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Prepared in cooperation with the Ohio Water Development Authority; the Ohio Department of Natural Resources, Ohio State Parks, and the City of Celina, Water Treatment Plant

Chemical and Biological Quality of Water in Grand Lake St. Marys, Ohio, 2011–12, with Emphasis on Cyanobacteria

Scientific Investigations Report 2014–5210

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

Cover: Sunset at docks on the northwest shore of Grand Lake St. Marys, St. Marys, Ohio, July 2011.
Inset: Algae at docks on the northeast shore of Grand Lake St. Marys, St. Marys, Ohio, July 2011.

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By D.H. Dumouchelle and E.A. Stelzer

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SALLY JEWELL, Secretary

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Conversion Factors, Datums, and Abbreviations

Multiply	By	To obtain
	Length	
inch (in.)	2.54	centimeter (cm)
centimeter (cm)	0.3937	inch (in.)
inch (in.)	25.4	millimeter (mm)
foot (ft)	0.3048	meter (m)
meter (m)	3.281	foot (ft)
mile (mi)	1.609	kilometer (km)
	Area	
square mile (mi^2)	259.0	hectare (ha)
square mile (mi^2)	2.590	square kilometer (km^2)
	Volume	
gallon (gal)	3.785	liter (L)
ounce	29.57	milliliