards were constructed by insertion of a PCR-amplified target sequence into a pCR4 TOPO *E. coli* plasmid vector (Life Technologies, Carlsbad, Calif.). The plasmid DNA was extracted and purified from *E. coli* cells by using the QuickLyse Miniprep Kit (Qiagen, Valencia, Calif.). Plasmid sequences were verified by DNA sequencing at The Ohio State University Plant-Microbe Genomics Facility, Columbus, Ohio. Plasmids for general *Dolichospermum* and the *Dolichospermum mcyE* toxin gene assays were synthesized by Integrated DNA Technologies (Coralville, Iowa). The copy number of each target was calculated by using the DNA concentration measured by the Qubit® dsDNA High Sensitivity Assay (Life Technologies, Carlsbad, Calif.) and the molecular weight of the plasmid. The same plasmid DNA standards were used for both the DNA and RNA assays. Sample results were reported as copies per 100 milliliters (copies/100 mL).

Guidelines for interpreting standard-curve data are available in the Applied Biosystems StepOne Plus Real-Time PCR Systems Reagent Guide (Applied Biosystems, 2010) and in Stelzer and others (2013). Standard-curve characteristics for this study are listed in table 3.

The limit of blank (LoB), limit of detection (LoD), and limit of quantification (LoQ) were calculated for each assay according to procedures in Francy and others (2014, appendix D8). The LoB is the lowest concentration that can be reported with 95 percent confidence to be above the concentrations of blanks, and it is used because blanks may sometimes produce results equal to low concentrations of the target. The LoD is the lowest concentration that can be detected with 95 percent confidence that it is a true detection and can be distinguished from the LoB. If the LoB is higher than the calculated LoD, then the LoB is used as the LoD. The LoQ is the lowest concentration of a gene that can be accurately quantified. The LoQ is calculated from the standard deviation of the LoD and is, therefore, higher than the LoD. LoB, LoD, and LoQ values are initially cycle threshold (Ct) values, converted to and reported as copies per reaction volume by use of an assay-specific standard curve. Sample results lower than the LoQ but above the LoD are reported as estimated values. Because original samples volumes and dilutions to overcome inhibition were often different for each sample or set of samples, the LoD was applied on a sample-by-sample basis to determine sample reporting limits (SRLs). The SRLs are the "less-than values" for each sample and assay and are reported as copies/100 mL.

Environmental Data Compilation

Environmental data were obtained from the nearest airport weather station, radar data, agency gage, and (or) other, local sources for the three lakes included in 2014 sampling (table 4). These environmental data were collected at locations that were within 25 mi from a study site, and most were within 10 mi. Definitions of factors and calculations for various data manipulations are listed in table 4 and described briefly below. The factors derived from mathematical manipulations of instantaneous or time-series data are referred to as "manipulated" in this report.

Weather data were obtained from the nearest National Weather Service (NWS) airport site (National Oceanic and Atmospheric Administration, 2014a). Hourly rainfall values were totaled for 24 hours up to 8 a.m. the day of sampling and designated as "day minus one" (Dm1); other manipulations were calculated by use of a software program called PROCESSNOAA, described in Francy and others (2013). The PROCESSNOAA software was also used to compile and process wind direction and speed data for 8 a.m. on the day of sampling and for the preceding 24-hour period. In order to obtain data from a wider area, hourly radar rainfall data were obtained from the NWS (National Oceanic and Atmospheric Administration, 2014b) for the Buckeye Lake drainage area; data manipulations for radar data were manually calculated (table 4).

Table 3. Standard curve information for quantitative polymerase chain reaction (qPCR) and quantitative reverse-transcription polymerase chain reaction (qRT-PCR) assays, 2013–14.

[LoD, limit of detection; LoQ, limit of quantification; copies/rxn, copies per reaction volume; DNA, deoxyribonucleic acid; RNA, ribonuceic acid]

Assay name	Analysis year	Dynamic range (copies/rxn)	Range of amplification efficiencies (percent)	Range of R^2 values	Limit of detection (LoD) (copies/rxn)	Limit of quantification (LoQ) (copies/rxn)
General assays						
Cyanobacteria	2013	16.2 – 4.19 x 10^6	89 – 95	0.998 – 1.00	33 – 180	72 – 180
Cyanobacteria	2014	19.2 – 9.17 x 10^6	89 – 96	0.996 – 1.00	26 – 180	72 – 180
Microcystis	2013	4.26 – 4.26 x 10^6	89 – 93	0.995 – 0.999	93 – 130	55 – 130
Microcystis	2014	17.2 – 3.57 x 10^6	88 – 92	0.990 – 1.00	27 – 72	55 – 72
Dolichospermum	2014	31.7 – 7.81 x 10^6	92 – 104	0.992 – 0.996	230	550
mcyE DNA toxin gene assays						
Microcystis	2013	15.1 – 2.43 x 10^6	98 – 101	0.999	30 – 122	39 – 122
Microcystis	2014	12.4 – 1.24 x 10^6	89 – 98	0.996 – 0.999	13	39
Planktothrix	2013	17.4 – 2.11 x 10^6	95 – 96	0.999	60 – 132	110 – 132
Planktothrix	2014	11.8 – 2.04 x 10^6	88 – 98	0.995 – 0.998	51 – 62	110
Dolichospermum[a]	2013	N/A	N/A	N/A	N/A	N/A
Dolichospermum	2014	37.6 – 6.92 x 10^6	91 – 99	0.997 – 0.999	46	120
mcyE RNA transcript assays						
Microcystis	2013	15.1 – 1.51 x 10^6	102 – 107	0.997 – 0.999	16	47
Microcystis	2014	14.9 – 1.49 x 10^6	94 – 98	0.998 – 1.00	16	47
Planktothrix	2013	21.1 – 2.11 x 10^6	95 – 99	0.997 – 1.00	61	130
Planktothrix	2014	24.5 – 2.45 x 10^6	94 – 104	0.991 – 0.999	61	130
Dolichospermum[b]	2013	N/A	N/A	N/A	N/A	N/A
Dolichospermum[b]	2014	N/A	N/A	N/A	N/A	N/A

[a] A standard was not developed for the *Dolichospermum mcyE* assays in 2013.

[b] *Dolichospermum mcyE* DNA was not detected in any samples in 2013–14; as a result, samples were not analyzed for *Dolichospermum mcyE* RNA.

Table 4. Definitions and manipulations of environmental factors computed for statistical analysis and compiled for three lakes included in 2014 sampling.

Acronym/Variable	Definition	Site-specific information sources
Weather data from the National Weather Service nearest airport site		
(http://www.ncdc.noaa.gov/qclcd/QCLCD?prior=N)		Buckeye Lake •Newark Heath Airport Harsha Lake •Cincinnati Municipal Lunken Field Maumee Bay State Park •Toledo Executive Airport
Airport Rain	Total rainfall, in inches, for the 24-hour period up to to 8 a.m. on the day of sampling (Dm1) and other manipulations listed below.	
Wind direction instantaneous	Wind direction, in degrees, at 8 a.m. on the day of sampling.	
Wind speed instantaneous	Wind speed, in miles per hour, at 8 a.m. on the day of sampling.	
Wind direction 24-hr and wind speed 24-hr	Wind direction and wind speed calculated by summing hourly wind vectors for the 24-hour period preceding sampling and determining the direction and speed of the resultant vector.	
Radar rainfall from 4- by 4-kilometer cells, National Weather Service		
(http://madis.noaa.gov/)		Buckeye Lake, 15 cells
Radar average	Average of radar-indicated precipitation for all cells for a selected site for the 24-hour period up to 8 a.m. on the day of sampling (for example, Dm1), and other manipulations listed below.	
Radar sum	Sum of radar-indicated precipitation for all cells for a selected site for the 24-hour period up to 8 a.m. on the day of sampling (for example, Dm1), and other manipulations listed below.	

Table 4. Definitions and manipulations of environmental factors computed for statistical analysis and compiled for three lakes included in 2014 sampling.—Continued

Acronym/Variable	Definition	Site-specific information sources
Water levels from National Oceanic and Atmospheric Administration (NOAA) or from U.S. Geological Survey (USGS) stations		
(http://tidesandcurrents.noaa.gov/ or http://waterdata.usgs.gov/nwis)		Buckeye Lake •USGS 395417082314200, Buckeye Lake near Millersport Harsha Lake •USGS 03247040, East Fork Lake near Bantam Maumee Bay State Park •NOAA lake levels Toledo station 9063085
Daily change	The change in lake level over the past 24 hours calculated as today's lake level for 10:00 a.m. (a typical sampling time) minus yesterday's lake level at 10:00 a.m.	
Weekly or biweekly change	The change in lake level over the past 7 or 14 days calculated as today's lake level for 10:00 a.m. (a typical sampling time) minus the lake level 1 or 2 weeks ago at 10:00 a.m.	
Average daily change	The average daily change in lake level for the past 7 or 14 days.	
Streamflow from U.S. Geological Survey stations		
(http://waterdata.usgs.gov/nwis)		Harsha Lake • 03246500, East Fork Little Miami River @ Williamsburg OH • 03247500, East Fork Little Miami River @ Perintown OH Maumee Bay State Park •04193500, Maumee River at Waterville (discharge)
Streamflow	Daily mean streamflow, in cubic feet per second, for the 24-hour period for the previous day before sampling (midnight to midnight).	
Maximum streamflow	Maximum daily streamflows averaged over 7, 14, or 30 days as indicated.	
Solar radiation from Ohio Agricultural Research and Development Center (OARDC)		
(http://www.oardc.ohio-state.edu/newweather/)		Maumee Bay State Park •OARDC North Central Station (solar radiation)
Solar Dm1	Solar radiation, in Langleys, for the 24-hour period for the previous day before sampling (midnight to midnight).	
Water-quality data from a United States Environmental Protection Agency (USEPA) operated continuous monitor		
(Monitor location: latitude 39° 01' 57", longitude -84° 08' 16")		Harsha Lake •USEPA Sonde (H. Joel Allen, U.S. Environmental Protection Agency, 2014, written commun.)
Water-quality data	Temperature, specific conductance, pH, dissolved oxygen, phycocyanin, and chlorophyll were recorded every 10 minutes from May 13–November 24, 2014.	
Instantaneous	Instantaneous measurements made at 10 a.m. (a typical sampling time).	
Average instanteous	Average of instantaneous measurements made at 10 a.m. for 3, 7, or 14 days as indicated.	
Dm1	Average of instantaneous measurements for the 24-hour period up to 10 a.m. on the day of sampling.	
Average Dm1	Average of Dm1 values for 3, 7, or 14 days as indicated.	
Data manipulations		
Dm1	Day minus 1 (yesterday); 24-hour totals for airport rainfall, radar rainfall, streamflow, solar radiation, and continuous monitor data.	
Dm2	Day minus 2 (2 days ago); Dm1 lagged one day.	
Dm3	Day minus 3 (3 days ago); Dm1 lagged two days.	
48W	Cumulated sums for the past 48 hours, giving the most weight to the most recent value, as follows: (2 * Dm1) + Dm2.	
72W	Cumulated sums for the past 72 hours, giving the most weight to the most recent value, as follows: (3 * Dm1) + (2 * Dm2) + Dm3.	
7-d Sum	Daily values summed for the last 7 days.	
14-d Sum	Daily values summed for the last 14 days.	

Water-level, streamflow, and solar-radiation data were compiled from various sources. Water-level data were obtained for the nearest offshore buoy (National Oceanic and Atmospheric Administration, 2014c) for MBSP or from the USGS National Water Information System Web site (NWISWeb) (U.S. Geological Survey, 2014) for stations below the dams at Harsha Lake and Buckeye Lake. Instantaneous water-level values were compiled for a typical sampling time (that is, 10 a.m.) using a "lake level spreadsheet" described in Francy and others (2013); data manipulations were calculated from the 10 a.m. values (table 4). Streamflow data for USGS stations were obtained from NWIS web (U.S. Geological Survey, 2014) and data manipulations were calculated from the daily mean value for the previous day. For Harsha Lake, discharge and water-level data were used to estimate an indicator of lake residence times as described in appendix 1. Solar-radiation data were obtained from the Ohio Agricultural Research and Development Center Weather System (OARDC) (http://www.oardc.ohio-state.edu/newweather/) and were compiled for the previous day (midnight to midnight).

The U.S. Environmental Protection Agency, Office of Research and Development, operates a continuous water-quality monitor at Harsha Lake during the recreational seasons. During 2014, a YSI EXO sonde (YSI Incorporated, Yellow Springs, Ohio) was suspended from a buoy deployed approximately 900 ft from a drinking-water intake (fig. 2*A*); the sonde was suspended in a compartment about 18 inches below the water's surface. Water-quality data were compiled for the 10 a.m. instantaneous measurement and for the 24-hour average up to 10 a.m. on the date of sampling; data manipulations were based on these values.

Field Quality Assurance and Quality Control

Standard field and data management quality-assurance practices for USGS water-quality activities in Ohio are described in Francy and Shaffer (2008). Standard laboratory quality-assurance/quality-control (QA/QC) practices for the OWML are described in Francy and others (2014). To quantify sampling and analytical bias and variability, the sampling crews collected field blanks, field concurrent replicates, and split samples.

Three field blanks were collected for nutrients and toxins, and five field blanks were collected for cyanobacterial genes. Field blank samples consisted of 2 L of inorganic-free water provided by the USGS NWQL for nutrients or sterile deionized water produced in the Ohio WSC for toxins and cyanobacterial gene analyses. The field blank water was taken through all steps of sample collection, processing, and handling as was done with regular samples under field conditions. Additional blanks were processed for cyanobacteria by molecular methods—one filter blank was processed every day that samples were filtered, and approximately 30 percent of the filter blanks were subsequently analyzed.

Field concurrent replicates are samples collected and analyzed in a manner such that the samples are thought to be virtually identical in composition. Field concurrent replicates were collected by filling two 5-L composite bottles with water at each of the subsample locations. Each composite bottle was subsequently processed as a separate sample and analyzed for nutrient and toxin concentrations and cyanobacteria by molecular and microscopy methods. Nine concurrent replicates were analyzed for nutrients, toxins, and cyanobacteria by microscopy (except for cylindrospermopsin, with six concurrent replicates). For molecular analysis of cyanobacteria, 12 concurrent replicates were collected in 2013–14. In 2014, each concurrent replicate was filtered, processed, and analyzed twice for cyanobacteria by molecular methods (split concurrent replicates).

Data Management and Analysis

Data on field parameters, wave heights, turbidity, water temperature, and nutrient, toxin, and cyanobacterial gene concentrations were entered into the USGS database for water-quality data available to the public through NWISWeb (U.S. Geological Survey, 2014) with codes for local collecting and analyzing agencies and station identification numbers for each sampling location (table 1). Data on phytoplankton abundance and community composition are presented as an online appendix (appendix 2).

Cyanobacterial community composition data were grouped into five categories for analysis: *Dolichospermum* spp. , *Microcystis* spp., *Planktothrix* spp., other microcystin producers, and non-microcystin producers. Cyanobacterial genera in the category "other microcystin producers" included *Anabaenopsis* spp., *Aphanocapsa* spp., *Phormidium*, spp., *Pseudanabaena* spp., *Snowella* spp., and *Woronichinia* spp. (Graham and others, 2008). Because microcystin was the predominant toxin detected during this study, all other cyanobacterial genera were grouped in the non-microcystin-producers category.

Because of the wide range of values, concentrations of cyanobacterial genes or transcripts and abundance or biovolume from community analysis were $\log_{10}$-transformed before data analysis or statistical testing. For summary statistics and data analysis, averages of concurrent replicate and split replicate samples were used for water-quality constituents, and geometric means were used for cyanobacterial abundance, biovolume, or gene concentrations. For examining differences in results from replicate pairs, relative percent differences (RPD) were used for water-quality and toxin data, and absolute value log differences (AVLD) were used for cyanobacterial molecular and microscopy data, calculated as follows:

$$RPD = 2\frac{\left|R_A - R_B\right|}{\left(R_A + R_B\right)} \quad (1)$$

$$AVLD = \left|\log(R_A) - \log(R_B)\right| \quad (2)$$

where

R_A is the concentration in replicate *A,* and

R_B is the concentration in replicate *B*.

If only one replicate was above the detection limit, the detection limit was used to calculate differences and the difference was reported as a greater-than value. If both replicates were below detection, differences were not calculated.

In 2014, concurrent replicates for cyanobacterial genes were used to prepare split replicates by filtering two or more well-mixed aliquots of water from each concurrent replicate bottle and then separately processing and analyzing two of the filters. So, for example, concurrent replicate *A* would have two split replicates designated R_{A_1} and R_{A_2}, and concurrent replicate *B* would have two split replicates designated R_{B_1} and R_{B_2}. The split replicates were used to compute AVLDs to evaluate variability both within and between concurrent replicate bottles. The between-bottle calculations (equation 4) included all possible between-bottle combinations.

The within-bottle AVLDs $\left(AVLD_w\right)$ were computed as follows:

$$AVLD_W = \left\{\begin{array}{l}\left|\log(R_{A_1}) - \log(R_{A_2})\right| \\ \left|\log(R_{B_1}) - \log(R_{B_2})\right|\end{array}\right\} \quad (3)$$

and the between-sample AVLDs $\left(ALVD_B\right)$ were computed as follows:

$$AVLD_B = \left\{\begin{array}{l}\left|\log(R_{A_1}) - \log(R_{B_1})\right| \\ \left|\log(R_{A_1}) - \log(R_{B_2})\right| \\ \left|\log(R_{A_2}) - \log(R_{B_1})\right| \\ \left|\log(R_{A_2}) - \log(R_{B_2})\right|\end{array}\right\} \quad (4)$$

The Kruskal-Wallis test with a post hoc Dunn's test was used to compare median AVLDs of qPCR, within-bottle, and between-bottle differences of replicate pairs for cyanobacterial molecular assays. Spearman's correlations were used to describe the correlations between microcystin and water-quality or environmental factors. Relations were considered to be statistically significant when p was less than or equal to (≤) 0.05.

Quality-Control Measures of Bias and Variability

QC samples were collected and analyzed for all constituents to characterize bias and variability. Because published data are scarce with regard to blanks and replicates for cyanobacteria by molecular methods, both qPCR and field QC samples were examined in more detail than the other constituents.

In the three field blanks collected for nutrient and toxin analyses, concentrations were below detection (table 5). Of the five field blanks collected for cyanobacterial genes, three had no detections; however, two blanks had detections for *Planktothrix mcyE* DNA and (or) general cyanobacteria (table 5). Fifteen filter blanks that were processed in the field in 2013–14, had four detections of various genes (data not shown). The detections in field and filter blanks were at or near sampling reporting limits and were at least one log value lower than the concentrations found in associated environmental samples. Out of eight laboratory extraction blanks analyzed during 2013–14, one was positive for both *Planktothrix mcyE* DNA and *Microcystis mcyE* DNA at concentrations at or near sample reporting limits (data not shown). Because the rare detections in blanks were very low concentrations, the limit of detection for each assay was established on the basis of Ct values of blanks, and sample results near reporting limits were qualified as estimates, it was unlikely that concentrations reported for environmental samples were appreciably affected by contamination.

Concurrent replicate summary data for nutrient and toxin analyses are listed in table 6, including the relative percent differences (RPD) calculated for each constituent. Median RPDs for nutrients ranged from 1 to 14 percent. Although the maximum RPDs for nitrite and orthophosphate were above 20 percent, they resulted from differences between two concentrations near the detection limits (for example, 0.003 and 0.002 mg/L for nitrite with an RPD of 40 percent). For total nitrogen, the maximum RPD and only RPD above 20 percent resulted from the difference between 1.03 and 0.81 mg/L; this difference (0.220 mg/L difference) was small in comparison with the range of environmental values. Median RPDs for microcystin and saxitoxin replicates were 20 and 23 percent, respectively.

Table 5. Quality-control field blank data for nutrients, toxins, and cyanobacterial genes, 2013–14.

[mg/L, milligram per liter; µg/L, micrograms per liter; copies/100 mL, copies per 100 milliliters; --, not done; <, less than; E, estimated value; ~, PCR duplicates do not agree; b, value was extrapolated at the low end (past the lowest concentration of the standard curve)]

Constituent	Buck Creek 7/23/13	Port Clinton 7/30/13	Harsha 6/12/14	Harsha 7/10/14	Maumee Bay State Park 10/7/14	Buckeye Lake 10/15/14
Nutrients (mg/L)						
Ammonia	<0.010	<0.010	<0.010	--	--	--
Nitrate + nitrite	<0.04	<0.04	<0.04			
Nitrite	<0.001	<0.001	<0.001	--	--	--
Orthophosphate	<0.004	<0.004	<0.004	--	--	--
Total phosphorus	<0.004	<0.004	<0.004	--	--	--
Total nitrogen	<0.05	<0.05	<0.05	--	--	--
Sulfate	ND	ND	<2.5	--	--	--
Toxins (µg/L)						
Microcystin	<0.10	<0.10	--	<0.10	--	--
Saxitoxin	<0.02	<0.02	--	<0.02	--	--
Cylindrospermopsin	<0.05	<0.05	--	--	--	--
Cyanobacterial genes (log copies/100 mL)						
Cyanobacteria	<2.94	<2.94	--	<3.49	Eb~3.74	<3.11
Microcystis	<3.42	<3.36	--	<3.51	<2.91	<3.13
Dolichospermum	--	--	--	<4.44	<3.84	<4.06
Microcystis mcyE DNA	<3.39	<3.37	--	<3.19	<2.59	<2.81
Planktothrix mcyE DNA	Eb~3.44	<3.42	--	<3.79	Eb~3.33	<3.41
Dolichospermum mcyE DNA	--	--	--	<3.74	<3.14	<3.36
Microcystis mcyE RNA	<2.00	<2.00	--	<3.80	<3.20	<3.42
Planktothrix mcyE RNA	<2.00	<2.00	--	<4.39	<3.78	<4.01

Table 6. Summary statistics for quality-control concurrent replicate data for nutrient and toxin concentrations, 2013–14.

[ND, not determined; RPD, relative percent difference]

Constituent	Detection limit	Number of sample pairs	Number of samples with at least one detection	Range of differences (in constituent units)	Median RPD	Maximum RPD
Nutrients, in milligrams per liter						
Ammonia nitrogen	<0.01	9	2	0–0.012	7	14
Nitrate + nitrite nitrogen	<0.04	9	4	0–0.005	1	3
Nitrite nitrogen	<0.001	9	4	0–0.011	5	40[a]
Dissolved orthophosphate	<0.004	9	2	0.001–0.003	14	22[a]
Total phosphorus	<0.004	9	9	0.001–0.013	5	10
Total nitrogen	<0.05	9	9	0.010–0.220	6	24
Sulfate		3	3	0–3.6	0	11
Toxins, in micrograms per liter						
Microcystin	<0.10	9	9	0–9.0	20	28
Saxitoxin	<0.02	9	2	0.01	23	>40[a]
Cylindrospermopsin	<0.05	6	0	ND	ND	ND

[a]Relative percent difference resulted from differences between two low concentrations near the detection limit.

Quality-control field concurrent replicate and split replicate samples were collected for cyanobacterial genes (table 7). The distributions of qPCR, within-bottle, and between-bottle differences for replicate cyanobacterial gene data are compared in boxplots (fig. 3). The qPCR replicates were extracts from the same filter that were analyzed twice by qPCR or qRT-PCR and provide a measure of part of the analytical variability. The within-bottle variability includes variability from sample filtration, reverse-transcription (for RNA) and DNA extraction and purification, qPCR, and the natural heterogeneity of the water matrix. The between-bottle variability includes variability through all these processes with the addition of sampling variability. A Kruskal-Wallis test was used to test the null hypothesis that AVLDs of paired qPCR, within-bottle, and between-bottle analyses came from the same distribution. If the null hypothesis was rejected, a post hoc Dunn's test was used to determine how the distributions differed from one another. Analyzing the data in this manner allows for greater scrutiny and identification of the source of variability.

The range and median magnitudes of AVLDs associated with qPCR replicates were appreciably smaller than within-bottle or between-bottle AVLDs for the five DNA assays (cyanobacteria, *Microcystis*, *Dolichospermum*, *Microcystis mcyE* DNA, and *Planktothrix mcyE* DNA), but not for AVLDs for the two RNA assays (*Microcystis* and *Planktothrix mcyE* RNA). The highest median AVLDs for qPCR replicates were for the two RNA assays (0.09 and 0.10 log copy/100 mL). The highest median within-bottle AVLD was found for *Microcystis* (0.35 log copy/100 mL), and the highest median between-bottle AVLD was found for *Microcystis mcyE* RNA (0.28 log copy/100 mL). More AVLD outliers occurred with the *mcyE* assays than for the general assays; the highest outlier was an AVLD of 1.85 log copies/100 mL for between-bottle comparisons of *Planktothrix mcyE* DNA. Distributions of within-bottle and between-bottle AVLDs were not statistically different for any assay, suggesting that sampling variability was small as compared to the combination of sample filtration, extraction and processing, and the heterogeneity of the matrix itself.

Concurrent replicate data for phytoplankton abundance are listed in table 8, including the AVLDs calculated for each concurrent replicate pair for the abundance and biovolume of cyanobacteria, *Dolichospermum*, *Microcystis*, *Planktothrix,* other microcystin producers, and non-microcystin producers. The AVLDs for measures of abundance ranged from 0 to 4.2 (median = 0.08; n = 63); 79 percent of comparisons had AVLDs less than 0.5. The AVLDs for measures of biovolume ranged from 0 to 5.5 (median = 0.13; n = 63); 76 percent of all comparisons had AVLDs less than 0.5. With few exceptions, replicates with AVLDs > 1.0 log (12 percent of all comparisons) were caused by rare taxa present in only one replicate or in low abundance or biovolume relative to the overall community (constituting less than 1 percent of the overall abundance or biovolume). Large AVLDs not affected by rare taxa likely were caused by the extreme spatial variability that may be present in cyanobacterial blooms that create challenges when collecting and processing replicate samples (Graham and others 2008, 2012).

Table 7. Absolute value log differences (AVLD) for quality-control field concurrent replicate and split replicate samples for cyanobacterial genes, in log copies per 100 milliliters, 2013–14.

[Within bottle, the AVLD between two split replicates where each bottle was analyzed twice and calculated from A1-A2 or B1-B2; Between bottle, the AVLD between two concurrent replicates where two bottles were collected at essentially the same time and calculated from A-B or A1-B1, A1-B2, A2-B1, A2-B2; --, not done or calculated; <, indicates less than value; >, indicates greater than value when one replicate was quantified and the second replicate was below detection; E, estimated value; b, value was extrapolated on the low end; ~, PCR duplicates did not agree; NTRU, nephelometric turbidity ratio unit]

Site	Replicate	Date	Turbidity, NTRU	Cyanobacteria			*Microcystis*			*Dolichospermum*			*Microcystis mcyE* DNA		
				Sample result	Within bottle	Between bottle	Sample result	Within bottle	Between bottle	Sample result	Within bottle	Between bottle	Sample result	Within bottle	Between bottle
Sandusky Bay	A	7/30/13	54.7	6.79	--	0.55	3.90	--	>0.11	--	--	--	<3.89	--	>1.03
	B		70.0	7.34		--	<3.79		--				4.92		--
Port Clinton	A	8/20/13	26.5	7.22	--	0.27	5.64	--	0.36	--	--	--	5.24	--	0.05
	B		27.5	6.95		--	6.01		--				5.19		--
Maumee Bay	A	9/18/13	113.0	7.53	--	0.71	7.37	--	0.75	--	--	--	7.23	--	0.45
	B		113.5	8.24		--	8.11		--				7.68		--
Buck Creek	A	9/14/13	6.0	7.68	--	0.25	4.44	--	0.09	--	--	--	4.48	--	0.71
	B		7.0	7.93		--	4.35		--				3.77		--
Harsha Main	A	7/23/13	16.1	7.34	--	0.07	5.44	--	0.06	--	--	--	4.97	--	0.11
	B		16.4	7.40		--	5.51		--				4.86		--
Deer Creek	A	8/12/13	17.2	8.31	--	0.07	4.23	--	0.21	--	--	--	4.42	--	>0.63
	B		19.2	8.24		--	4.01		--				<3.79		--
Harsha Main	A1	6/12/14	15.2	7.83	0.17	0.82	7.72	0.11	0.83	8.95	0.15	0.71	5.98	0.08	0.55
	A2			7.66		0.14	7.61		0.17	8.80		0.07	5.90		0.02
	B1		15.6	7.01	0.68	0.65	6.89	0.66	0.72	8.24	0.64	0.56	5.43	0.57	0.47
	B2			7.69		0.03	7.55		0.06	8.88		0.08	6.00		0.10
Onion Island	A1	7/24/14	46.2	8.54	0.19	0.17	5.23	0.35	0.50	7.10	0.37	0.50	4.55	0.21	0.22
	A2			8.73		--	4.88		--	6.73		--	4.76		--
	B1		44.0	8.37	--	0.36	4.73	--	0.15	6.60	--	0.13	4.33	--	0.43
	B2			--		--	--		--	--		--	--		--
Maumee Bay	A1	8/26/14	77.0	7.81	0.15	0.10	7.67	0.17	0.11	6.91	0.05	0.41	7.78	0.11	0.32
	A2			7.96		0.44	7.84		0.42	6.96		0.10	7.89		0.56
	B1		73.7	7.91	0.34	0.05	7.78	0.31	0.06	6.50	0.31	0.46	8.10	0.24	0.21
	B2			8.25		0.29	8.09		0.25	6.81		0.15	8.34		0.45
Harsha Main	A1	10/3/14	3.9	7.83	0.01	0.03	5.79	0.05	0.18	7.58	0.04	0.10	3.78	0.18	0.02
	A2			7.82		0.05	5.74		0.02	7.62		0.19	3.60		0.08
	B1		3.9	7.80	0.08	0.02	5.61	0.16	0.13	7.68	0.09	0.06	3.80	0.06	0.20
	B2			7.88		0.06	5.77		0.03	7.77		0.15	3.86		0.26
Maumee Bay	A1	10/7/14	35.6	8.00	0.71	0.40	7.89	0.61	0.37	7.46	0.29	0.52	6.91	0.08	0.00
	A2			7.29		0.02	7.28		0.09	7.17		0.23	6.83		0.11
	B1		--	7.60	0.38	0.31	7.52	0.46	0.24	6.94	0.75	0.23	6.91	0.11	0.08
	B2			7.98		0.69	7.98		0.70	7.69		0.52	6.80		0.03
Fairfield	A1	10/14/14	52.7	9.19	0.08	0.02	5.43	0.42	0.25	7.03	0.11	0.30	4.18	0.22	0.07
	A2			9.27		0.08	5.01		0.65	6.92		0.20	3.96		0.38
	B1		--	9.17	0.10	0.10	5.68	0.40	0.67	6.73	0.10	0.19	4.11	0.45	0.15
	B2			9.27		0.00	6.08		1.07	6.83		0.09	4.56		0.60

Table 7. Absolute value log differences (AVLD) for quality-control field concurrent replicate and split replicate samples for cyanobacterial genes, in log copies per 100 milliliters, 2013–14.—Continued

Site	Replicate	Date	Turbidity, NTRU	*Planktothrix mcyE* DNA			*Microcystis mcyE* RNA			*Microcystis mcyE* RNA		
				Sample result	Within bottle	Between bottle	Sample result	Within bottle	Between bottle	Sample result	Within bottle	Between bottle
Sandusky Bay	A	7/30/13	54.7	4.14	--	1.85	<3.00	--	--	3.85	--	0.03
	B		70.0	5.99		--	<3.00		--	3.88		--
Port Clinton	A	8/20/13	26.5	<4.08	--	--	4.02	--	0.30	<3.13	--	--
	B		27.5	<3.74		--	3.72		--	<3.13		--
Maumee Bay	A	9/18/13	113.0	<3.78	--	--	3.35	--	0.09	<3.17	--	--
	B		113.5	<3.78		--	3.44		--	<3.17		--
Buck Creek	A	9/14/13	6.0	5.41	--	0.15	<2.70	--	--	2.77	--	>0.07
	B		7.0	5.56		--	<2.70		--	<2.70		--
Harsha Main	A	7/23/13	16.1	5.5	--	0.01	<3.00	--	--	<3.00	--	--
	B		16.4	5.51		--	<3.00		--	<3.00		--
Deer Creek	A	8/12/13	17.2	5.24	--	0.17	<2.87	--	--	<2.87	--	--
	B		19.2	5.07		--	<2.87		--	<2.87		--
Harsha Main	A1	6/12/14	15.2	4.27	0.69	0.64	Eb3.98	0.23	>0.38	<4.18	--	--
	A2			4.96		0.02	Eb~3.75		0.33	<4.18		--
	B1		15.6	Eb~3.63	0.62	1.33	<3.60	>0.05	>0.15	<4.18	--	--
	B2			4.25		0.71	Eb~3.65		0.10	<4.18		--
Onion Island	A1	7/24/14	46.2	7.81	0.22	0.22	<3.80	--	--	5.72	0.26	0.23
	A2			8.03			<3.80		--	5.98		
	B1		44.0	7.59		0.44	<3.80	--	--	5.95	--	0.03
	B2			--			--		--	--		
Maumee Bay	A1	8/26/14	77.0	4.96	0.19	0.07	5.05	1.39	0.08	<4.78	--	--
	A2			5.15		0.24	6.44		0.41	<4.78		--
	B1		73.7	5.03	0.17	0.12	5.13	0.33	1.31	<4.78	--	--
	B2			5.20		0.05	5.46		0.98	<4.78		--
Harsha Main	A1	10/3/14	3.9	5.74	0.11	0.14	<3.60	--	--	<4.18	--	--
	A2			5.63		0.24	<3.60		--	<4.18		--
	B1		3.9	5.60	0.10	0.03	<3.60	--	--	<4.18	--	--
	B2			5.50		0.13	<3.60		--	<4.18		--
Maumee Bay	A1	10/7/14	35.6	Eb~3.99		0.35	Eb~3.99	0.03	0.04	<4.48	--	--
	A2			<3.88		0.64	Eb~4.02		0.29	<4.48		--
	B1		--	4.34	0.29	>0.46	Eb~3.95	0.33	0.07	<4.48	--	--
	B2			4.63		>0.75	Eb4.28		0.26	<4.48		--
Fairfield	A1	10/14/14	52.7	8.51	0.08	0.05	<3.60	--	--	5.86	0.05	0.05
	A2			8.59		0.08	<3.60		--	5.81		0.02
	B1		--	8.46	0.13	0.13	<3.60	--	--	5.91	0.03	0.1
	B2			8.59		0.00	<3.60		--	5.88		0.07

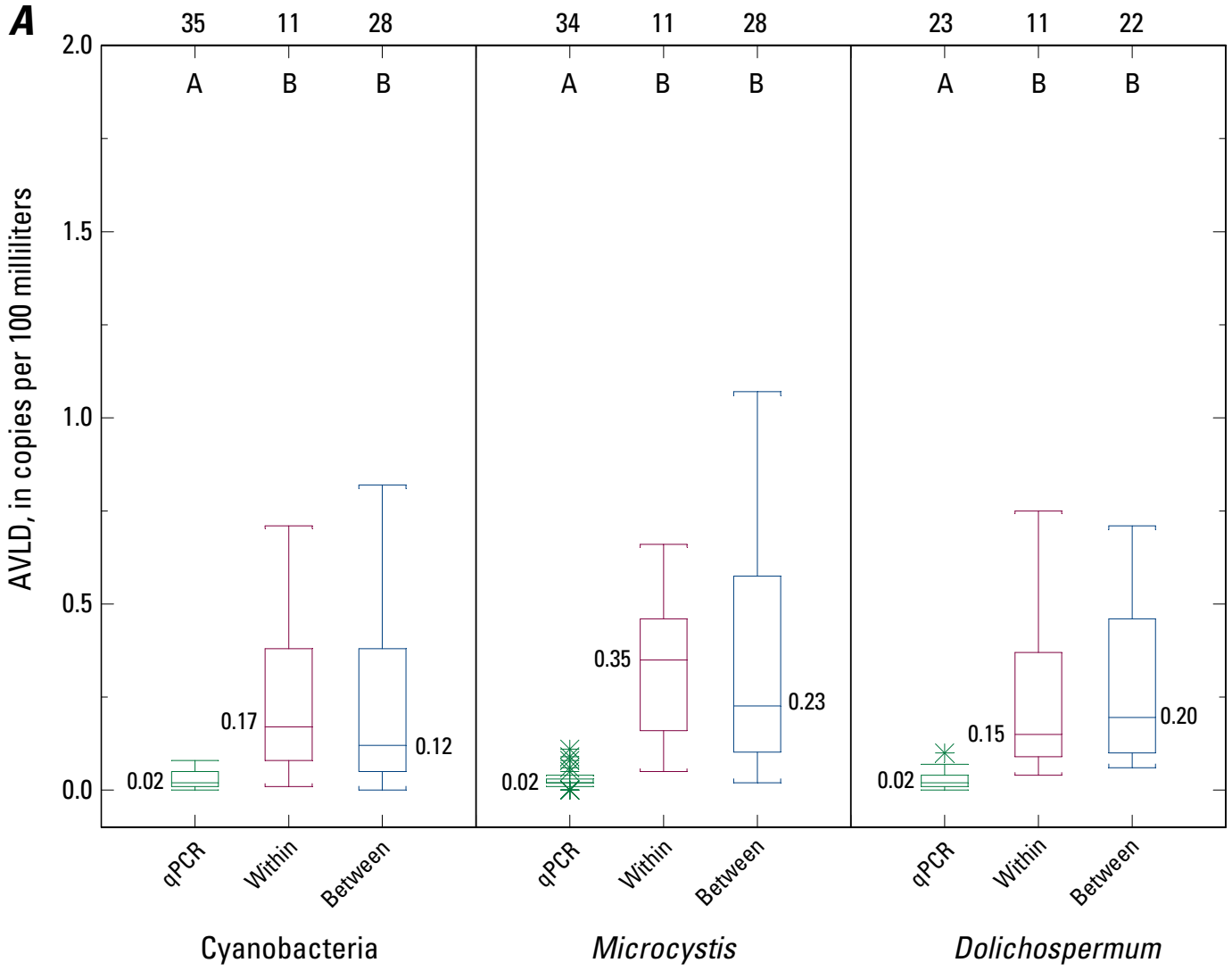


Figure 3. Comparison of quantitative polymerase chain reaction (qPCR) within-bottle, and between-bottle absolute value log differences (AVLDs) from field and laboratory replicate samples. *A*, cyanobacteria, *Microcystis*, and *Dolichospermum*. *B*, *Microcystis* (*Micro*) and *Planktothrix* (*Plankto*) *mcyE* toxin genes (deoxyribonucleic acid [DNA]) or transcripts (ribonucleic acid [RNA]), 2013–14. (The qPCR results are quantitative reverse-transcriptase PCR results for RNA assays. Points replace boxplot if data values are too few to display a statistical distribution.)

[1]Outliers are defined as values outside 1.5 times the interquartile range beyond the ends of the box.

[2]The whiskers are the largest and smallest values within 1.5 times the interquartile range beyond the 75th and 25th percentiles, respectively.

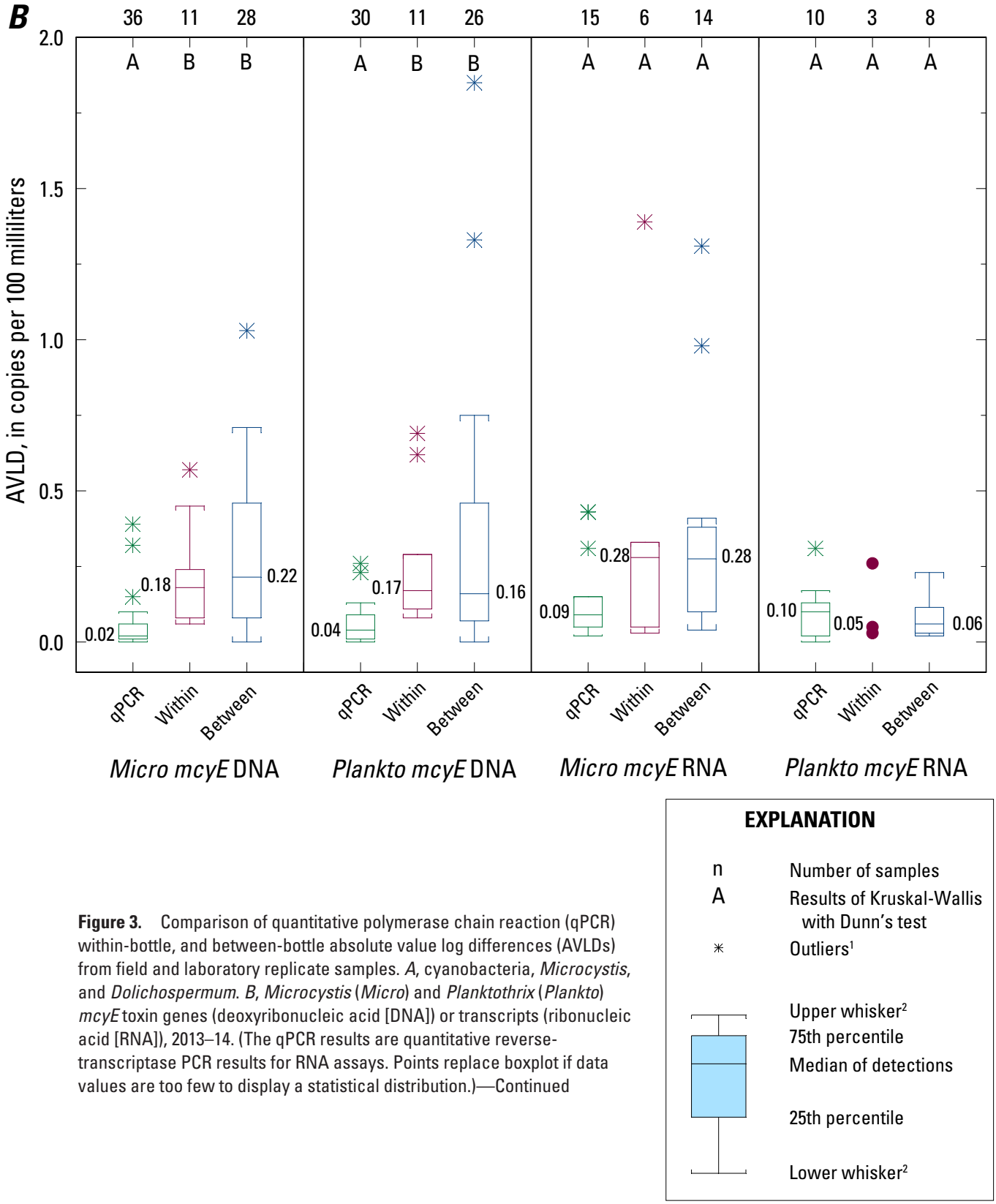


Figure 3. Comparison of quantitative polymerase chain reaction (qPCR) within-bottle, and between-bottle absolute value log differences (AVLDs) from field and laboratory replicate samples. *A*, cyanobacteria, *Microcystis*, and *Dolichospermum*. *B*, *Microcystis* (*Micro*) and *Planktothrix* (*Plankto*) *mcyE* toxin genes (deoxyribonucleic acid [DNA]) or transcripts (ribonucleic acid [RNA]), 2013–14. (The qPCR results are quantitative reverse-transcriptase PCR results for RNA assays. Points replace boxplot if data values are too few to display a statistical distribution.)—Continued

[1]Outliers are defined as values outside 1.5 times the interquartile range beyond the ends of the box.

[2]The whiskers are the largest and smallest values within 1.5 times the interquartile range beyond the 75th and 25th percentiles, respectively.

Table 8. Absolute value log differences (AVLD) for quality-control field concurrent replicate samples for phytoplankton abundance and biovolume, 2013–14.

[A value of 1 was added to all replicates where cyanobacteria were not detected to allow calculation of AVLDs. Abbreviations: cyano, cyanobacteria; MP, microcystin producing; cells/mL, cells per milliliter; µm³/mL, micrometer cubed per milliliter; --, not applicable]

Site	Date	Replicate	Total phytoplankton (cells/mL)	AVLD	Cyano density (cells/mL)	AVLD	*Dolichospermum* (cells/mL)	AVLD	*Microcystis* (cells/mL)	AVLD
Harsha Main	7/23/13	A	411,700	0.09	411,263	0.09	25,716	0.06	119,247	0.16
Harsha Main		B	335,262	--	334,785	--	22,650	--	83,419	--
Bay View East	7/30/13	A	258,225	0.08	221,118	0.06	2,995	0.19	1	0.00
Bay View East		B	309,865	--	255,392	--	4,599	--	1	--
Deer Creek	8/12/13	A	716,544	0.03	681,969	0.03	1	0.00	1	0.00
Deer Creek		B	674,920	--	639,592	--	1	--	1	--
Port Clinton	8/20/13	A	203,152	0.23	187,295	0.26	1	0.00	24,462	0.13
Port Clinton		B	119,601	--	102,231	--	1	--	33,197	--
Buck Creek	9/14/13	A	555,551	0.08	540,728	0.08	1	2.37	4,278	0.03
Buck Creek		B	673,916	--	654,896	--	232	--	3,993	--
Maumee Bay	9/18/13	A	306,610	0.01	297,660	0.01	378	0.59	293,333	0.01
Maumee Bay		B	313,111	--	303,675	--	1,483	--	301,935	--
Harsha Main	6/12/14	A	187,724	0.03	178,195	0.03	36,090	0.21	71,675	0.11
Harsha Main		B	199,515	--	191,547	--	58,932	--	55,987	--
Onion Island	7/24/14	A	2,502,858	0.01	2,438,739	0.01	9,411	0.83	1,644	3.22
Onion Island		B	2,537,454	--	2,510,774	--	1,380	--	1	--
Maumee Bay Cove 3	8/26/14	A	146,801	0.79	136,214	0.80	1	0.00	132,585	0.67
Maumee Bay Cove 3		B	899,975	--	864,444	--	1	--	616,325	--

Site	Date	Replicate	*Planktothrix* (cells/mL)	AVLD	Other MP cyano (cells/mL)	AVLD	Non-MP cyano (cells/mL)	AVLD	Total phytoplankton (µm³/mL)	AVLD
Harsha Main	7/23/13	A	43,897	0.09	15,362	0.10	207,041	0.10	7,272,361	0.09
Harsha Main		B	53,398	--	12,070	--	163,248	--	8,924,266	--
Bay View East	7/30/13	A	64,254	0.20	118,840	0.01	35,029	0.13	11,835,153	0.11
Bay View East		B	102,028	--	122,990	--	25,774	--	15,074,576	--
Deer Creek	8/12/13	A	1	0.00	358,703	0.07	323,266	0.01	20,308,441	0.01
Deer Creek		B	1	--	306,984	--	332,608	--	20,805,468	--
Port Clinton	8/20/13	A	1	0.00	149,221	0.34	13,612	1.17	10,158,947	0.11
Port Clinton		B	1	--	68,104	--	930	--	12,991,710	--
Buck Creek	9/14/13	A	1	0.00	63,598	0.13	472,852	0.08	15,147,002	0.03
Buck Creek		B	1	--	84,988	--	565,683	--	16,194,078	--
Maumee Bay	9/18/13	A	1	0.00	1	0.00	3,949	1.19	37,380,127	0.01
Maumee Bay		B	1	--	1	--	257	--	37,894,710	--
Harsha Main	6/12/14	A	7,700	0.27	17,734	0.07	44,996	0.06	7,152,643	0.13
Harsha Main		B	4,144	--	20,668	--	51,816	--	9,612,932	--
Onion Island	7/24/14	A	1,999,925	0.01	72,070	0.89	355,688	0.11	21,203,577	0.34
Onion Island		B	2,038,854	--	9,380	--	461,159	--	46,619,613	--
Maumee Bay Cove 3	8/26/14	A	1	4.20	2,651	1.89	978	1.46	6,240,738	0.89
Maumee Bay Cove 3		B	15,889	--	204,118	--	28,112	--	48,119,179	--

Table 8. Absolute value log differences (AVLD) for quality-control field concurrent replicate samples for phytoplankton abundance and biovolume, 2013–14.—Continued

[A value of 1 was added to all replicates where cyanobacteria were not detected to allow calculation of AVLDs. Abbreviations: cyano, cyanobacteria; MP, microcystin producing; cells/mL, cells per milliliter; µm³/mL, micrometer cubed per milliliter; --, not applicable]

Site	Date	Replicate	Cyano biovolume (µm³/mL)	AVLD	*Dolichospermum* (µm³/mL)	AVLD	*Microcystis* (µm³/mL)	AVLD	*Planktothrix* (µm³/mL)	AVLD
Harsha Main	7/23/13	A	6,648,941	0.11	1,763,470	0.02	3,009,369	0.10	930,873	0.44
Harsha Main		B	8,533,124	--	1,665,292	--	2,394,223	--	335,506	--
Bay View East	7/30/13	A	1,319,092	0.14	195,992	0.19	1	0.00	788,518	0.20
Bay View East		B	1,817,237	--	300,988	--	1	--	1,252,074	--
Deer Creek	8/12/13	A	14,474,752	0.00	1	0.00	1	0.00	1	0.00
Deer Creek		B	14,540,839	--	1	--	1	--	1	--
Port Clinton	8/20/13	A	3,041,606	0.10	1	0.00	2,766,573	0.13	1	0.00
Port Clinton		B	3,866,975	--	1	--	3,754,446	--	1	--
Buck Creek	9/14/13	A	12,511,760	0.02	1	4.26	483,820	0.03	1	0.00
Buck Creek		B	12,976,943	--	18,256	--	451,566	--	1	--
Maumee Bay	9/18/13	A	34,285,595	0.00	29,666	0.59	33,175,190	0.01	1	0.00
Maumee Bay		B	34,304,835	--	116,475	--	34,148,042	--	1	--
Harsha Main	6/12/14	A	4,661,168	0.15	1,750,917	0.25	1,873,389	0.11	163,289	0.51
Harsha Main		B	6,562,248	--	3,097,985	--	1,446,707	--	50,857	--
Onion Island	7/24/14	A	15,711,573	0.46	221,751	0.39	76,607	4.88	12,565,890	0.54
Onion Island		B	44,800,475	--	90,329	--	1	--	43,235,398	--
Maumee Bay Cove 3	8/26/14	A	4,507,127	0.96	1	0.00	4,442,956	0.96	1	5.53
Maumee Bay Cove 3		B	41,083,306	--	1	--	40,338,363	--	336,946	--

Site	Date	Replicate	Other MP cyano (µm³/mL)	AVLD	Non-MP cyano (µm³/mL)	AVLD
Harsha Main	7/23/13	A	46,796	1.77	898,433	0.66
Harsha Main		B	790	--	4,137,312	--
Bay View East	7/30/13	A	210,008	0.01	124,574	0.42
Bay View East		B	217,341	--	46,834	--
Deer Creek	8/12/13	A	5,658,542	0.06	8,816,210	0.04
Deer Creek		B	4,889,374	--	9,651,465	--
Port Clinton	8/20/13	A	55,226	0.25	219,807	1.18
Port Clinton		B	98,002	--	14,528	--
Buck Creek	9/14/13	A	1,198,799	0.03	10,829,141	0.01
Buck Creek		B	1,299,306	--	11,207,816	--
Maumee Bay	9/18/13	A	1	0.00	1,080,739	1.43
Maumee Bay		B	1	--	40,318	--
Harsha Main	6/12/14	A	1,161	2.73	872,413	0.19
Harsha Main		B	623,678	--	1,343,022	--
Onion Island	7/24/14	A	441,815	0.28	2,405,510	0.29
Onion Island		B	230,144	--	1,244,605	--
Maumee Bay Cove 3	8/26/14	A	174	3.34	63,997	0.36
Maumee Bay Cove 3		B	379,885	--	28,112	--

A General Survey of Toxin Concentrations, Water-Quality Factors, and Cyanobacteria at Eight Sites in 2013 and Site Selection for 2014

A total of 46 water-quality samples (6 of those included concurrent replicate samples) were collected from 8 sites at 8 lakes during 2013 to facilitate an initial assessment and aid in the selection of sites for more intensive sampling in 2014. Criteria for selection for 2014 sampling included sites with (1) a wide range of toxin concentrations, (2) at least one detection of the *mcyE* toxin gene, and (or) (3) statistically significant correlations between toxin concentrations and cyanobacterial gene concentrations or optical sensor (chlorophyll and phycocyanin) measurements.

Wide ranges of physical and water-quality conditions, toxin concentrations, and cyanobacteria were measured in 2013 (table 9). Nutrient constituents were detected in the majority of samples except for orthophosphate, which was detected in only 20 percent of samples. Cylindrospermopsin was not detected in any of the 46 samples, and microcystin was detected in 37 samples (80 percent). There were five detections of saxitoxin (11 percent), all well below the Ohio EPA Recreational Public Health Advisory level of 0.8 µg/L. Cyanobacterial gene or transcript percentages of detections ranged from 11 percent for *Microcystis mcyE* RNA to 96 percent for cyanobacteria. *Microcystis* and *Planktothrix mcyE* DNA median concentrations were 1 to 2 logs higher than the associated median RNA concentrations. The general *Dolichospermum* assay was not available, and *Dolichospermum mcyE* DNA was not detected in any samples in 2013 (data not shown). Cyanobacteria constituted 0 to 99 percent of the total phytoplankton community biovolume ("relative cyanobacteria biovolume," table 9) and 0 to 100 percent of the phytoplankton community abundance.

Findings by site are described below for microcystin concentrations (fig. 4), *Microcystis* and *Planktothrix mcyE* DNA concentrations and DNA and RNA percentages of detections (fig. 5), cyanobacterial biovolume (figs. 6–8), and Spearman's Rank correlations between microcystin concentrations and other factors (table 10). For microcystin, eight samples exceeded the Ohio EPA Recreational Public Health Advisory level of 6 µg/L, and five samples exceeded 20 µg/L (fig. 4). Cyanobacterial community dynamics were unique to each site during summer 2013 (figs. 6, 7*A*, and 8*A*). Cyanobacterial abundance reflects the total number of algal cells present in a sample, whereas biovolume is an indicator of algal biomass. Overall patterns in cyanobacterial abundance and biovolume generally were similar; therefore, only biovolume is discussed in detail below. Spearman's correlation analysis was done to identify statistically significant monotonic relations ($p \leq 0.05$) between water-quality and environmental factors and microcystin concentrations and to help select sites for sampling in 2014.

Buck Creek

At Buck Creek, microcystin was detected in 100 percent of samples; however, concentrations were low, ranging from 0.23 to 1.3 µg/L with a median of 0.46 µg/L (fig. 4). *Microcystis* and *Planktothrix mcyE* DNA were detected in 100 percent of samples, *Planktothrix mcyE* RNA was found 80 percent of samples, and *Microcystis mcyE* RNA was not detected (fig. 5). Non-microcystin-producing taxa typically dominated (fig. 6*A,* >51 percent relative community composition), but *Planktothrix* dominated (76 percent) in early July. Seasonal microcystin dynamics at Buck Creek generally matched patterns in cyanobacterial biovolume until July, when cyanobacterial biovolume began to increase but microcystin concentrations decreased.

Statistically significant correlations were found between microcystin concentrations and three water-quality factors—phycocyanin, Secchi depth (negative), and N to P ratios (table 10). Positive significant correlations were found between microcystin concentration and *Planktothrix* DNA or RNA, but not with *Planktothrix* biovolume. Although investigating the role of N to P ratios in influencing microcystin production by cyanobacteria at Buck Creek would have been an interesting study, microcystin concentrations well below Ohio EPA advisory levels made this a lower priority site for sampling in 2014.

Buckeye Crystal

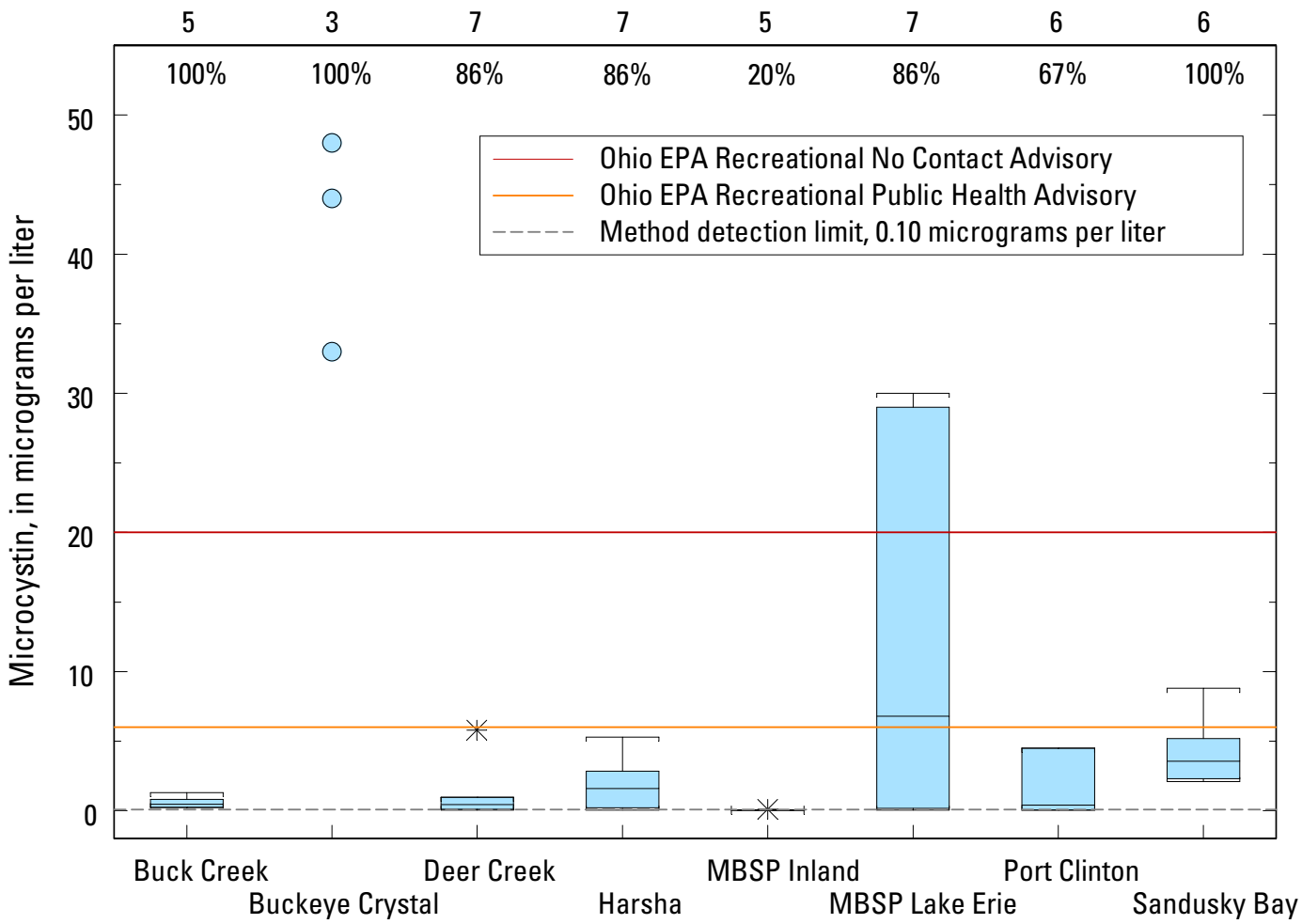
Buckeye Lake was added to the study in August 2013 because of high microcystin concentrations reported by Ohio EPA (Ohio Environmental Protection Agency, 2014). In the three samples collected by the USGS in August–October, 2013, microcystin concentrations were consistently high, ranging from 33 to 48 µg/L (fig. 4). Among all sites sampled in 2013, *Planktothrix mcyE* DNA (fig. 5*B*) and RNA (data not shown) concentrations were highest at Buckeye Crystal. Although Buckeye Crystal had the highest cyanobacterial biovolumes and microcystin concentrations during 2013 (fig. 6*B*), potential microcystin-producing taxa represented <1 percent of the relative community composition in all samples. High microcystin concentrations without an apparent microcystin-producing taxon present may have been caused by a currently unidentified microcystin producer or a producer spatially separated from microcystin occurring in the dissolved phase (Graham and others, 2010).

Although correlation analysis could not be done because of the small dataset, phycocyanin was observed to increase with increasing microcystin concentrations (data not shown), and *Planktothrix mcyE* RNA was found in every sample. Buckeye Lake, therefore, was included in the 2014 sampling to represent a lake with high microcystin concentrations, potentially produced by *Planktothrix.*

Table 9. Summary statistics for water-quality measurements, toxins, cyanobacterial gene and transcript concentrations, and cyanobacterial abundance and biovolume in 46 samples collected at 8 Ohio recreational sites, 2013.

[<, indicates less-than value; --, not applicable; °C, degree Celsius; µS/cm, microsiemens per centimeter at 25 degrees Celsius, RFU, relative fluorescence unit; NTRU, nephelometric turbidity ratio unit; mg/L, milligram per liter; µg/L, microgram per liter; %, percent; mL, milliliter; µm³, micrometer cubed]

	Minimum	Maximum	Median	Percentage of detections
Water-quality measurements				
pH	7.7	9.8	8.8	--
Water temperature, °C	8.6	28.1	22.4	--
Specific conductance, µS/cm	277	942	499	--
Dissolved oxygen	2.3	14.2	10.1	--
Chlorophyll sensor, RFU	1.0	10.5	2.8	--
Phycocyanin sensor, RFU	0.03	34.5	3.8	--
Turbidity, NTRU	5.9	114	26.2	--
Secchi depth, feet	0.3	3.2	1.4	--
Ammonia, mg/L	<0.01	0.265	0.017	76
Nitrate + nitrite, mg/L	<0.04	5.63	0.093	59
Orthophosphate, mg/L	<0.004	0.100	<0.004	20
Total phosphorus, mg/L	0.03	0.259	0.088	100
Total nitrogen, mg/L	0.52	6.69	1.61	100
N to P ratio (total)	6.51	117.4	16.3	100
Toxins (µg/L)				
Cylindrospermopsin	<0.05	--	--	0
Microcystin	<0.10	48.0	0.90	80
Saxitoxin	<0.02	0.15	<0.02	11
Cyanobacterial genes and transcripts (log copies/100 mL)				
Cyanobacteria	<4.16	9.25	7.34	96
Microcystis	<3.57	8.42	4.47	80
Microcystis mcyE DNA	<3.35	7.46	4.15	78
Planktothrix mcyE DNA	<3.65	8.09	5.11	65
Microcystis mcyE RNA	<2.18	4.02	2.97	11
Planktothrix mcyE RNA	<2.63	5.74	3.05	35
Cyanobacterial biovolume (µm³/mL)				
Relative cyanobacteria biovolume (%)	0	99	47	98
Cyanobacteria	0	530,000,000	2,500,000	98
Dolichospermum	0	4,200,000	0	43
Microcystis	0	34,000,000	4,056	50
Planktothrix	0	7,500,000	0	30
Other microcystin producers	0	5,300,000	12,000	67
Non-microcystin producers	0	530,000,000	310,000	87
Cyanobacterial abundance (cells/mL)				
Relative cyanobacteria abundance (%)	0	100	93	98
Cyanobacteria	0	1,300,000	220,000	98
Dolichospermum	0	140,000	0	43
Microcystis	0	300,000	44	50
Planktothrix	0	960,000	0	30
Other microcystin producers	0	330,000	5,700	67
Non-microcystin producers	0	1,300,000	20,000	87



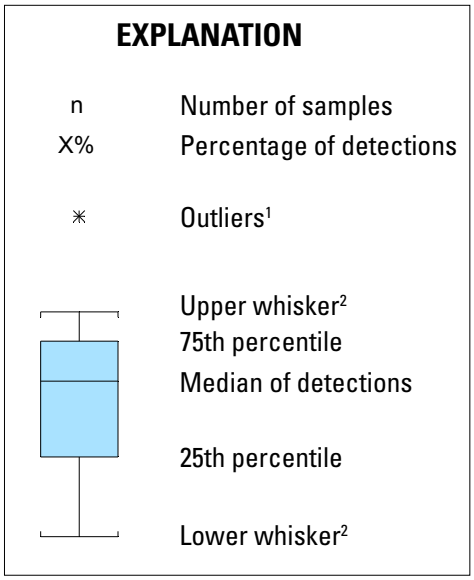


Figure 4. Concentrations of microcystin and percentages of detections at Ohio recreational sites, 2013. (An Ohio Environmental Protection Agency [Ohio EPA] Recreational No Contact Advisory for microcystin means that the public is recommended to avoid all contact with the water; it is in effect if someone or an animal dies or becomes ill as a direct result of cyanotoxin exposure and microcystin is greater than 20 micrograms per liter or other thresholds are reached. Points replace boxplot if data values are too few to display a statistical distribution.)

[1]Outliers are defined as values outside 1.5 times the interquartile range beyond the ends of the box.

[2]The whiskers are the largest and smallest values within 1.5 times the interquartile range beyond the 75th and 25th percentiles, respectively.

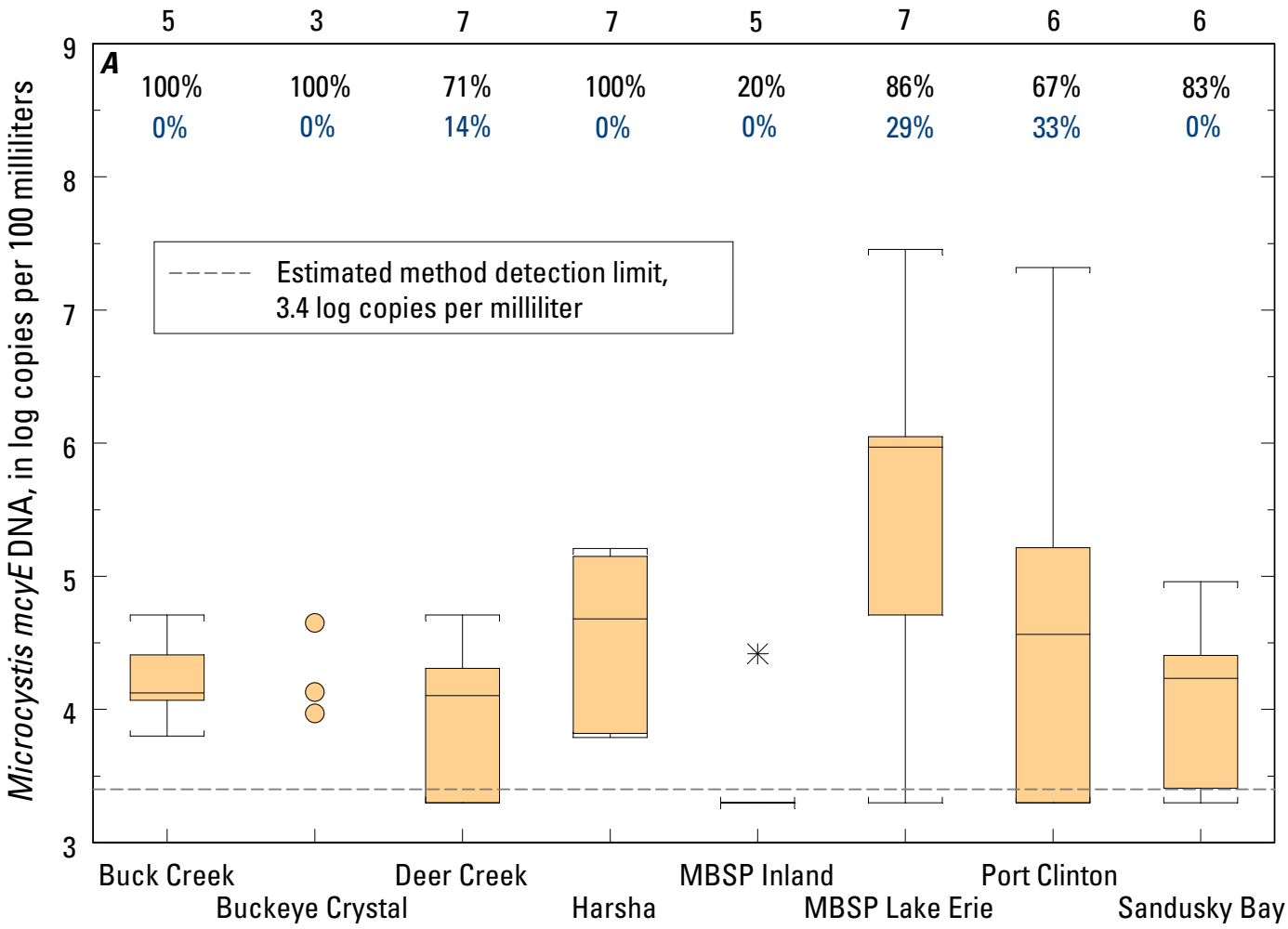


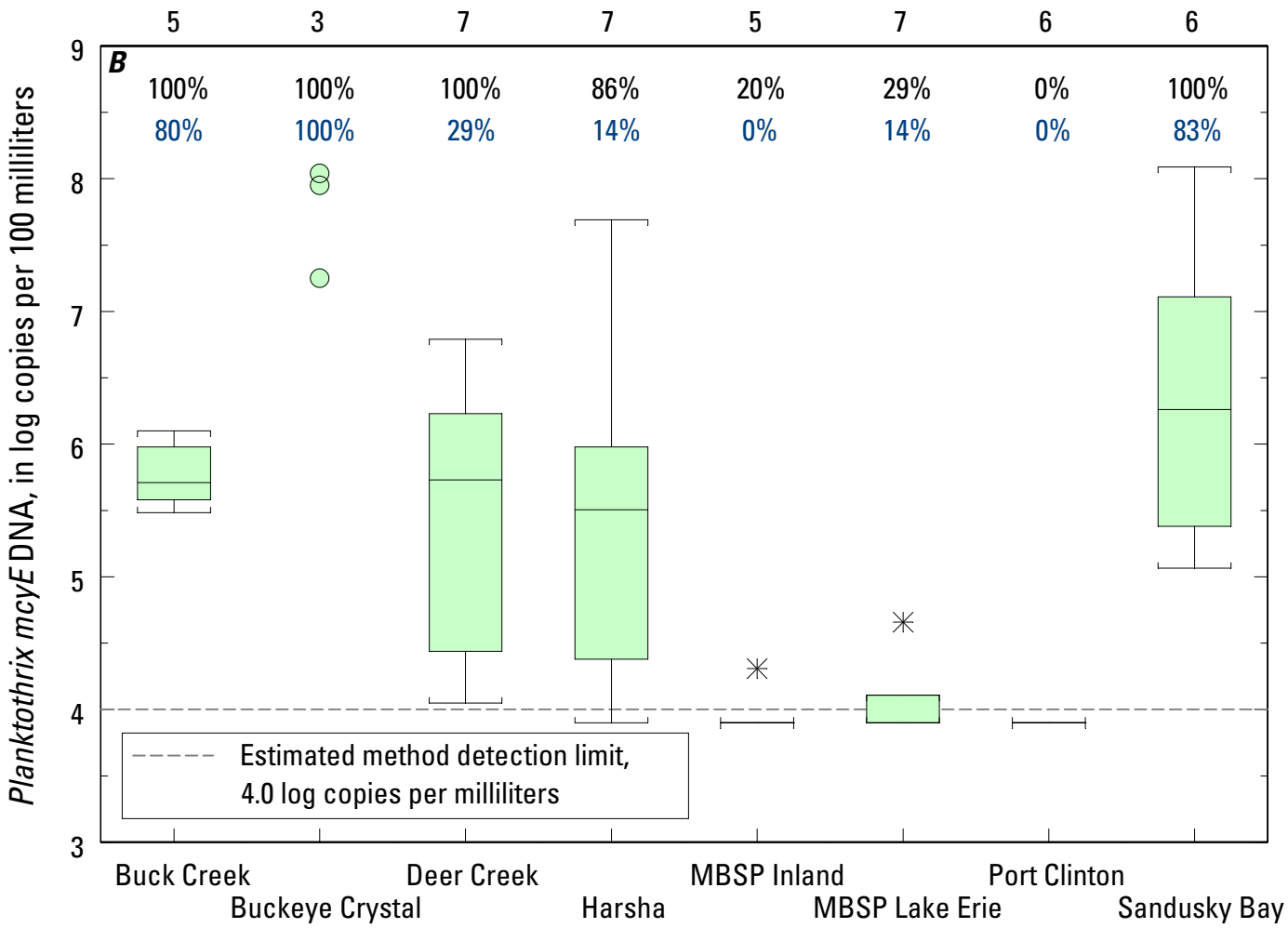
Figure 5. Concentrations of cyanobacterial toxin genes and percentages of detections at Ohio recreational sites, 2013. *A. Microcystis mcyE* deoxyribonucleic acid (DNA), *B. Planktothrix mcyE* DNA. (Percentages of detection of *Microcystis* and *Planktothrix mcyE* ribonucleic acid [RNA] are shown on each graph; however, the associated concentrations in boxplots are not included. Points replace boxplot if data values are too few to display a statistical distribution.)

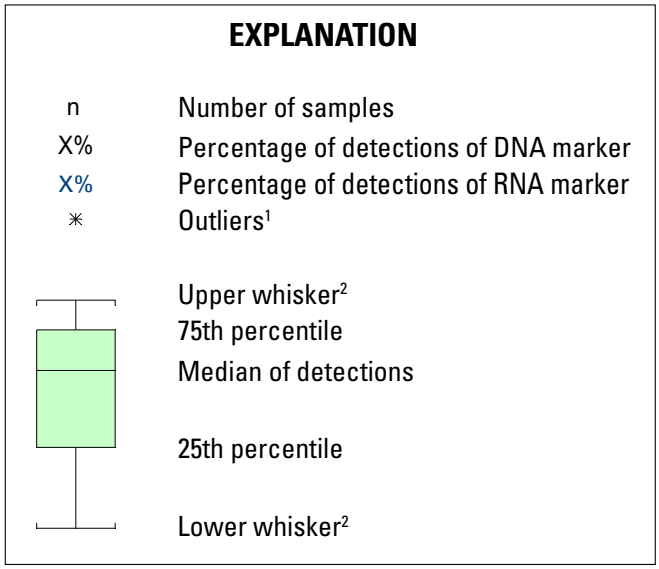
EXPLANATION

n	Number of samples
X%	Percentage of detections of DNA marker
X%	Percentage of detections of RNA marker
✳	Outliers[1]
	Upper whisker[2]
	75th percentile
	Median of detections
	25th percentile
	Lower whisker[2]

[1]Outliers are defined as values outside 1.5 times the interquartile range beyond the ends of the box.

[2]The whiskers are the largest and smallest values within 1.5 times the interquartile range beyond the 75th and 25th percentiles, respectively.





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[2]The whiskers are the largest and smallest values within 1.5 times the interquartile range beyond the 75th and 25th percentiles, respectively.

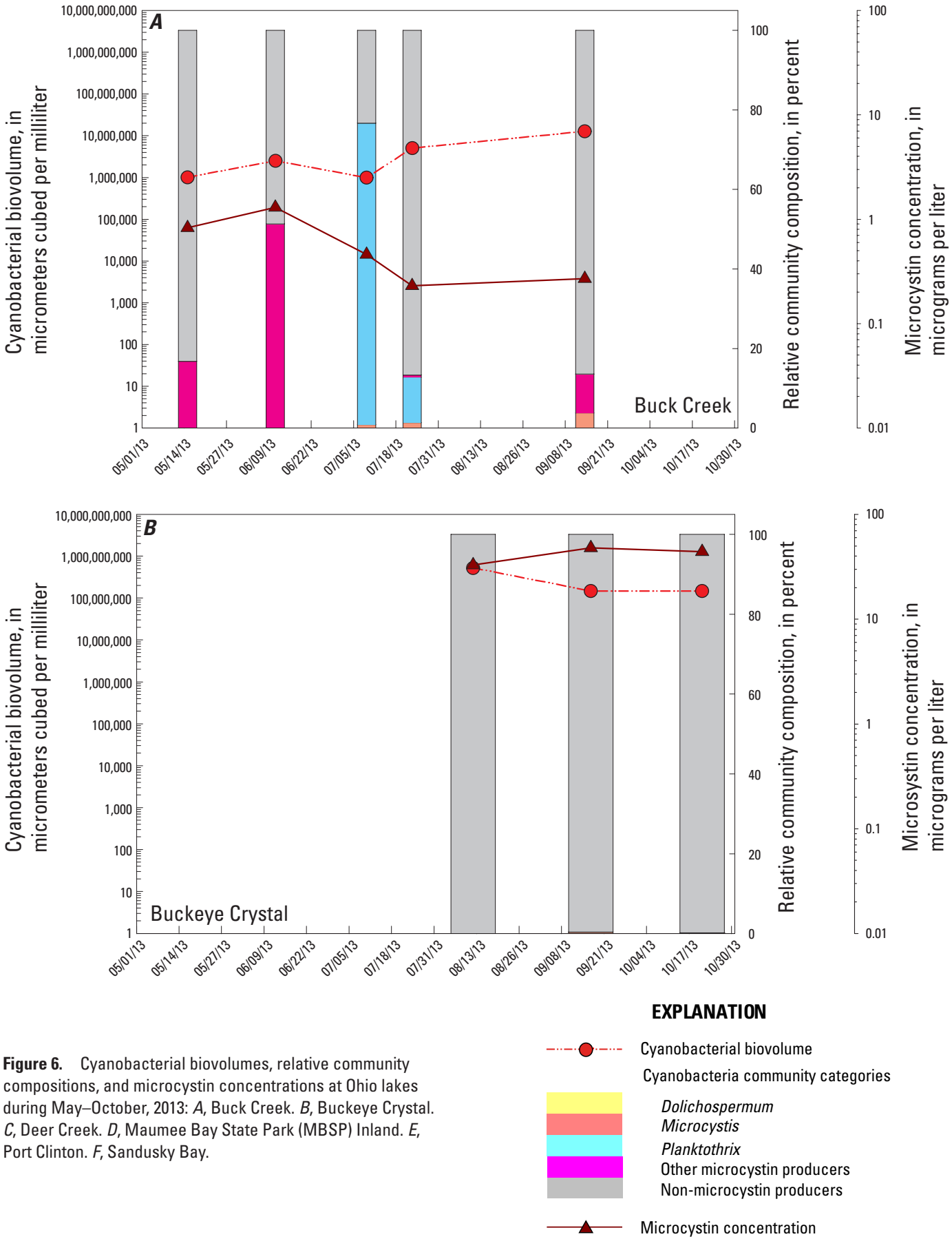
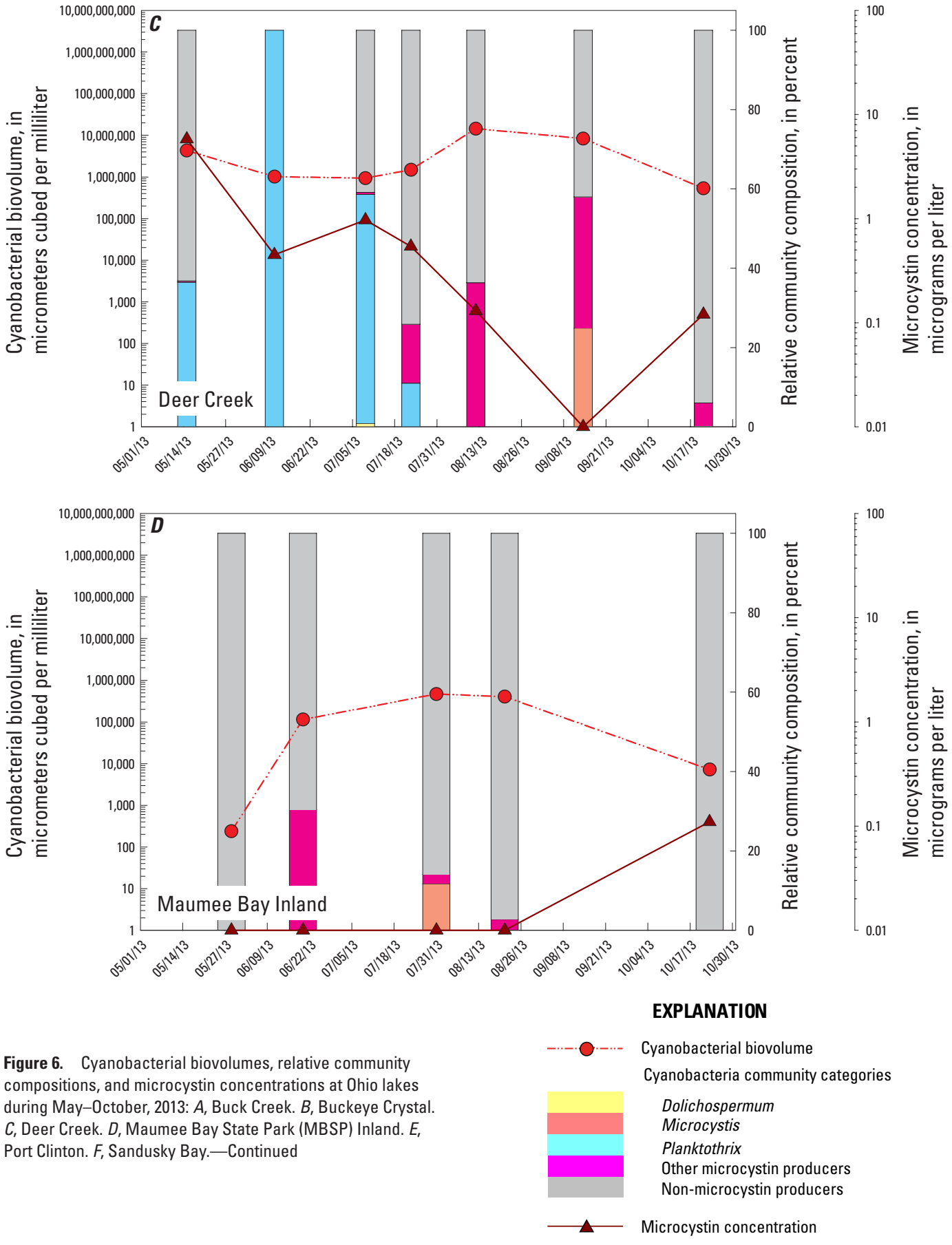


Figure 6. Cyanobacterial biovolumes, relative community compositions, and microcystin concentrations at Ohio lakes during May–October, 2013: *A*, Buck Creek. *B*, Buckeye Crystal. *C*, Deer Creek. *D*, Maumee Bay State Park (MBSP) Inland. *E*, Port Clinton. *F*, Sandusky Bay.



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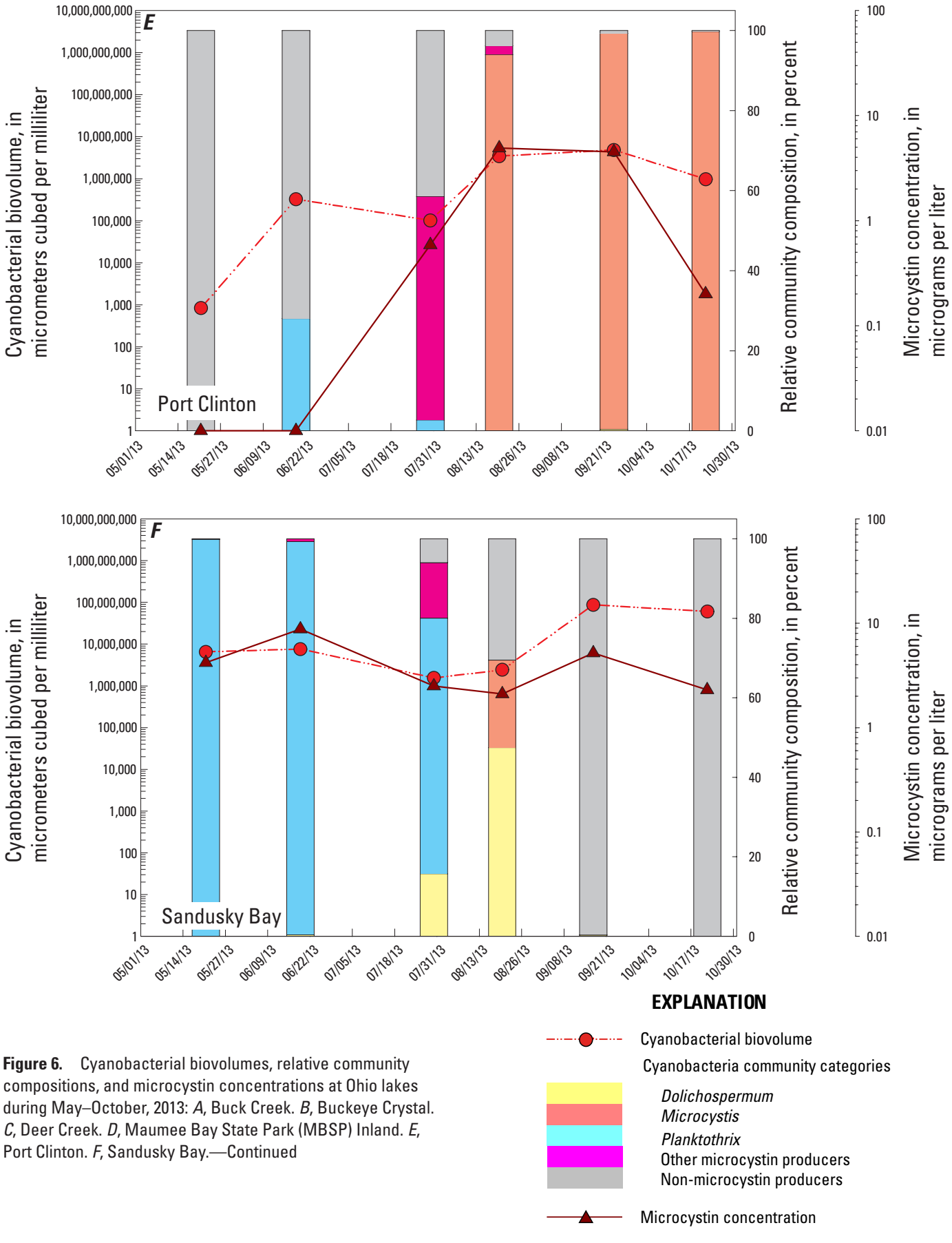

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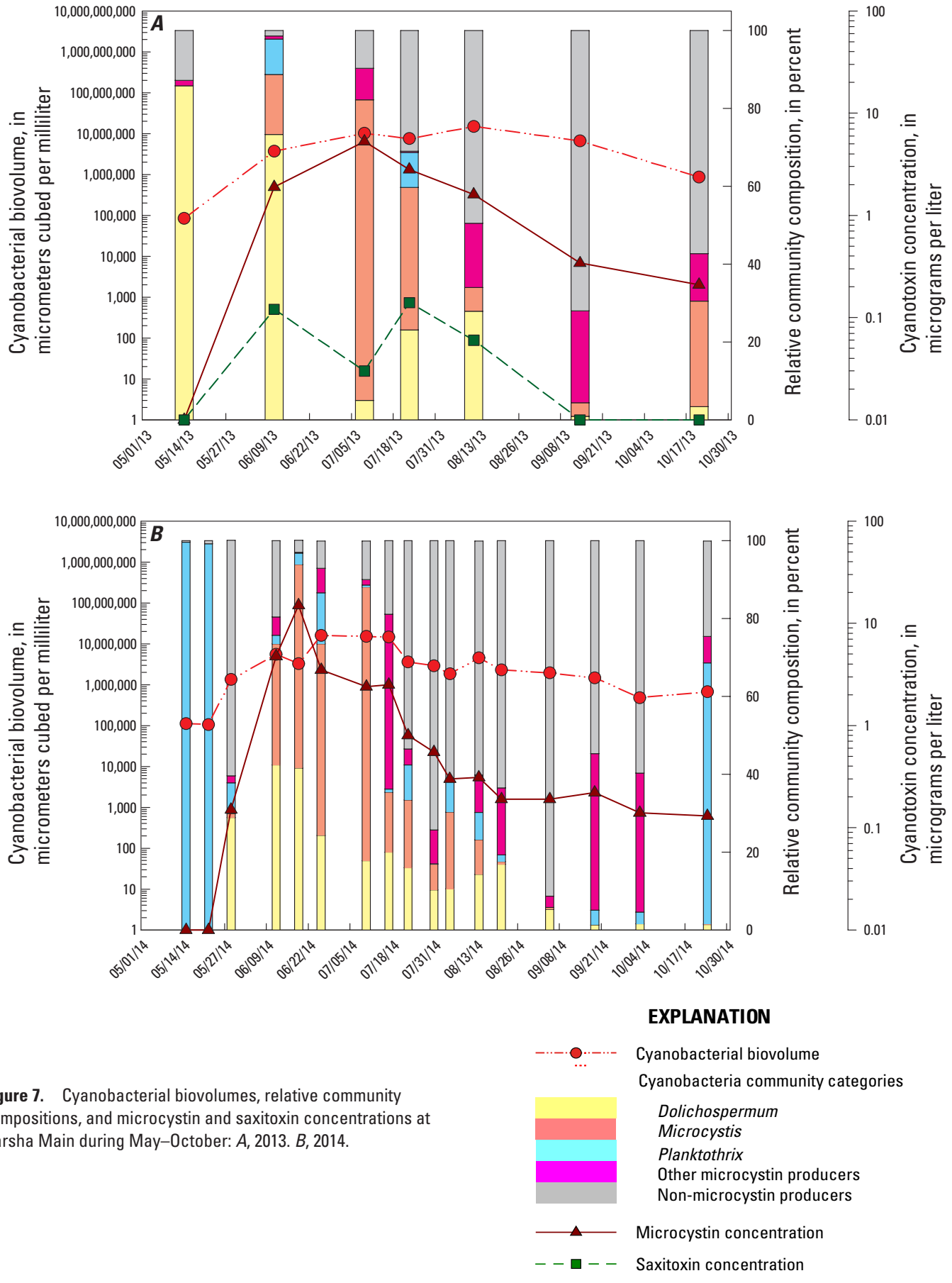


Figure 7. Cyanobacterial biovolumes, relative community compositions, and microcystin and saxitoxin concentrations at Harsha Main during May–October: *A*, 2013. *B*, 2014.

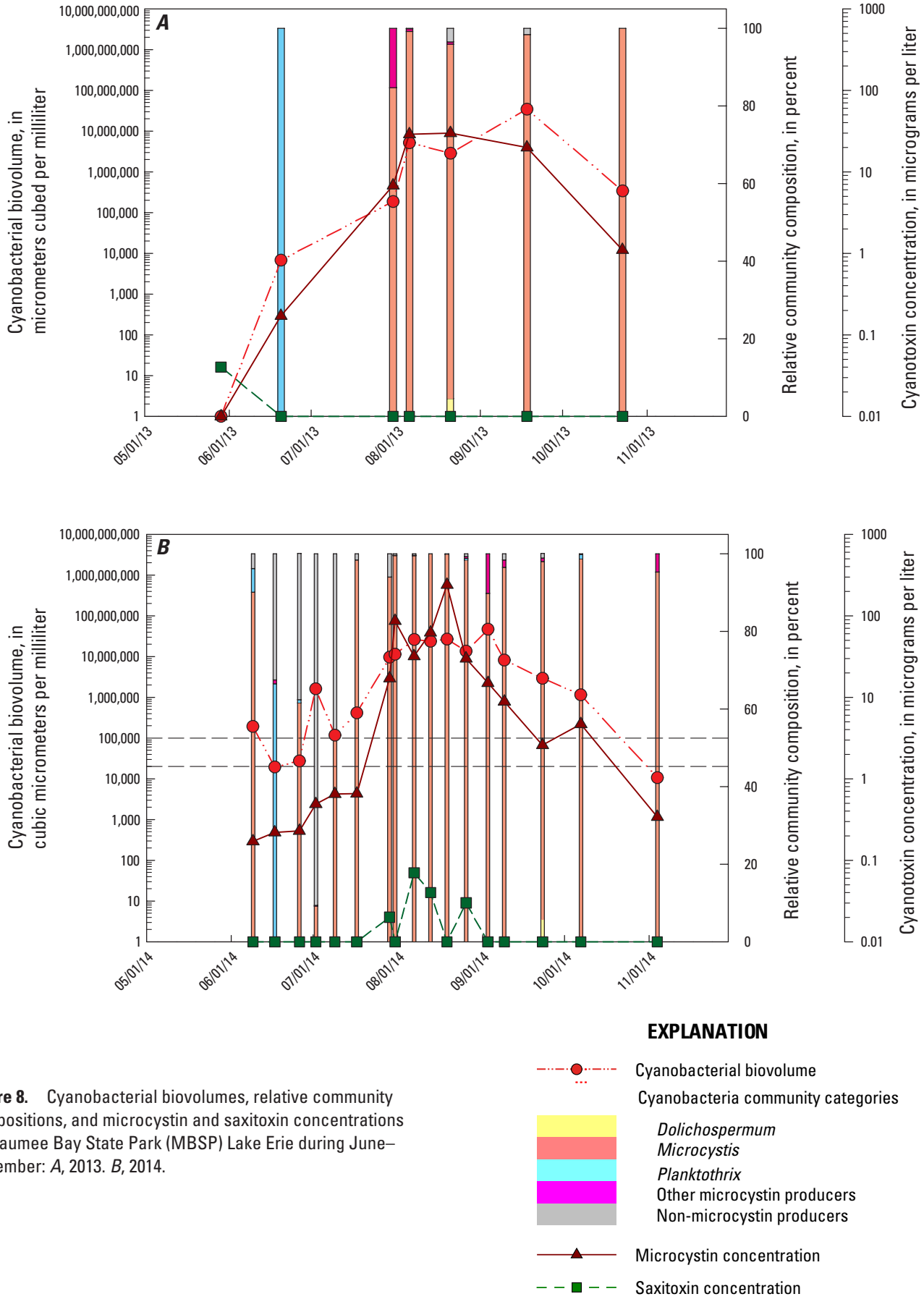


Figure 8. Cyanobacterial biovolumes, relative community compositions, and microcystin and saxitoxin concentrations at Maumee Bay State Park (MBSP) Lake Erie during June–November: *A*, 2013. *B*, 2014.

Table 10. Spearman Rank correlations (rho) between microcystin concentrations and water-quality factors, cyanobacterial gene and transcript concentrations, and cyanobacterial abundance and biovolume at six Ohio recreational sites, 2013.

[Relations that were significiant at p<0.05 are shaded and in bold; --, not determined because the factor lacked any detected values; °C, degree Celsius; µS/cm, microsiemens per centimeter at 25 degrees Celsius, RFU, relative fluorescence unit; NTRU, nephelometric turbidity ratio unit; mg/L, milligram per liter; mL, milliliter; µm^3, micrometer cubed]

Variable	Buck Creek n=5	Deer Creek n=7	Harsha Main n=7	Maumee Bay Lake Erie n=7	Port Clinton n=6	Sandusky Bay n=6
Water-quality variables						
pH	-0.60	-0.41	**0.75**	0.46	0.50	0.37
Water temperature, _C	-0.50	0.21	**0.93**	0.11	0.24	-0.26
Conductivity, µS/cm	0.00	0.04	**-0.75**	0.39	0.50	-0.31
Dissolved oxygen	-0.70	0.46	**0.79**	-0.04	**0.91**	-0.09
Chlorophyll sensor, RFU	0.10	**-0.75**	0.57	0.39	0.09	-0.37
Phycocyanin sensor, RFU or cells/mL	**0.90**	-0.29	**0.89**	0.64	0.50	0.77
Turbidity	0.70	0.32	**0.82**	0.54	0.76	-0.20
Secchi depth, feet	**-0.87**	-0.02	**-0.87**	-0.21	-0.47	0.31
Ammonia, mg/L	-0.10	-0.36	**-0.89**	**-0.90**	-0.71	**-1.00**
Nitrate + nitrite, mg/L	0.78	**0.96**	**-0.71**	-0.46	**-0.76**	0.04
Orthophosphate, mg/L	--	-0.41	**-0.81**	-0.61	-0.13	0.41
Total phosphorus, mg/L	0.46	-0.50	-0.43	0.54	-0.79	-0.26
Total nitrogen, mg/L	0.80	**0.93**	0.25	-0.21	-0.62	0.35
N to P ratio (total)	**0.90**	**0.93**	0.68	-0.46	-0.32	0.37
Cyanobacterial genes and transcripts (log copies/100 mL)						
Cyanobacteria	**-0.90**	0.46	0.05	0.39	0.79	-0.26
Microcystis	**-1.00**	0.11	0.71	0.57	0.69	-0.14
Microcystis mcyE DNA	0.40	**0.85**	0.25	**0.79**	**0.93**	-0.60
Microcystis mcyE RNA	--	-0.61	--	0.13	**0.84**	--
Planktothrix mcyE DNA	**0.90**	**0.96**	-0.14	-0.49	--	0.12
Planktothrix mcyE RNA	**0.90**	0.33	-0.41	-0.41	--	0.71
Cyanobacterial biovolume (µm^3/mL)						
Cyanobacteria	-0.50	-0.14	0.71	**0.82**	0.76	0.43
Dolichospermum	-0.35	0.41	0.54	**0.87**	0.54	-0.25
Microcystis	-0.87	-0.61	**0.96**	**0.81**	**0.82**	**-0.85**
Planktothrix	-0.45	**0.85**	0.49	-0.41	-0.40	0.64
Other microcystin producers	0.50	-0.54	0.18	0.65	0.57	0.15
Non microcystin cyanobacteria	-0.50	-0.29	0.32	**0.79**	0.18	-0.37
Cyanobacterial abundance (cells/mL)						
Cyanobacteria	-0.30	-0.14	0.71	**0.86**	0.50	**0.94**
Dolichospermum	-0.35	0.41	0.57	**0.87**	0.54	-0.37
Microcystis	**-0.97**	-0.61	**1.00**	**0.81**	**0.81**	**-0.85**
Planktothrix	-0.45	**0.93**	0.49	-0.41	-0.40	0.64
Other microcystin producers	0.50	-0.25	0.61	0.65	0.57	0.15
Non microcystin cyanobacteria	-0.10	-0.25	0.57	**0.79**	-0.15	-0.26

Deer Creek

At Deer Creek, microcystin was found in six out of seven samples (86 percent), with concentrations ranging from <0.10 to 5.8 µg/L and a median of 0.45 µg/L (fig. 4). *Microcystis* and *Planktothrix mcyE* DNA were detected in 71 and 100 percent of samples, respectively, and RNA was detected in 1 or 2 samples (fig. 5). *Planktothrix* dominated cyanobacterial communities during May through early July, and non-microcystin producers generally dominated the remainder of the season (fig. 6*C*). Maximum cyanobacterial biovolumes occurred in August, well after the maximum microcystin concentration that occurred on May 15, 2013.

Phycocyanin was not significantly correlated with microcystin concentrations, and chlorophyll had a significant negative correlation with microcystin concentrations (table 10). Some significant relations were found between microcystin concentrations and nutrient concentrations or cyanobacterial genes; however, the correlations were highly influenced by one point—the sample with 5.8 µg/L collected on May 15. Because only one sample collected early in the season was found to have >1 µg/L microcystin and because key correlations were not significant or were influenced by a single point, Deer Creek was considered a low priority site for sampling in 2014.

Harsha Lake

At Harsha Main, microcystin was detected in six out of seven samples, with concentrations ranging from <0.10 to 5.3 µg/L and a median of 1.6 µg/L (fig. 4). It was the only site where saxitoxin was found in the majority of samples. Saxitoxin concentrations were strongly correlated with microcystin concentrations (rho = 0.74, p = 0.06). *Microcystis* and *Planktothrix mcyE* DNA were detected in 100 and 86 percent of samples, respectively, and *Planktothrix mcyE* RNA was detected in one sample (fig. 5). Cyanobacterial community dynamics were complex at Harsha Main during 2013 (fig. 7*A*). *Dolichospermum* dominated (>70 percent) in May and June, *Microcystis* dominated (77 percent) in early July, and non-microcystin-producing taxa gradually replaced *Dolichospermum* and *Microcystis* and were dominant (>60 percent) in September and October. Although microcystin concentrations declined rapidly after the observed maximum concentration in early July, the decline in cyanobacterial biovolume was not observed until October. Seasonal saxitoxin patterns did not match seasonal patterns in cyanobacterial biovolume, and saxitoxin was not always detected when *Dolichospermum* (a saxitoxin producer) was dominant.

Microcystin concentrations were significantly correlated with many water-quality factors, including phycocyanin, and to *Microcystis* biovolume (table 10). The frequent detection of saxitoxin, significant correlations with many water-quality factors, and a complex community structure made this an interesting site to investigate in 2014.

Maumee Bay State Park Inland

The MBSP inland lake site was confirmed to be a negative control site, with microcystin detected in one sample (0.11 µg/L, fig. 4) and *Microcystis* and *Planktothrix mcyE* DNA (fig. 5) each detected once, but in another sample. It was the only site where both *Microcystis* and *Planktothrix mcyE* RNA were not detected. The median phycocyanin concentration was the lowest among the 2013 sampling sites (1.4 relative fluorescence units [RFU]). Maximum cyanobacterial biovolumes were one or more orders of magnitude lower than at the other study sites; cyanobacterial community composition at this site was dominated (>70 percent) by taxa in the non-microcystin-producers category (fig. 6*D*).

Because there was only one detection of microcystin, correlation analysis was not done. Sampling at a negative control site was not included in 2014.

Maumee Bay State Park Lake Erie

The widest range of microcystin concentrations was found at MBSP Lake Erie. Microcystin was detected in six out of seven samples, with concentrations ranging from <0.10 to 30 µg/L and a median of 6.8 µg/L (fig. 4). Four samples and two samples exceeded the Ohio EPA advisory levels of 6 and 20 µg/L microcystin, respectively. A wide range of *Microcystis mcyE* DNA concentrations was found in 86 percent of samples; however, *Microcystis mcyE* RNA and *Planktothrix mcyE* DNA and RNA were found in only 1 or 2 samples (fig. 5). *Planktothrix* dominated cyanobacterial communities in June, and *Microcystis* constituted >85 percent of the relative community composition for the remainder of the season (fig. 8*A*). The maximum cyanobacterial biovolume occurred in September, and maximum microcystin concentrations occurred about 1 month earlier. Saxitoxin was found in one sample collected in late May, at a concentration of 0.04 µg/L.

Statistically significant correlations were found between microcystin concentrations and ammonia (negative) and *Microcystis mcyE* DNA (table 10). Microcystin concentrations were significantly correlated with biovolume and abundance of cyanobacteria, *Microcystis*, and *Dolichospermum*, but not with *Planktothrix* and non-microcystin-producing cyanobacteria. With a wide range of toxin concentrations, MBSP Lake Erie was a good site to include as a system dominated by *Microcystis* with the presence of *Dolichospermum*, a potential saxitoxin producer.

Port Clinton

At Port Clinton, microcystin was detected in four out of six samples with concentrations ranging from <0.10 to 4.5 µg/L and a median of 0.40 µg/L (fig. 4). As was the case at MBSP Lake Erie, a wide range of *Microcystis mcyE* DNA concentrations was found at Port Clinton, and *Microcystis mcyE* RNA was detected in two samples; however, *Planktothrix mcyE* DNA

and RNA were not found in any Port Clinton samples (fig. 5). Non-microcystin producers dominated early in the season, and *Microcystis* constituted >90 percent of the cyanobacterial community at Port Clinton from August through October (fig. 6*E*). Seasonal microcystin dynamics at Port Clinton matched patterns in cyanobacterial biovolume, and maximum observed microcystin concentrations occurred in August and September.

Statistically significant correlations were found between microcystin and dissolved oxygen and *Microcystis mcyE* DNA and RNA (table 10). Similar to MBSP Lake Erie, Port Clinton offered an opportunity to study a system dominated by *Microcystis*.

Sandusky Bay

At Sandusky Bay, microcystin was detected in 100 percent of samples, with concentrations ranging from 2.1 to 8.8, µg/L and a median of 3.6 µg/L (fig. 4). *Microcystis* and *Planktothrix mcyE* DNA were found in 83 and 100 percent of samples, respectively, unlike the two Lake Erie sites (MBSP Lake Erie and Port Clinton) where *Planktothrix mcyE* DNA was seldom found (fig. 5). The highest percentage of detection of *Planktothrix mcyE* RNA (83 percent) was found at Sandusky Bay. *Planktothrix* dominated cyanobacterial communities from May through July, no one category of taxa was dominant in August, and non-microcystin producers dominated in September through October (fig. 6*F*). Maximum cyanobacterial biovolumes at Sandusky Bay occurred in September, and the maximum microcystin concentration occurred 3 months earlier.

Microcystin was strongly negatively correlated to ammonia concentrations and positively significantly correlated to cyanobacterial abundance (table 10). Similar to Buckeye Lake, the Sandusky Bay site offered an opportunity to study a *Planktothrix*-dominated system.

Toxins, Water-Quality Factors, and Cyanobacteria at Three Recreational Lakes, 2014

A total of 65 water-quality samples (6 of those included concurrent replicate/split replicate samples) were collected during 2014 to identify factors affecting the cyanobacterial community composition, toxin concentrations, and cyanobacterial gene concentrations. Three lakes were selected for weekly sampling in 2014—Buckeye Lake, Harsha Lake, and Maumee Bay. The three lakes represent a range of lake types found throughout Ohio—Buckeye Lake is a shallow manmade canal lake, Harsha Lake is a flood-control and water-supply reservoir, and Maumee Bay is in the western basin of Lake Erie.

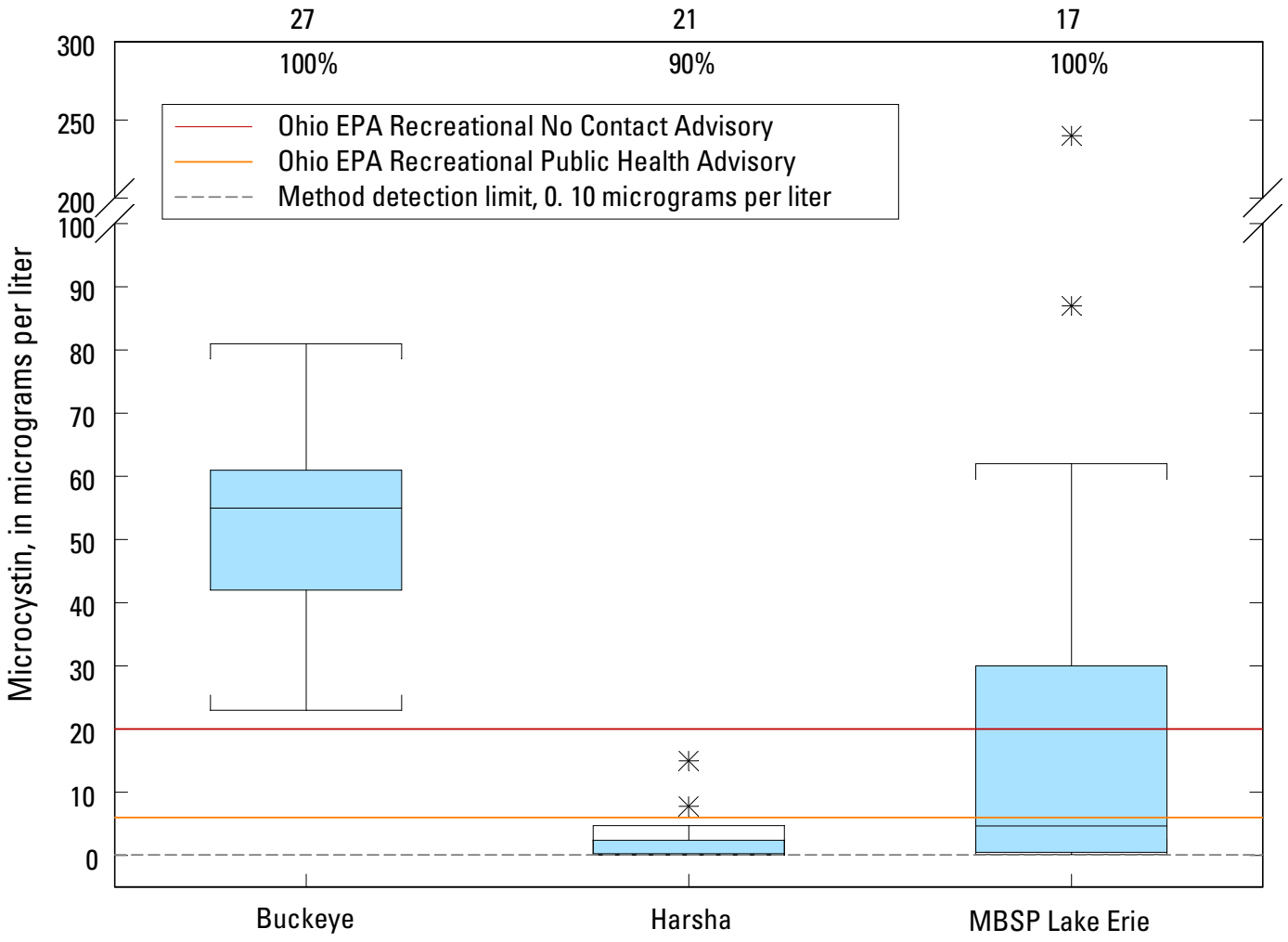
At Buckeye Lake, the beach site was moved from Buckeye Crystal to Fairfield in 2014, because Fairfield is open to lake circulation and more representative of shoreline lake conditions (fig. 2*A*). A second site, Buckeye Onion Island, was added because it is a popular boater swim area. At Harsha Lake, samples were routinely collected at Harsha Main (on the south side of the lake); but on 4 days, samples were collected at Harsha Campers because of occasional bloom sightings on the north side of the lake (fig. 2*B*). Because in-lake summary statistics were similar, data from Buckeye Lake Fairfield and Onion Island and from Harsha Main and Harsha Campers were combined in this section. At Maumee Bay, one site—MBSP Lake Erie—was included in 2014 sampling.

Findings by lake are described below for toxin concentrations (table 11, fig. 9), water-quality measurements (table 11), cyanobacterial gene and transcript concentrations (table 11), and cyanobacterial biovolume (table 11, figs. 7, 8, and 10). Samples were analyzed for microcystin and saxitoxin but not for cylindrospermopsin because it was not detected in any samples during 2013. At all lakes, microcystin concentrations ranged from <0.10 to 240 µg/L (table 11), and saxitoxin was detected in four samples from MBSP Lake Erie. Measured values of pH, water temperature, specific conductance, and dissolved oxygen were as expected for lake-water samples, except for an unexplained high specific conductance result (727 microsiemens per centimeter) on June 16 at MBSP Lake Erie (table 11). Among the cyanobacterial gene assays, *Dolichospermum mcyE* DNA was not detected in any samples in 2014; as a result, samples were not analyzed for *Dolichospermum mcyE* RNA. As was done for 2013, only biovolume is discussed in detail below because overall patterns in cyanobacterial abundance and biovolume generally were similar.

Buckeye Lake

At Buckeye Lake in 2014, microcystin concentrations were consistently high, ranging from 23 to 81 µg/L (fig. 9, table 11). Among all lakes sampled in 2014, the median phycocyanin measurement was highest at Buckeye Lake (24.5 RFU). The highest median total phosphorus and total nitrogen concentrations were also found at Buckeye Lake (0.229 and 3.13 mg/L); however, orthophosphate was not detected in any samples. The N to P ratios varied somewhat at Buckeye Lake (11–54). Among the three lakes sampled, the highest median concentration (9.18 log copies/100 mL) and narrowest range of general cyanobacterial genes was found at Buckeye Lake, as was the highest median concentration of *Planktothrix mcyE* DNA (8.43 log copies/100 mL). In 2014, Buckeye Lake was the only lake where *Planktothrix mcyE* RNA was detected. As was the case in 2013, *Microcystis* RNA was not detected at Buckeye Lake in 2014. *Dolichospermum* was found in 100 percent of samples from Buckeye Lake in 2014; this assay was not performed in 2013.

Cyanobacteria consistently dominated the phytoplankton community (64–99 percent relative cyanobacterial biovolume) in Buckeye Lake in 2014 (table 11). Cyanobacteria and microcystin dynamics between the Buckeye Fairfield and Onion Island sites were similar; therefore, only results from Buckeye Fairfield are discussed in detail. During 2014, cyanobacterial biovolume was relatively consistent, differing by approximately tenfold or less (fig. 10); it was lowest in late June (6 million μm^3/mL) and highest in mid-August and November (66 and 78 million μm^3/mL, respectively). The



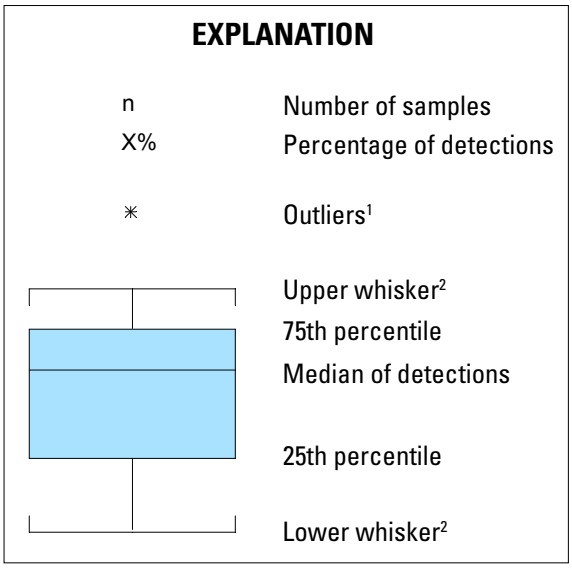


Figure 9. Concentrations of microcystin above the detection limit (0.10 microgram per liter) and percentages of detections at Ohio recreational sites, 2014. (Ohio EPA, Ohio Environmental Protection Agency. A Recreational No Contact Advisory for microcystin means that the public is recommended to avoid all contact with the water. It is in effect if someone or an animal dies or becomes ill as a direct result of cyanotoxin exposure and microcystin is greater than 20 micrograms per liter or other thresholds are reached.)

[1]Outliers are defined as values outside 1.5 times the interquartile range beyond the ends of the box.

[2]The whiskers are the largest and smallest values within 1.5 times the interquartile range beyond the 75th and 25th percentiles, respectively.

Table 11. Summary statistics for water-quality measurements, cyanobacterial gene and transcript concentrations, and cyanobacterial abundance and biovolume in 65 samples collected at 3 Ohio recreational lakes, 2014.

[°C, degree Celsius; µS/cm, microsiemens per centimeter at 25 degrees Celsius, RFU, relative fluorescence unit; NTRU, nephelometric turbidity ratio unit; mg/L, milligram per liter; µg/L, microgram per liter; mL, milliliter; µm^3, micrometer cubed<, indicates less than value; --, not applicable; ND, not determined; %, percent; DNA, deoxyribonucleic acid; RNA, ribonuceic acid]

	Buckeye Lake Fairfield and Onion Island, n=27				Harsha Main and Campers, n=21				Maumee Bay Lake Erie, n=17			
	Minimum	Maximum	Median	Percentage of detections	Minimum	Maximum	Median	Percentage of detections	Minimum	Maximum	Median	Percentage of detections
Water-quality variables												
pH	7.8	9.5	8.8	--	7.3	10.0	9.1	--	7.6	9.9	8.7	--
Water temperature, °C	9.2	27.6	24.5	--	16.7	29.5	25.6	--	7.7	25.5	22.1	--
Specific conductance, µS/cm	281	345	307	--	245	325	265	--	305	727	396	--
Dissolved oxygen	4.1	16.7	8.4	--	ND	ND	ND	--	6.3	12.7	8.5	--
Chlorophyll sensor, RFU	1.8	7.1	3.4	--	0.4	6.5	2.2	--	1.3	68.0	41	--
Phycocyanin sensor, RFU	9.5	33.8	24.5	--	0	25.7	7.3	--	0.8	66	9.5	--
Turbidity, NTRU	27	64	49	--	3.6	27	7.6	--	7.2	203	46	--
Secchi depth, feet	0.5	1.3	0.7	--	1.2	3.0	2.2	--	0.3	2.5	0.9	--
Ammonia, mg/L	<0.01	0.16	<0.01	48	<0.01	0.23	<0.01	29	<0.01	0.12	0.02[a]	63[a]
Nitrate + nitrite, mg/L	<0.04	0.626	<0.04	24	<0.04	1.2	<0.04	29	<0.04	3.83	0.787[a]	88[a]
Nitrite, mg/L	<0.001	0.054	<0.001	28	<0.001	0.076	0.001	48	<0.001	0.058	0.016[a]	69[a]
Orthophosphate, mg/L	<0.004	--	--	0	<0.004	0.213	0.005	48	<0.004	0.043	0.011[a]	75[a]
Total phosphorus, mg/L	0.059	0.311	0.229	100	0.045	0.349	0.096	100	0.047	0.363	0.102[a]	100[a]
Total nitrogen, mg/L	2.07	3.66	3.13	100	0.66	2.4	1.12	100	1.03	4.52	1.96[a]	100[a]
N to P ratio (total)	11	54	14	100	6.8	17.3	12	100	7.4	53	16[a]	100[a]
Sulfate (mg/L)	15.4	24.9	18.2	100	14.5	20.3	17.1	100	18	48.4	36.2	100
Toxins (µg/L)												
Microcystin	23	81	55	100	<0.10	15	0.30	89	0.17	240	4.7	100
Saxitoxin	<0.02	--	--	0	<0.02	--	--	0	<0.02	0.07	<0.02	24
Cyanobacterial genes and transcripts (log copies/100 mL)												
Cyanobacteria	8.55	9.60	9.18	100	6.6	8.81	8.19	100	5.86	8.15	7.47	100
Microcystis	4.08	5.94	5.29	100	3.72	8.53	7.44	100	4.52	8.14	6.27	100
Microcystis mcyE DNA	<2.94	4.79	4.34	88	<2.99	7.66	5.22	90	3.87	8.03	6.40	100
Microcystis mcyE RNA	<3.42–<4.90	--	--	0	<3.42	4.55	<3.61	19	<3.72	5.52	4.45	76
Planktothrix mcyE DNA	7.81	8.86	8.43	100	<3.58	5.62	5.13	90	<3.71	7.13	<4.18	41
Planktothrix mcyE RNA	4.31	6.88	5.92	100	<4.01–<4.88	--	--	0	<4.31–<4.78	--	--	0
Dolichospermum	4.91	8.53	6.72	100	4.83	9.63	8.35	100	<4.36	8.38	5.94	65
Dolichospermum mcyE DNA	<3.36–<3.74	--	--	0	<3.36–<3.79	--	--	0	<3.66–<4.14	--	--	0

Table 11. Summary statistics for water-quality measurements, cyanobacterial gene and transcript concentrations, and cyanobacterial abundance and biovolume in 65 samples collected at 3 Ohio recreational lakes, 2014.—Continued

	Buckeye Lake Fairfield and Onion Island, n=27				Harsha Main and Campers, n=21				Maumee Bay Lake Erie, n=17			
	Minimum	Maximum	Median	Percentage of detections	Minimum	Maximum	Median	Percentage of detections	Minimum	Maximum	Median	Percentage of detections
Cyanobacterial biovolume (µm³/mL)												
Relative cyanobacteria biovolume (%)	64	99	93	100	12	98	54	100	<1	98	30	100
Total cyanobacteria	6,100,000	78,000,000	31,000,000	100	100,000	29,000,000	2,000,000	100	11,000	46,000,000	2,900,000	100
Dolichospermum	0	760,000	0	41	0	13,000,000	380,000	90	0	170,000	--	35
Microcystis	0	8,500,000	0	33	0	12,000,000	190,000	76	0	42,000,000	2,700,000	94
Planktothrix	5,900,000	78,000,000	30,000,000	100	0	2,100,000	91,000	86	0	20,000	--	47
Other microcystin producers	0	320,000	20,000	85	0	6,600,000	57,000	81	0	4,700,000	200	53
Non-microcystin producers	0	9,900,000	750,000	89	420	3,300,000	1,100,000	100	0	1,500,000	10,000	76
Cyanobacterial abundance (cells/mL)												
Relative cyanobacterial abundance (%)	96	100	99	100	66	99	96	100	13	99	89	100
Total cyanobacteria	430,000	8,400,000	2,000,000	100	16,000	900,000	270,000	100	1,100	1,100,000	87,000	100
Dolichospermum	0	12,000	0	41	0	200,000	13,000	90	0	2,100	--	35
Microcystis	0	130,000	0	33	0	220,000	6,100	76	0	640,000	41,000	94
Planktothrix	430,000	7,900,000	2,000,000	100	0	99,000	8,500	86	0	2,100	--	47
Other microcystin producers	0	91,000	6100	85	0	470,000	31,000	81	0	430,000	1,000	53
Non-microcystin producers	0	1,200,000	88,000	89	6,500	440,000	110,000	100	0	9,900	400	76

[a]Values were calculated from 16 samples, because one cooler was lost in shipment.

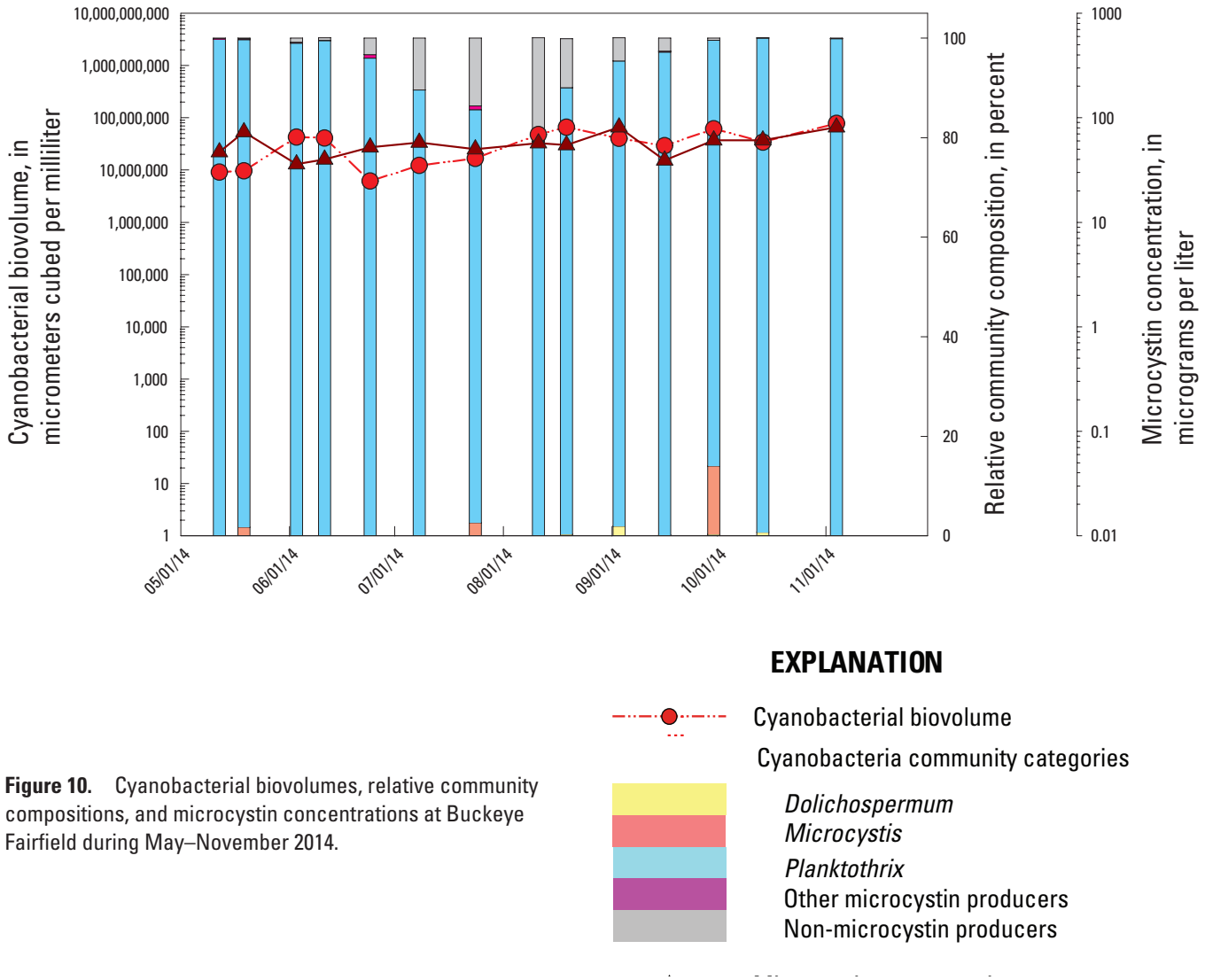


Figure 10. Cyanobacterial biovolumes, relative community compositions, and microcystin concentrations at Buckeye Fairfield during May–November 2014.

relative community composition fluctuated little between May and November and was consistently dominated by *Planktothrix* (fig. 10). Similarly, microcystin concentrations fluctuated little throughout the season, with the highest observed concentrations occurring in early September and November (81 µg/L). Cyanobacterial community composition at the Buckeye Lake Crystal site in August–October, 2013 was dominated by non-microcystin-producing taxa, and *Planktothrix* was not present (fig. 6*B*). Despite differences in cyanobacterial community composition between 2013 and 2014, microcystin concentrations in Buckeye Lake were within the same range (figs. 6*B* and 10).

Harsha Lake

At Harsha Lake during 2014, two samples exceeded the Ohio EPA Recreational Public Health Advisory level of 6 µg/L microcystin, and no samples exceeded 20 µg/L (fig. 9). Saxitoxin was not detected at Harsha Lake in 2014, even though it was detected at low concentrations in 2013. Among all lakes sampled in 2014, the median phycocyanin measurement was lowest (7.3 RFU) and the N to P ratios were relatively consistent and low (6.8–17.3) at Harsha Lake (table 11). All cyanobacteria genes (DNA) were detected at a high frequency at Harsha Lake; however, *Microcystis mcyE* RNA was found in only 19 percent of the samples, and *Planktothrix mcyE* RNA was not found in any samples. The highest median concentration of *Dolichospermum* was found at Harsha Lake (8.35 log copies/100 mL).

Cyanobacteria consistently dominated the phytoplankton community in Harsha Lake during May through October 2014 based on abundance but not biovolume. Cyanobacteria constituted 12 to 98 percent of the phytoplankton community based on biovolume and 66 to 99 percent based on abundance (table 11). The difference in relative abundance of cyanobacteria based on abundance and biovolume is caused by the presence of small taxa in the non-microcystin-producer category that were numerically abundant but did not contribute substantially to algal biomass. Cyanobacteria and microcystin dynamics between the Harsha Main and Harsha Campers sites were similar; therefore, only results from the Harsha Main site are discussed in detail.

Cyanobacterial biovolume steadily increased from May to late June, remained constant from early to mid-July, and then slowly decreased from August through October (fig. 7*B*). The highest observed cyanobacterial biovolume was about 16 million μm^3/mL. Similar to what was observed in 2013 (fig. 7*A*), cyanobacterial community composition at the Harsha Main site in 2014 was complex and varied throughout the season. *Planktothrix* dominated the cyanobacterial community in May (99 percent of total cyanobacterial biovolume) and late October (67 percent) but represented a relatively small percentage (less than 15 percent) of the community during the rest of the season (fig. 7*B*). *Dolichospermum* and *Microcystis* were a substantial percentage of the cyanobacterial community from late May through August. Non-microcystin-producing taxa were dominant (53 to 91 percent of total) from late July through early October. Despite the complexities in cyanobacterial community composition, microcystin dynamics generally reflected seasonal patterns in cyanobacterial biovolume, with the lowest concentrations (<0.1 µg/L) occurring in May and the highest in late June (15 µg/L).

The highest observed cyanobacterial biovolumes in Harsha Main were similar in 2013 and 2014 (about 14 and 16 million μm^3/mL, respectively) and occurred at about the same time of year (late June/mid-July). However, there were substantial differences in community composition between 2013 and 2014, particularly early in the season (fig. 7). During 2013, *Dolichospermum* was dominant in May, whereas in 2014, *Dolichospermum* was present but never dominant. Likewise, during 2014, *Planktothrix* was dominant in May but did not represent a substantial portion of the cyanobacterial community in 2013. Midsummer and fall communities were generally similar between years, with the exception of *Planktothrix* dominance in late October 2014. Saxitoxin was detected in Harsha Main during June through August 2013 but was not detected in 2014 (fig. 7).

Maumee Bay State Park Lake Erie

As in 2013, the widest range of microcystin concentrations among all sites was found at MBSP Lake Erie in 2014 (fig. 9). At MBSP Lake Erie in 2014, eight samples exceeded the Ohio EPA Recreational Public Health Advisory level of 6 µg/L microcystin, and five samples were > 20 µg/L. Concentrations of saxitoxin in four samples where it was detected ranged from 0.02 to 0.07 µg/L, all well below the Ohio EPA Recreational Public Health Advisory level of 0.8 µg/L. Among all lakes sampled in 2014, ranges of values for chlorophyll, phycocyanin, turbidity, and Secchi depth were widest at MBSP Lake Erie. Dissolved nitrogen constituents (ammonia, nitrite plus nitrite, and nitrite) were detected most often in MBSP Lake Erie samples (63–88 percent of samples). In addition, among all lakes, *Microcystis mcyE* DNA (100 percent) and *Microcystis mcyE* RNA transcripts (76 percent) were most often detected in samples from MBSP Lake Erie in 2014.

Unlike Buckeye and Harsha Lakes, cyanobacteria did not consistently dominate the phytoplankton community in MBSP Lake Erie. Based on biovolume, cyanobacteria constituted <1 to 98 percent of the phytoplankton community (table 11). Cyanobacterial biovolume increased and decreased in June and early July, generally increased from mid-July to the highest observed biovolume in early September (46 million μm^3/mL), and declined steadily from September to November (fig. 8*B*). The cyanobacterial community composition was variable in the five samples collected during June to early July, with *Microcystis* dominating in three samples (>52 percent of total cyanobacterial biovolume), *Planktothrix* dominating in one sample (66 percent), and non-microcystin producers dominating in one sample (90 percent of total). *Microcystis* dominated (>90 percent of total cyanobacterial biovolume) from mid-July through November. Microcystin concentrations generally reflected seasonal dynamics in cyanobacterial biovolume, although increases were steadier, and the highest observed microcystin concentration (240 µg/L) occurred earlier than the highest observed cyanobacterial concentration (fig. 8*B*). Saxitoxin was occasionally detected in August and early September at the MBSP Lake Erie site, even though *Microcystis*, not known to be a saxitoxin producer, represented more than 98 percent of the total cyanobacterial biovolume during this time.

The highest observed biovolumes at MBSP Lake Erie were found on September 18 in 2013 and September 3 in 2014 (35 and 46 million μm^3/mL, respectively) (fig. 8). Cyanobacterial community dynamics also were similar both years, with *Planktothrix* dominance early in the season shifting to *Microcystis* dominance by mid-July. Seasonal dynamics in microcystin concentration were generally similar both years, with the highest observed concentrations occurring in August. Despite relatively similar cyanobacterial biovolumes and community dynamics during 2013 and 2014, maximum microcystin concentrations in 2014 (240 µg/L) were nearly an order of magnitude higher than in 2013 (30 µg/L; fig. 8). This difference may be due to the more intensive sampling regime in 2014 (for example, the maximum concentration in 2013 was missed) or may have been caused by subtle differences in cyanobacterial community composition. Saxitoxin was detected during both years but occurred more frequently and later in the year during 2014 than 2013 (fig. 8).

Relations between Cyanobacterial Gene Concentrations and Community Composition

Molecular methods for quantifying concentrations of cyanobacterial genes and transcripts may be useful in understanding the community composition and potential for toxin production. There is little information, however, on how well results from molecular methods compare with traditional microscopy methods for cyanobacterial community composition. Any discrepant results between the two methods may be due to differences in what is actually being measured,

as well as differences in sample volumes analyzed. Molecular methods detect specific gene fragments that do not have to be from an intact, viable cell and therefore might not be accounted for in the phytoplankton identification process. The traditional microscopy method is based on observation, and misidentification may occasionally occur due to human error. Microscopy methods are also limited to the volume of sample that can be analyzed in order to avoid overcrowding the field of view for counting. Not having that limitation, analysis with molecular methods can be done on larger sample volumes.

Spearman's correlation coefficients were computed to determine how well concentrations from the molecular methods correlated with counts from traditional microscopy methods. General cyanobacteria and genus-specific counts for *Microcystis*, *Dolichospermum*, and *Planktothrix* were compared to general cyanobacteria and genus-specific gene concentrations where applicable, except for *Planktothrix*. *Planktothrix* counts were compared to *Planktothrix mcyE* DNA gene concentrations because a general *Planktothrix* gene assay was not included in this study. Results from all sites and both years (2013 and 2014) were used. Microscopy counts were significantly correlated with gene concentrations for cyanobacteria (rho = 0.81, p = <0.0001), *Microcystis* (rho = 0.67, p = <0.0001), *Dolichospermum* (rho = 0.79, p = <0.0001), and *Planktothrix* (rho = 0.70, p = <0.0001). Scatterplots showing each relation are provided in figures 11*A–D*. Among the scatterplots, the *Microcystis* comparison was most evenly scattered around the 1:1 line; the *Dolichospermum* comparison indicated a positive bias for the molecular method.

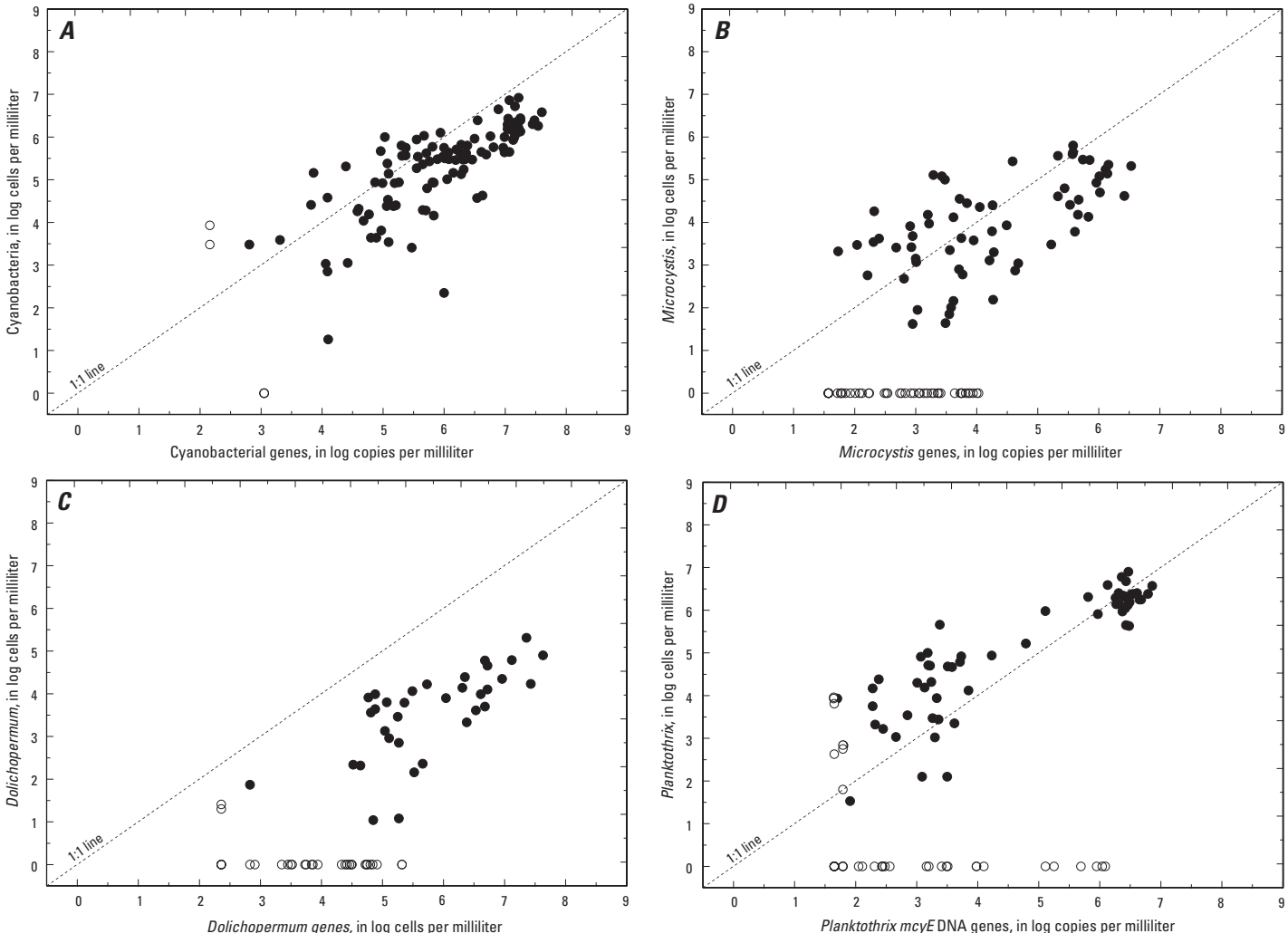


Figure 11. Relations between cyanobacterial gene concentrations and community compositions at 12 recreational sites, 2013–14. *A*, Cyanobacteria. *B*, *Microcystis*. *C*, *Dolichospermum*. *D*, *Planktothrix*. Open circles indicate nondetects (nondetects for the community composition data was set to 1 cell per milliliter, and nondetects for gene concentrations were set at their reporting limits). Cyanobacterial gene concentrations were plotted as log copies per milliliter in this figure to allow for comparison with cyanobacterial community composition. (DNA, deoxyribonucleic acid)

Factors Affecting Toxin Concentrations, Cyanobacterial Community Composition, and Cyanobacterial Gene Concentrations at Four Recreational Sites, 2013–14

Spearman's correlation coefficients were determined for each site to quantitatively evaluate the relations between environmental and water-quality factors and microcystin concentrations. For 2014, correlation results at Buckeye Fairfield and Buckeye Onion Island were segregated because of different sampling site configurations—Fairfield is a beach site and Onion Island is an open-water site. Because only three samples were collected at Buckeye Crystal during 2013, these data were not included in the correlation analysis. At Harsha, only 2014 data from Harsha Main were used for correlation analysis because (1) different sondes were used in 2013 and 2014 and the phycocyanin and chlorophyll data could not be combined from both years and (2) only four samples were collected from Harsha Campers in 2014. At MBSP State Park, data from 2013 and 2014 were combined for the correlation analysis.

Findings by site described below include Spearman's Rank correlations between microcystin concentrations and other factors (tables 12 and 13) and scatterplots of some key factors (figs. 12–16). Two different predictive modeling scenarios are presented: (1) A daily predictive model developed by use of easily or continuously measured water-quality factors and available environmental data that does not require a site visit for sample collection, and (2) a long-term predictive model that provides advanced warning of the potential for cyanoHAB events (a few days to several weeks) and includes results from samples collected and analyzed for factors that require laboratory analysis in addition to daily predictive model factors. Accordingly, correlation results for factors were grouped by the type of prediction(s) that could be developed in the future. The highest Spearman's correlations between microcystin and water quality, environmental, or cyanobacterial factors are presented for daily or long-term predictions in descending order (tables 12 and 13). Factors were considered statistically significant at $p \leq 0.05$. Scatterplots (figs. 12–16) were used to confirm these relations and ensure that they were not influenced by outlier data points and to identify factors that were not statistically significant but may be used in future models developed with more robust datasets.

Buckeye Fairfield

At Buckeye Fairfield, statistically significant correlations between microcystin concentrations and factors for daily predictions were found for three factors—wind direction and two radar factors (table 12). A closer examination of the data showed that some of the highest microcystin concentrations were found on days with no wind, and some of the lowest microcystin concentrations on days with northerly winds (fig. 12*A*). Wind direction may be used as categorical variable, possibly combined with wind speed, which was negatively related to microcystin concentrations (fig. 12*B*). The negative relation between Radar sum Dm1 (radar rainfall sum for the 24-hour period before sampling) and microcystin was influenced by one point (fig. 12*C*); however, radar data may be useful as a variable in a model as more data are collected. Although day of the year was not significantly correlated to microcystin concentrations, a scatterplot shows increasing concentrations of microcystin throughout the season (fig. 12*D*). Noticeably absent from the list of statistically significant factors were those expected to be related to microcystin concentrations—chlorophyll (rho = 0.21, p = 0.4299) and phycocyanin (rho = −0.01, p = 0.9813).

No statistically significant correlations were found between microcystin concentrations and factors for long-term predictions (table 12); however, weak associations can be seen on scatterplots between microcystin and total phosphorus (fig. 12*E*), ammonia (fig. 12*F*), *Microcystis mcyE* DNA and *Planktothrix mcyE* DNA (fig. 12*G*), and *Planktothrix* biovolume (fig. 12*H*). The lack of microcystin concentrations on the low end (< 20 µg/L) most likely limited the identification of promising factors for predictive models.

Buckeye Onion Island

At Buckeye Onion Island, statistically significant correlations between microcystin concentrations and factors for daily predictions were found for phycocyanin, lake level change in 24 hours, and Secchi depth (table 12). Scatterplots confirm that these are promising factors for daily predictive models (figs. 13*A–C*). The two lowest microcystin concentrations were associated with the two highest radar rainfall amounts (RadarSum Dm2, radar in the 24-hour period 2 days before sampling) and the inverse relation was influenced by these two points (fig. 13*D*). Chlorophyll was not significantly correlated to microcystin concentrations (rho = −0.12, p = 0.7379).

For long-term predictions at Buckeye Onion Island, three factors were significantly correlated with microcystin concentrations (table 12). *Planktothrix mcyE* DNA was significantly correlated with microcystin concentrations, but *Planktothrix mcyE* RNA was not (fig. 13*E*). Total nitrogen was significantly correlated to microcystin concentrations (fig. 13*F*), but total phosphorus was not (rho = 0.26, p = 0.3544). The two lowest microcystin concentrations were associated with the two highest ammonia concentrations (fig. 13*G*), although this correlation was not significant. Among the factors for phytoplankton community composition, non-microcystin-producing cyanobacteria had the highest correlation with microcystin concentrations (fig. 13*H*) with an expected inverse relation.

Table 12. Highest Spearman's correlations (rho) between microcystin and water quality, environmental, or cyanobacterial factors for daily or long-term predictions, in descending order, at Buckeye Fairfield and Onion Island (2014), Harsha Main (2014), and Maumee Bay State Park (MBSP) Lake Erie beach (2013–14).

[Lake level values are for USGS Buckeye Lake nr Millersport (Buckeye Lake) or NOAA Toledo station (Maumee Bay); discharge values are for the Maumee River at Waterville (Maumee Bay); airport values are for the Newark Heath Airport (Buckeye Lake), Cincinnati Municipal Airport (Harsha Main), and Toledo Executive Airport (Maumee Bay); A, average of two or more values]

Buckeye Fairfield (n=17)			Buckeye Onion Island (n=10)			Harsha Main (n=17)			Maumee Bay State Park Lake Erie (n=24)		
Variable	rho	p	Variable	rho	p	Variable	rho	p	Variable	rho	p
Factors for daily predictions (also used for long-term predictions)											
Wind direction, airport, 8 a.m.	−0.64	0.0060	Phycocyanin	0.79	0.0065	Phycocyanin	0.93	<0.0001	Phycocyanin	0.85	<0.0001
Radar sum Dm1[af]	−0.49	0.0474	Secchi depth	−0.76	0.0101	pH	0.87	<0.0001	Turbidity	0.80	<0.0001
Radar sum 48W[df]	−0.48	0.0505	Lake level change in 24 hours	−0.71	0.0222	Turbidity	0.73	<0.0001	pH	0.76	<0.0001
Radar ave Dm1[ag]	−0.46	0.0641	Turbidity	0.62	0.0558	Algae category	0.72	0.0010	Discharge Dm3h	−0.69	0.0002
Wind Speed, airport, 8 a.m.	−0.46	0.0644	Lake level chg 7 day	−0.53	0.1147	Secchi depth	−0.69	0.0022	Secchi depth	−0.67	0.0004
Day of the year	0.44	0.0760	Barometric Press, airport, 8 a.m.	0.55	0.0959	Chlorophyll	0.63	0.0065	Algae category	0.62	0.0012
Radar sum 72W[ef]	−0.45	0.0681	Radar sum Dm2[bf]	−0.49	0.1540	Water temperature	0.59	0.0124	Discharge, 14 day or 30 day, average or peak	A −0.53	0.0085
Radar ave 48W[dg]	−0.45	0.0713	Radar ave Dm2[bg]	−0.41	0.2411	Wave height	−0.47	0.0598	Day of the year	0.47	0.0204
Lake level	−0.38	0.1300				Wind Speed, airport, 8 a.m.	−0.41	0.1052	Discharge Dm2h, 7 day average or peak	A −0.42	0.0432
						Rain airport Dm3[c]	0.40	0.1079	Conductivity	−0.37	0.0772
						Barometric Press, airport, change 24 hrs	0.33	0.1990	Lake level change in 24 hours	−0.36	0.0854
									Wind speed, airport, 24 hrs	−0.35	0.0965
									Chlorophyll	0.32	0.1223
Factors for long-term predictions											
Total phosphorus	0.46	0.0653	*Planktothrix mcyE* DNA	0.73	0.0168	*Microcystis mcyE* DNA	0.92	<0.0001	*Microcystis* biovolume	0.87	<0.0001
Ammonia	−0.42	0.0897	Total nitrogen	0.72	0.0180	*Microcystis* or *Dolichospermum*, qPCR	0.90	<0.0001	Cyanobacteria biovolume or *Microcystis* abundance	0.86	<0.0001
Total nitrogen	0.40	0.1112	Cyanobacteria, qPCR	0.71	0.0221	Cyanobacteria, biovolume	0.90	<0.0001	Cyanobacteria abundance	0.84	<0.0001
Microcystis mcyE DNA	0.35	0.1683	Ammonia	−0.58	0.0815	*Microcystis* or *Dolichospermum*, abundance or biovolume	0.89	<0.0001	*Microcystis mcyE* DNA	0.82	<0.0001
Cyanobacteria, biovolume	0.35	0.1718	*Planktothrix mcyE* RNA	0.51	0.1328	Cyanobacteria, abundance	0.63	0.0065	*Microcystis* qPCR	0.73	<0.0001
Planktothrix mcyE DNA	0.34	0.1786	Non-microcystin producing cyanobacteria	−0.45	0.1921	Cyanobacteria, qPCR	0.57	0.0168	Total phosphorus	0.78	<0.0001

Table 12. Highest Spearman's correlations (rho) between microcystin and water quality, environmental, or cyanobacterial factors for daily or long-term predictions, in descending order, at Buckeye Fairfield and Onion Island (2014), Harsha Main (2014), and Maumee Bay State Park (MBSP) Lake Erie beach (2013–14).—Continued

[Lake level values are for USGS Buckeye Lake nr Millersport (Buckeye Lake) or NOAA Toledo station (Maumee Bay); discharge values are for the Maumee River at Waterville (Maumee Bay); airport values are for the Newark Heath Airport (Buckeye Lake), Cincinnati Municipal Airport (Harsha Main), and Toledo Executive Airport (Maumee Bay)]

Buckeye Fairfield (n=17)			Buckeye Onion Island (n=10)			Harsha Main (n=17)			Maumee Bay State Park Lake Erie (n=24)		
Variable	rho	p	Variable	rho	p	Variable	rho	p	Variable	rho	p
Factors for long-term predictions—continued											
Planktothrix, biovolume	0.32	0.2062	*Planktothrix*, abundance	0.36	0.3088	Ammonia, Nitrate + Nitrite	−0.53	0.0300	Ammonia	−0.78	<0.0001
						Orthophosphate	−0.48	0.0514	Nitrate + Nitrite	−0.64	0.0009
						N to P ratio	0.45	0.0714	N to P ratio	−0.63	0.0011
						Other microcystin producers, biovolume or abundance	0.40	0.1138	*Microcystis mcyE* RNA	0.58	0.0109
									Cyanobacteria, qPCR	0.49	0.0148
									Dolichospermum, qPCR	0.41	0.1002

[a] Radar or rain Dm1 was the amount of rainfall in the 24-hour period before sampling.

[b] Radar or rain Dm2 was the amount of rainfall, in the 24-hour period 2 days before sampling.

[c] Radar or rain Dm3 was the amount of rainfall, in the 24-hour period 3 days before sampling.

[d] Radar or rain 48W was the amount of radar or rainfall in the 48-hour period before sampling, with the most recent rainfall receiving the most weight and calculated as (2*Dm1) + Dm2.

[e] Radar or rain 72W was the amount of radar or rainfall in the 72-hour period before sampling, with the most recent rainfall receiving the most weight and calculated as (3*Dm1) + (2*Dm2) + Dm3.

[f] Radar sum was the sum of radar rainfall from15 cells for the time period specified.

[g] Radar ave was the hourly maximum values among 15 cells divided by the number of cells for the time period specified.

[h] Discharge, daily mean for 2 days (Dm2) or 3 days before the sampling date (Dm3).

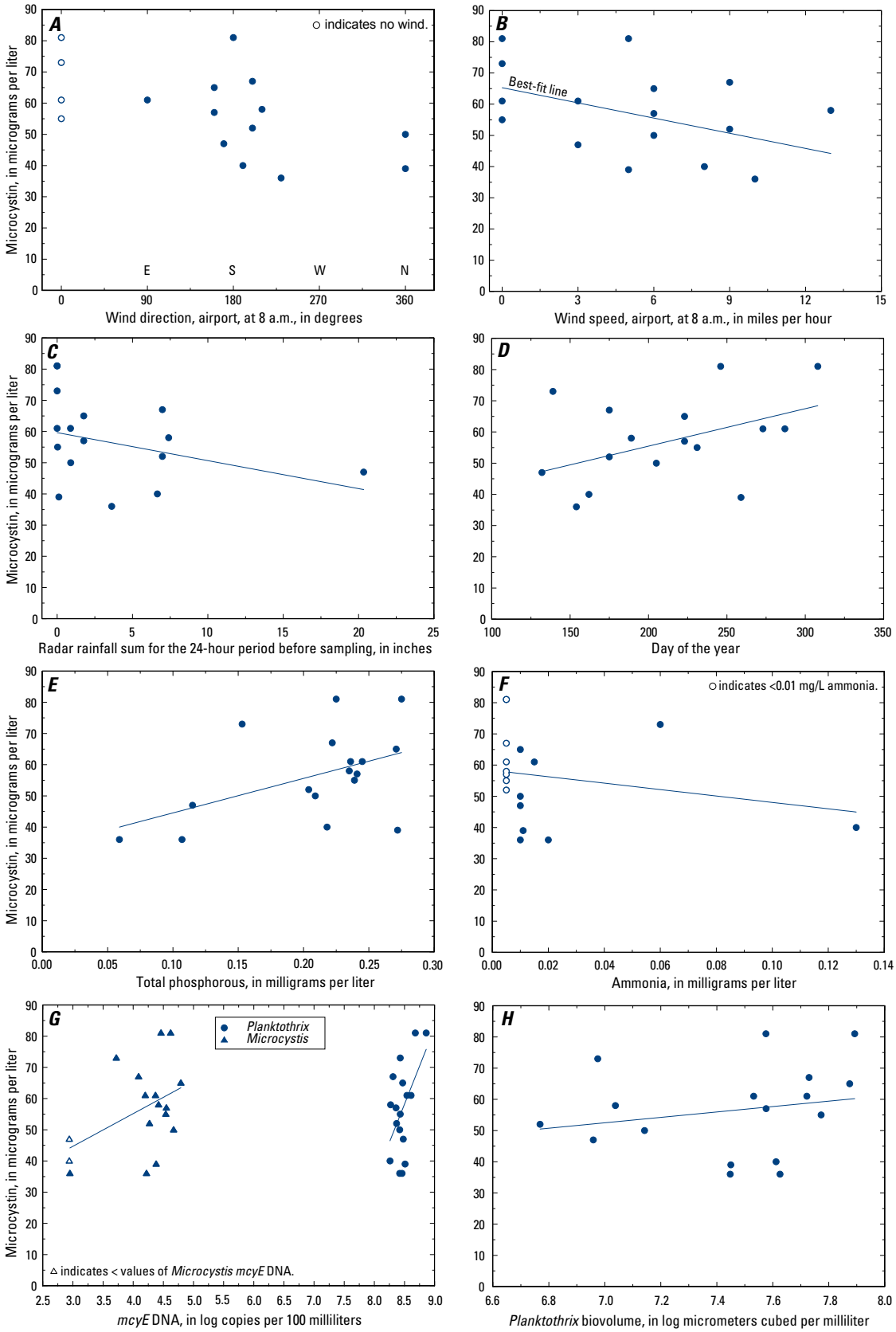


Figure 12. Relations between environmental and water-quality factors and microcystin concentrations at Buckeye Fairfield, 2014, for potential daily or long-term predictions of microcystin concentrations. *A*, Wind direction. *B*, Wind speed. *C*, Radar rainfall sum for the 24-hour period before sampling. *D*, Day of the year. *E*, Total phosphorous. *F*, Ammonia. *G*, *Planktothrix* and *Microcystis mcyE* deoxyribonucleic acid (DNA). *H*, *Planktothrix* biovolume.

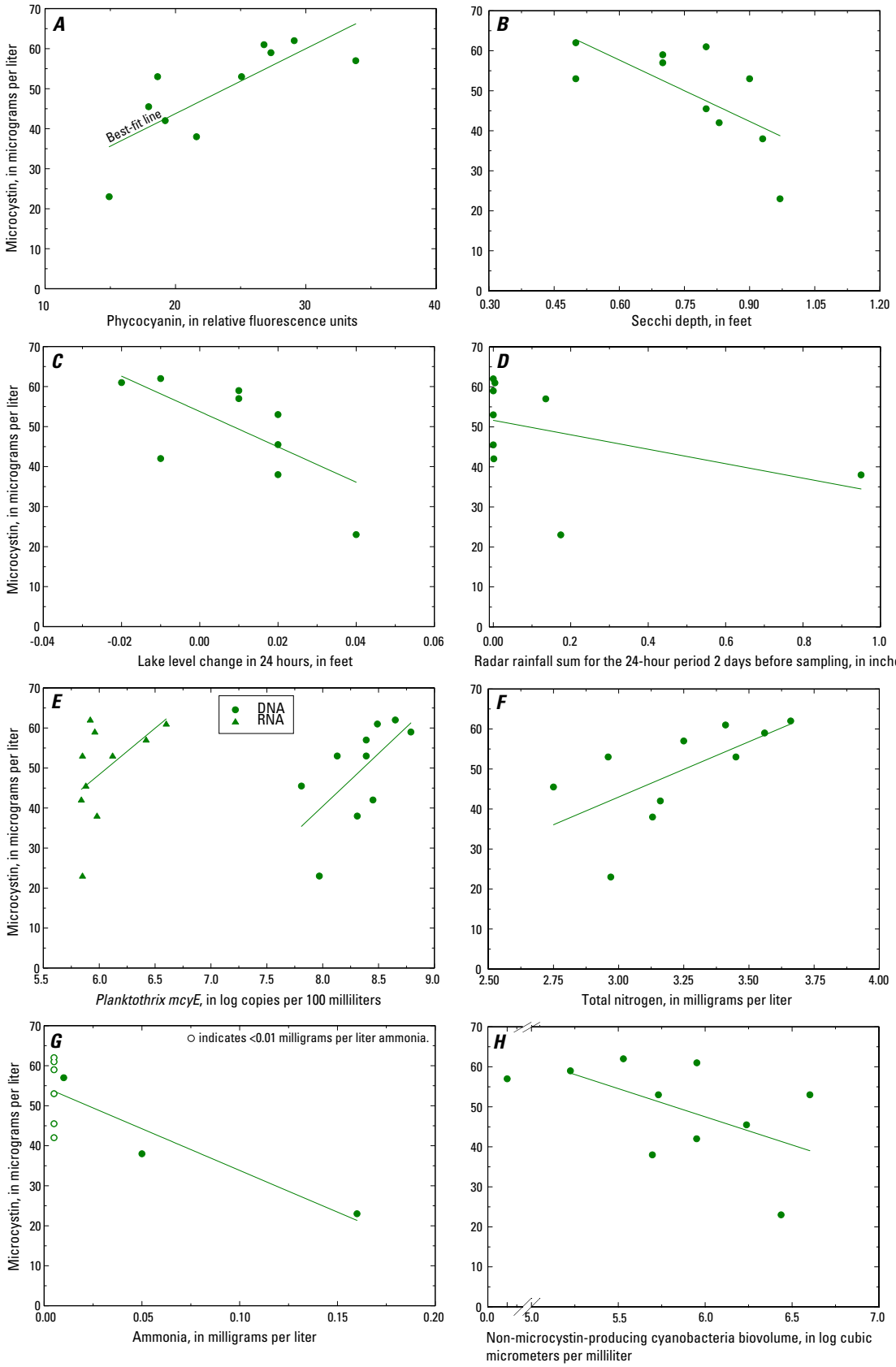


Figure 13. Relations between environmental and water-quality factors and microcystin concentrations at Buckeye Onion Island, 2014, for potential daily or long-term predictions of microcystin concentrations. *A*, Phycocyanin. *B*, Secchi depth. *C*, Lake level change in 24 hours. *D*, Radar rainfall sum for the 24-hour period 2 days before sampling. *E*, *Planktothrix mcyE* deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). *F*, Total nitrogen. *G*, Ammonia. *H*, Non-microcystin-producing cyanobacterial biovolume.

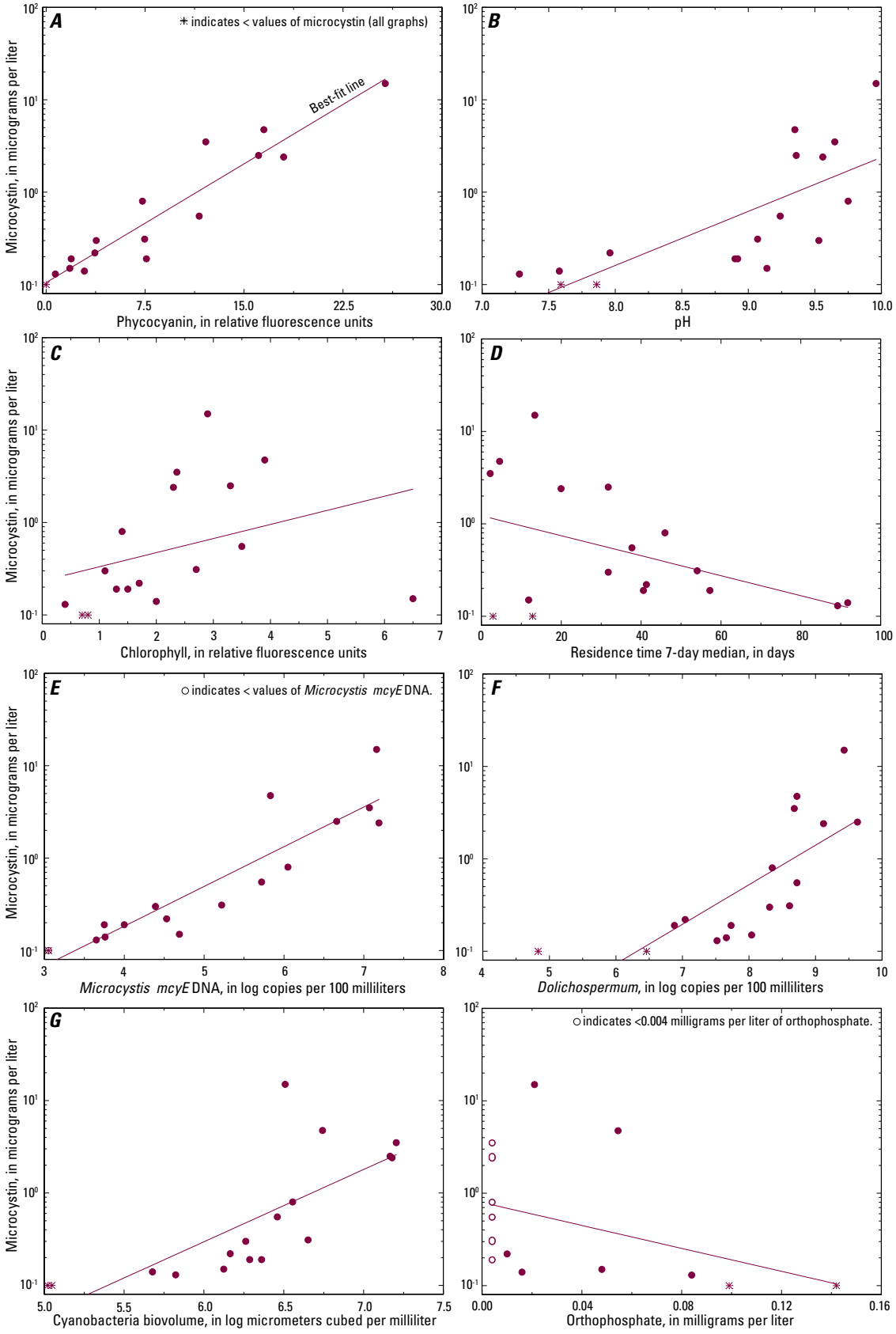


Figure 14. Relations between environmental and water-quality factors and microcystin concentrations at Harsha Main, 2014, for potential daily or long-term predictions of microcystin concentration. *A*, Phycocyanin. *B*, pH. *C*, Chlorophyll. *D*, Residence time 7-day median. *E*, *Microcystis mcyE* deoxyribonucleic acid (DNA). *F*, *Dolichospermum*. *G*, Cyanobacterial biovolume. *H*, Orthophosphate.

Table 13. Highest Spearman's correlations (rho) between microcystin concentrations and sonde continuous water-quality measurements, in descending order, Harsha Main, 2014.

[Continuous data from a U.S. Environmental Protection Agency–operated sonde, suspended from a buoy at latitude 390157, longitude −840816; A, average of two or more values; shaded rows indicate strongest correlation for each parameter]

Parameter	Manipulation (s)	rho	p
Phycocyanin	7-day average	0.98	<0.0001
Phycocyanin	7-day average instantaneous 10 a.m.	0.97	<0.0001
Phycocyanin	24-hour average, 3-day average	0.94	<0.0001
Phycocyanin	14-day average, 3-day average instantaneous 10 a.m.	0.90	<0.0001
Phycocyanin	14-day average instaneous 10 a.m., instantaneous 10 a.m.	0.89	<0.0001
Dissolved oxygen	14-day average	0.88	<0.0001
pH	7-day average	0.83	<0.0001
pH	Instantaneous 10 a.m.	0.82	0.0003
pH	24-hour average	0.76	0.0007
pH	3-day average, 14-day average	0.73	A0.0016
Temperature, water	Instantaneous 10 a.m.	0.73	0.0031
Dissolved oxygen	7-day average	0.67	0.0047
Temperature, water	3-day average, 7-day average, 24-hour average	A0.57	A0.0213
Dissolved oxygen	24-hour average, 3-day average	0.56	A0.0269
Chlorophyll	24-hour average	0.53	0.0358
Dissolved oxygen	Instantaneous 10 a.m.	0.46	0.1013
Temperature, water	14-day average	0.41	0.1316

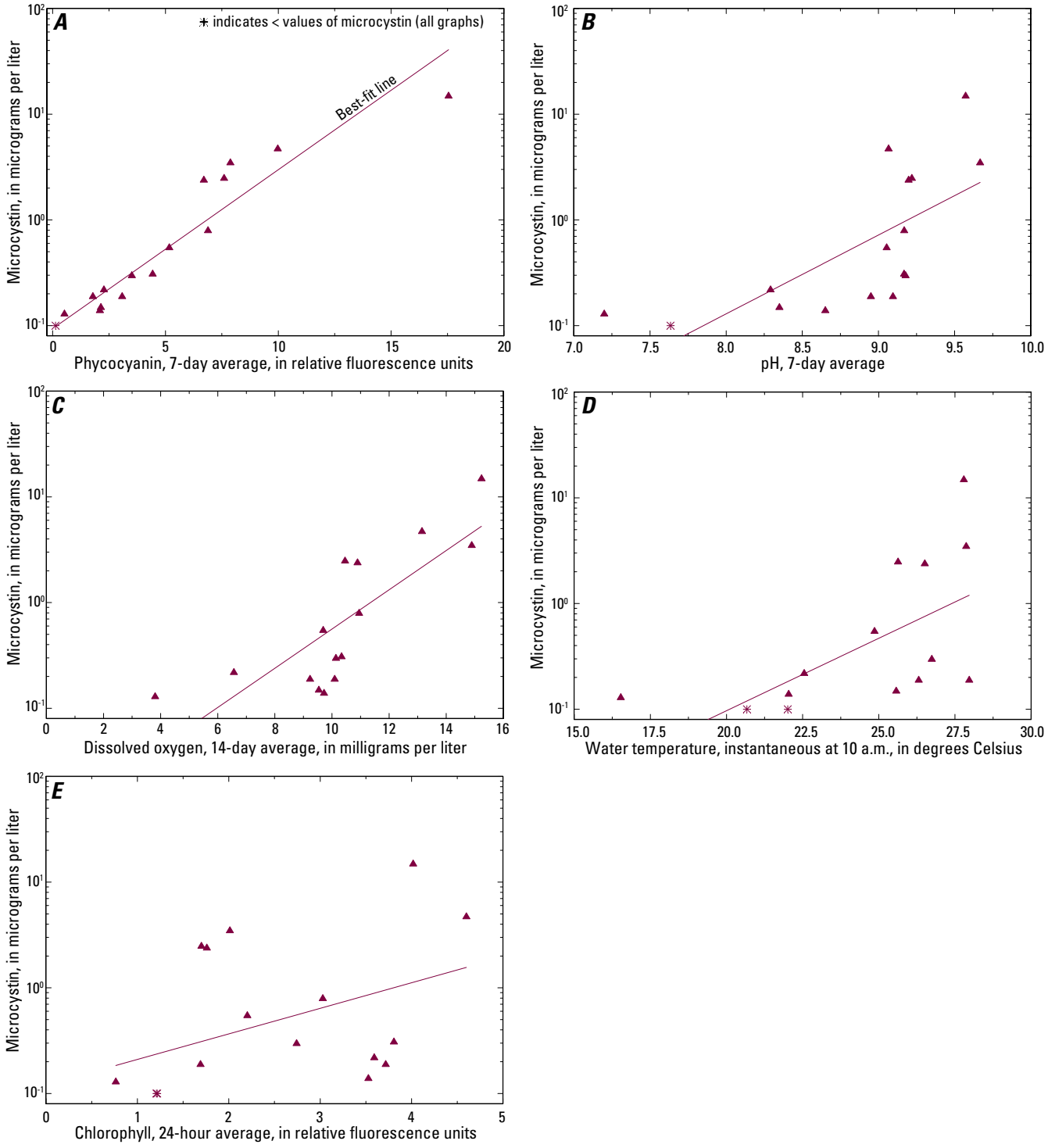


Figure 15. Relations between continuous water-quality measurements and microcystin concentrations at Harsha Lake, 2014, for potential daily predictions of microcystin concentrations. *A*, Phycocyanin, 7-day average. *B*, pH, 7-day average. *C*, Dissolved oxygen, 14-day average. *D*, Temperature, water, instantaneous 10 a.m. *E*, Chlorophyll, 24-hour average.

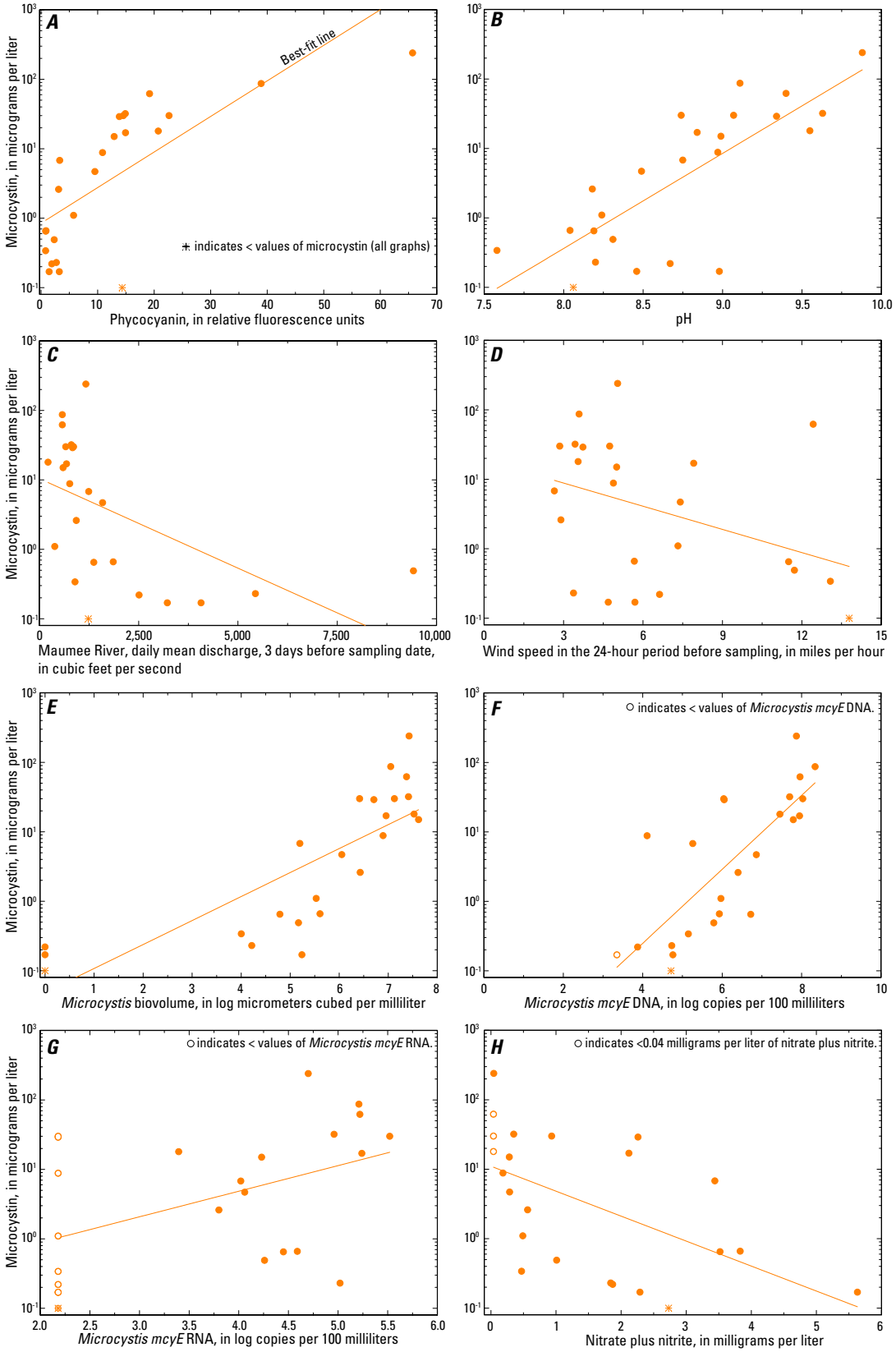


Figure 16. Relations between environmental and water-quality factors and microcystin concentrations at Maumee Bay State Park (MBSP) Lake Erie beach, 2013–14, for potential daily or long-term predictions of microcystin concentrations. *A*, Phycocyanin. *B*, pH. *C*, Maumee River daily mean discharge 3 days before sampling date. *D*, Wind speed in the 24-hour period before sampling. *E, Microcystis* biovolume. *F*, *Microcystis mcyE* deoxyribonucleic acid (DNA). *G*, *Microcystis mcyE* ribonucleic acid (RNA). *H*, Nitrate plus nitrite.

Harsha Main

At Harsha Main for daily predictions, statistically significant correlations were found between microcystin concentrations and several field measurements (table 12). Phycocyanin (fig. 14*A*), pH (fig. 14*B*), and turbidity were strongly correlated with microcystin concentrations; chlorophyll was less strongly related and included an influential outlier (fig. 14*C*). Although the correlation of 7-day median residence time with microcystin concentrations was not statistically significant (rho = −0.26, p = 0.3157), the scatterplot suggests a potential inverse relation (fig. 14*D*).

For long-term predictions at Harsha Main, several factors were significantly correlated with microcystin concentrations (table 12). Among the molecular assays, *Microcystis mcyE* DNA (fig. 14*E*), *Microcystis*, and *Dolichospermum* (fig. 14*F*) were strongly correlated with microcystin concentrations. Among the community analysis factors, cyanobacterial biovolume was most strongly correlated with microcystin (fig. 14*G*). Higher orthophosphate concentrations were associated with lower microcystin concentrations (fig. 14*H*); although not shown, similar relations were found for ammonia and nitrate plus nitrite. None of the factors for *Planktothrix* and total phosphorus or total nitrogen were significantly related to microcystin concentrations.

In addition to discrete water-quality measurements recorded at Harsha Main at the time of sampling, continuous water-quality data were available from a sonde located 1 mi north of Harsha Main (fig. 2*B*) and operated by the U.S. Environmental Protection Agency. These measurements can potentially be used for daily predictions of microcystin concentrations. Statistically significant correlations with microcystin concentrations were found for many manipulated factors (factors derived from mathematical manipulation of time-series data) for phycocyanin and pH, plus a few manipulated factors for dissolved oxygen and temperature (table 13). The highest correlations for each parameter were the 7-day average phycocyanin (fig. 15*A*) and pH (fig. 15*B*), 14-day average dissolved oxygen (fig. 15*C*), and 10 a.m. instantaneous temperature (fig. 15*D*) measurements. Only one chlorophyll measurement was significantly correlated with microcystin concentrations—the 24-hour average (fig. 15*E*). None of the specific conductance factors were significantly correlated with microcystin concentrations.

Maumee Bay State Park Lake Erie

At MBSP Lake Erie, there were several statistically significant correlations between microcystin concentrations and factors for daily predictions (table 12). As was found at Harsha Lake, phycocyanin (fig. 16*A*), pH (fig. 16*B*), and turbidity were strongly correlated with microcystin concentrations at MBSP Lake Erie, but not chlorophyll. Several discharge factors (Maumee River at Waterville, river mouth is approximately 3.5 mi from the beach) had significant negative correlations with microcystin concentrations, including those for 14-day or 30-day averages or peaks; the strongest correlation was with daily mean discharge 3 days before the sampling date (discharge Dm3) (fig. 16*C*). Although not statistically significant, wind speed in the 24-hour period before sampling (fig. 16*D*) was inversely associated with microcystin concentrations.

For long-term predictions at MBSP Lake Erie, *Microcystis* (fig. 16*E*) and cyanobacterial biovolume and abundance were strongly correlated with microcystin concentrations (table 12). Among the molecular assays, *Microcystis mcyE* DNA was the most strongly correlated (fig. 16*F*), and *Microcystis mcyE* RNA (fig. 16*G*) had a weaker, but significant, correlation to microcystin concentrations. Several nutrient constituents, including nitrate plus nitrite (fig. 16*H*), had significant negative correlations with microcystin concentrations.

Summary and Conclusions

Harmful cyanobacterial blooms (cyanoHABs) are a major water-quality issue for Lake Erie and inland lakes in Ohio. In freshwaters, microcystins are commonly produced by cyanobacteria in the genera *Microcystis, Planktothrix,* and *Dolichospermum* (*Anabaena*) that contain in their genomes the microcystin synthetase (*mcy*) gene cluster. A variety of factors such as light, temperature, nutrient concentrations, wind patterns, turbidity, pH, and lake mixing have been shown to influence the proliferation of cyanobacteria and their toxin production. Predicting when and where a bloom will occur is important to protect the public that uses and consumes a water source; however, predictions are complicated and likely site specific because of the many factors affecting toxin production. Monitoring for a variety of environmental and water-quality factors, for concentrations of cyanobacteria by molecular assays, and for algal pigments such as chlorophyll or phycocyanin may provide data that can be used to predict the occurrence of cyanoHABs.

To better understand the relations between cyanobacterial community composition, toxin production, and environmental and water-quality factors and help support predictive capabilities for cyanoHABs, lake-water samples were collected at Ohio recreational sites during May–November in 2013 and 2014. Samples were analyzed for dissolved and total nutrients, toxins, phytoplankton abundance and biovolume, and cyanobacterial genes by molecular methods. Field crews measured physical water-quality parameters at the time of sampling. Environmental data were obtained from the nearest airport weather station, radar data, agency gage, and (or) from local sources for the three lakes included in 2014 sampling.

Quantitative polymerase chain reaction (qPCR) and quantitative reverse transcriptase PCR (qRT-PCR) are two commonly used molecular methods. Quantitative PCR is used to quantify deoxyribonucleic acid (DNA) gene sequences, and qRT-PCR is used to quantify ribonucleic acid (RNA) transcripts. In this study, molecular

assays for cyanobacteria were done to enumerate (1) general cyanobacteria; (2) general *Microcystis* and *Dolichospermum* (*Anabaena*); (3) *mcyE* toxin genes for *Microcystis, Dolichospermum* (*Anabaena*), and *Planktothrix* targeting DNA; and (4) *mcyE t*ranscripts for *Microcystis, Dolichospermum (Anabaena)*, and *Planktothrix* targeting RNA. The DNA assays for the *mcyE* gene provide data on cyanobacteria that have the potential to produce microcystin, whereas the RNA assays provide data on cyanobacteria that are actively transcribing the toxin gene.

Quality-control (QC) samples were collected and analyzed for all constituents to characterize bias and variability, with QC samples for cyanobacterial molecular methods examined in more detail than the other constituents. Field and laboratory blanks indicated that it was unlikely that the concentrations reported for environmental samples were appreciably affected by contamination. Concurrent replicate data for nutrients and toxins resulted in median relative percent differences (RPDs) ranging from 0 percent for sulfate to 23 percent for saxitoxin. For cyanobacterial molecular methods, absolute value log differences (AVLDs) were calculated for concurrent (two bottles), split concurrent (two bottles analyzed twice each), and qPCR replicates. The qPCR variability (extracts from the same filter that were analyzed twice by qPCR or qRT-PCR) was small and statistically lower than within- or between-bottle variability for the five DNA assays, but not for the two RNA assays. The inherent variability in the qRT-PCR RNA assays should be considered when making conclusions based on these data. Because within-bottle and between-bottle variability were not statistically different for any molecular assay, sampling variability appeared to have been small in comparison with the combined variability associated with sample filtering, extraction and purification, and the matrix itself. For phytoplankton abundance and biovolume, 79 and 76 percent of concurrent replicate pairs, respectively, had AVLDs less than 0.5 cell per milliliter or micrometer cubed per milliliter. The AVLDs greater than 1.0 generally were caused by the occurrence of rare taxa and, in some cases, were likely caused by the extreme spatial variability in cyanobacterial blooms.

During 2013, 46 water-quality samples collected monthly at 8 sites at 8 lakes were used to complete an initial assessment and select sites for more intensive sampling during 2014. Criteria for selection for 2014 sampling included sites with a (1) wide range of toxin concentrations, (2) at least one detection of the *mcyE* toxin gene, and (or) (3) statistically significant correlations between toxin concentrations and cyanobacterial gene concentrations or optical sensor measurements. The eight sites (with median microcystin concentrations in parenthesis) were Buck Creek (0.46 microgram per liter [µg/L]), Buckeye Lake Crystal (44 µg/L), Deer Creek (0.45 µg/L), Harsha Main (1.6 µg/L), Maumee Bay State Park (MBSP) Inland (<0.10 µg/L, negative control site), MBSP Lake Erie (6.8 µg/L), Port Clinton (0.40 µg/L), and Sandusky Bay (3.6 µg/L). Samples from MBSP Lake Erie had the widest range of microcystin concentrations and one detection of saxitoxin; samples from Harsha Main included four detections of saxitoxin. The *Microcystis mcyE* DNA gene was found in the majority of samples from all sites except for MBSP State Park Inland. The *Planktothrix mcyE* DNA gene was found in the majority of samples collected at the inland lake sites (Buckeye Lake Crystal, Harsha Main, Buck Creek, and Deer Creek) and Sandusky Bay but in only a few samples from MBSP Inland and the two Lake Erie sites (MBSP Lake Erie and Port Clinton). Statistically significant correlations were found between microcystin concentrations and at least one cyanobacterial gene or optical sensor measurement at Buck Creek, Deer Creek, Harsha Main, MBSP Lake Erie, and Port Clinton, but not Sandusky Bay. Correlations were not done for data collected at Buckeye Lake Crystal and MBSP Inland because there were two few data points or too few detections of microcystin, respectively.

During 2014, 65 water-quality samples were collected to identify factors affecting the cyanobacterial community composition, toxin concentrations, and cyanobacterial gene concentrations. Three lakes were included for weekly sampling in 2014—Buckeye Lake, Harsha Lake, and Maumee Bay. Summary-statistic data from the two Buckeye Lake sites and the two Harsha Lake sites were combined. Median microcystin concentrations were 55, 0.30, and 4.7 µg/L at Buckeye Lake, Harsha Lake, and MBSP Lake Erie, respectively. A total of 37 samples exceeded the Ohio EPA Recreational Public Health Advisory level of 6 µg/L microcystin (27 from Buckeye Lake, 2 from Harsha Lake, and 8 from MBSP Lake Erie) and 32 samples were >20 µg/L (27 from Buckeye Lake and 5 from MBSP Lake Erie). Median phycocyanin measurements were 24.5, 7.3, and 9.5 relative fluorescence units at Buckeye Lake, Harsha Lake, and MBSP Lake Erie, respectively. There were four detections of saxitoxin, all at MBSP Lake Erie, ranging from 0.02 to 0.07 µg/L.

Throughout the 2014 season, the cyanobacterial community, as determined by molecular and microscopy methods, and the dominance associated with peak microcystin concentrations were unique to individual lakes. *Microcystis mcyE* DNA was detected in 88–100 percent of lake samples, with the highest median found in samples from MBSP Lake Erie (6.4 log copies/100 mL); *Microcystis mcyE* RNA was not found in any samples from Buckeye Lake, in 19 percent of samples from Harsha, and in 76 percent of samples from MBSP Lake Erie. *Planktothrix mcyE* DNA was found in 41–100 percent of lake samples, with the highest median found in samples from Buckeye Lake (8.43 log copies/100 mL); *Planktothrix mcyE* RNA was detected only in samples from Buckeye Lake (100 percent). The highest median concentration of *Dolichospermum* was found at Harsha Lake (8.35 log copies/100 mL), and *Dolichospermum mcyE* DNA was not detected in any samples in 2014. In terms of cyanobacterial biovolume,

Buckeye Lake was dominated consistently by *Planktothrix*; microcystin concentrations were also relatively consistent, with the highest observed concentrations occurring in early September and November (81 µg/L). At Harsha Lake, *Planktothrix* dominated the cyanobacterial community in May and late October, *Dolichospermum* and *Microcystis* were a substantial percentage from late May through August, and non-microcystin-producing taxa were dominant from late July through early October. At Harsha, microcystin concentrations were lowest in May (<0.1 µg/L) and highest in late June (15 µg/L). MBSP Lake Erie was generally dominated by non-microcystin producers early in the season and *Microcystis* from mid-July through early November; the maximum microcystin concentration occurred in mid-August (240 µg/L).

Spearman's correlation coefficient (rho) was computed to assess the relations between environmental and water-quality factors and microcystin concentrations at Buckeye Fairfield, Buckeye Onion Island, Harsha Main, and MBSP Lake Erie. Only 2014 data were used for correlation analysis except at MBSP Lake Erie, where data from 2013 and 2014 were combined for the correlation analysis. Correlation results for explanatory factors were grouped into two categories based on their potential application to (1) a daily predictive model developed from easily or continuously measured water-quality factors and available environmental data that do not require a site visit for sample collection, and (2) a long-term predictive model that provides advanced warning of the potential for cyanoHAB events and that (in addition to daily predictive model factors) includes results from water samples that require laboratory analyses. Factors were considered statistically significant at $p < 0.05$, and the highest rho values for each site for daily and long-term predictions are included in this discussion.

At Buckeye Fairfield, there were three statistically significant correlations between microcystin concentrations and factors suitable for daily predictions—wind direction (rho = −0.64) and two radar rainfall factors—and none for factors suitable for long-term predictions. The lack of microcystin concentrations on the low end (<20 µg/L) most likely limited the identification of promising factors for predictive models. At Buckeye Onion Island, statistically significant correlations were found for three factors for daily predictions and three factors for long-term predictions—phycocyanin (rho = 0.79), Secchi depth, lake level change in 24 hours, *Planktothrix mcyE* DNA (rho = 0.73), total nitrogen, and cyanobacteria by qPCR.

At Harsha Main and MBSP Lake Erie, there were similarities and differences among the factors significantly correlated to microcystin concentrations. Statistically significant correlations were found between microcystin concentrations and 7 and 15 factors for daily predictions, and 13 and 12 factors for long-term predictions at Harsha Main and MBSP Lake Erie, respectively. At both sites for daily factors, the highest correlations were found for phycocyanin (rho= 0.93 and 0.85), pH, and turbidity. Several measurements of discharge from a nearby river were among the daily factors with statistically significant negative correlations to microcystin concentrations at MBSP Lake Erie. Among long-term factors for the cyanobacterial community at both sites, *Microcystis mcyE* DNA (rho = 0.92 at Harsha Main), *Microcystis* biovolume (rho = 0.87 at MBSP Lake Erie) or abundance, cyanobacteria or *Microcystis* by qPCR, and cyanobacterial biovolume or abundance were all significantly correlated to microcystin. Ammonia and nitrate plus nitrite had significant inverse relations to microcystin concentrations at both sites, but total phosphorus was significantly correlated only at MBSP Lake Erie. A statistically significant correlation for *Dolichospermum* by qPCR was found only at Harsha Main. In addition to discrete water-quality measurements recorded at Harsha Main at the time of sampling, many manipulated measurements available from a sonde operated by the U.S. Environmental Protection Agency were strongly correlated to microcystin concentrations; the highest correlation was for the relation between microcystin concentrations and the 7-day average phycocyanin (rho = 0.98).

Weaker correlations to microcystin concentrations were found for RNA than for DNA assay results at all sites. This may have occurred because RNA was produced before microcystin was elevated, and an inadequate time series of samples collected on consecutive days precluded lagging the data to observe that correlation. RNA samples, however, provide valuable information because RNA assays target only living cells that are actively expressing the genes for microcystin production, whereas DNA assays target both living and dead cells, as well as free DNA (Sipari and others, 2010). Therefore, monitoring for RNA transcripts may be used as a precursor to toxin production in future studies when identifying environmental factors that trigger microcystin production.

The results of this study identified the water-quality and environmental factors that were statistically related to microcystin concentrations at four very different Ohio recreational sites—a small beach (Buckeye Fairfield) and open-water site (Buckeye Onion Island) on a shallow canal lake, a beach on a flood-control and water-supply reservoir (Harsha Main), and a beach along a large bay to Lake Erie (MBSP Lake Erie). The significant factors are promising for use in site-specific predictive models, both to provide real-time swimming advisories to the public (daily predictions) or to provide advanced warning of the potential for a cyanoHAB (long-term predictions). Daily predictions may also be used to focus sample collection efforts on days when toxins are likely to be elevated. Long-term predictions may also be used to understand changes to the cyanobacterial community composition, identify factors that may lead to increased toxin production, and help with management and remediation decisions. This was a small study with only weekly data collection. In order to develop accurate, site-specific models to predict toxin concentrations at freshwater lake sites, data need to be collected more frequently and for consecutive days in future studies. Models may need to be revised periodically to adapt to changes in phytoplankton populations at each lake.

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Abbreviations, Acronyms, and Definitions

AVLD, absolute value log difference. The AVLD was determined by calculating the absolute value of the difference between concentration results for two replicate samples that were $\log_{10}$ transformed.
Copies/100 mL, copies per 100 milliliters. The unit used to report cyanobacterial gene or transcripts results determined by qPCR or qRT-PCR.
Copies/rxn, copies per reaction volume. For qPCR and qRT-PCR assays targeting cyanobacterial genes, the unit used to report limits of blank, detection, and quantification.
CyanoHABs, cyanobacterial harmful algal blooms.
CCSWD, Clermont County Soil and Water Conservation District.
DNA, deoxyribonucleic acid. Molecule that contains the genetic instructions for most living organisms. In this report, DNA is the genetic material for cyanobacteria.
Discharge Dm1, Dm2, or Dm3, mean daily discharge value for the previous day (Dm1) or value lagged for 2 days (Dm2) or 3 days (Dm3).
ECGHD, Erie County General Health District, Sandusky, Ohio
ELISA, enzyme linked immunosorbent assay. A method for detecting toxins in water samples.
Field blank. A blank solution used to determine potential contamination during all steps of sample collection, transport, and processing. Field blanks are similar to equipment blanks except they are processed under actual field conditions and transported to the lab along with environmental samples.
Field concurrent replicates. Environmental samples collected and analyzed in a manner such that the samples are thought to be virtually identical in composition. Concurrent replicates are collected to determine sampling and (or) analytical variability.
LoB. For qPCR and qRT-PCR assays targeting cyanobacterial genes, the lowest concentration that can be reported with 95 percent confidence to be above the concentrations of blanks.
LoD. For qPCR and qRT-PCR assays targeting cyanobacterial genes, the lowest concentration that can be detected with 95 percent confidence that it is a true detection and can be distinguished from the LoB.
LoQ. For qPCR and qRT-PCR assays targeting cyanobacterial genes, the lowest concentration of a gene that can be accurately quantified.
MBSP Lake Erie, Maumee Bay State Park Lake Erie beach, one of the sites in this study.
mcy. For toxin production to occur, the microcystin synthetase gene cluster (*mcy*) must be present in the genome of cyanobacteria.
mcyE. The *mcyE* gene is part of the *mcy* gene cluster.
OGRL, U.S. Geological Survey Organic Geochemistry Research Laboratory.
QA/QC, quality assurance and quality control.
qPCR, quantitative polymerase chain reaction. A technique used to amplify DNA many orders of magnitude so that it can be detected and quantified. This method was used to detect cyanobacteria.
qRT-PCR, quantitative reverse transcriptase polymerase chain reaction. A technique used to convert RNA to DNA and then magnify DNA many orders of magnitude so that it can be detected and quantified. This method was used to detect *mcyE* RNA transcripts in cyanobacteria that are actively expressing the *mcyE* gene.
RPD, relative percent difference.
Rainfall Dm1, Dm2, Dm3, 48W, or 72W. Hourly rainfall values were totaled for 24 hours up to 8 a.m. the day of sampling and designated as day minus 1 (Dm1), lagged for 1 day (Dm2), and lagged for 2 days (Dm3) or weighted for 48 hours (48W) or 72 hours (72W) cumulative rainfall.
RNA, ribonucleic acid. Molecule that contains the genetic instructions for transcription of genes. In this study, genus-specific *mcyE* RNA transcripts for *Microcystis*, *Dolichospermum* (*Anabaena*), and *Planktothrix* were targeted.
UT LEC, University of Toledo, Lake Erie Center, Oregon, Ohio.
USGS OWML, U.S. Geological Survey Ohio Water Microbiology Laboratory in Columbus, Ohio.
USGS NWQL, U.S. Geological Survey National Water Quality Laboratory in Denver, Colo.
USGS NWISWeb, U.S. Geological Survey National Water Information System, a public system for retrieving data collected for USGS projects.

Appendixes

Appendix 1. Calculations of Lake Residence Times at Harsha Lake

G.F. Koltun, U.S. Geological Survey

A table listing Harsha Lake elevations versus storage at 1-foot (ft) intervals was provided by the U.S. Army Corps of Engineers (Jade L. Young, U.S. Army Corps of Engineers, written commun., 2015). The data provided were interpolated to 0.01-ft intervals by means of linear interpolation, assuming that storage changes as a linear function of elevation within 1-ft intervals.

Lake-level measurements made daily at 11 a.m. (EDT) at USGS 03247040, East Fork Lake near Bantam, were used in combination with the interpolated elevation-storage tables to determine the change in storage between consecutive-day measurements and to estimate the average storage for the same periods. The change in storage (in cubic feet [ft^3]) was divided by 86,400 (the number of seconds in a 24-hour period) to compute the average net inflow (in cubic feet per second [ft^3/s]) to Harsha Lake between lake-level measurements. Positive net inflows indicate that more water entered the reservoir than was discharged or otherwise lost due to processes such as evaporation and infiltration. Negative net inflows indicate the opposite. The average storage for a 24-hour period was estimated by taking the average of storages associated with consecutive 11 a.m. lake-level measurements.

The total inflow to Harsha Lake was estimated on the basis of daily mean streamflows measured at USGS streamgage number 03246500 on the East Fork Little Miami River at Williamsburg, Ohio, located about 5 miles upstream from Harsha Lake. The East Fork Little Miami River drains approximately 72 percent of the total area draining to Harsha Lake. The inflow from the remaining 28 percent of the drainage was estimated by assuming the same streamflow yield (in cubic feet per second per square mile [$ft^3/s/mi^2$]) as determined for the streamgage location. The measured inflow from the gage was added to the estimated inflow from the remainder of the contributing drainage area to estimate the total inflow to Harsha Lake.

The daily mean outflow plus losses (if any) from Harsha Lake was estimated by subtracting the storage-based net inflow from the estimated total inflow. With the exception of 1 day, estimated total inflows exceeded the storage-based net inflows. When storage-based net inflows exceed estimated total inflow, this suggests that water is entering the reservoir at a rate exceeding that of outflow (plus losses).

The residence time was estimated for each 24-hour period by dividing the average reservoir storage (ft^3) by the estimated total outflow plus losses (ft^3/s), and then converting the resulting time to units of decimal days. This estimate of residence time effectively represents the time required to drain the reservoir given that 24-hour period's rate of outflow plus losses. When total outflow plus losses is negative, the implication is that the effective residence time for parcels of water entering the reservoir during that period is infinite (at least until outflows plus losses once again exceed inflows). Actual residence times for a parcel of water entering the reservoir during a particular period likely would be different than estimated depending on flow patterns in the reservoir and on changes in environmental conditions over the parcel's residence time in the reservoir.

Appendix 2. Phytoplankton Abundance and Community Composition at Ohio Recreational Lake Sites, 2013–14

Available for download at http://pubs.usgs.gov/sir/20155120/

Madison Publishing Service Center, Wisconsin
Manuscript approved for publication August 28, 2015
Edited by Mike Eberle
Design and layout by Cory Hurd

USGS

Francy and others—**Factors for Use in Predictive Models for Microcystin Concentrations at Ohio Recreational Sites**—Scientific Investigations Report 2015–5120

ISSN 2328-0328 (online)
http://dx.doi.org/10.3133/sir20155120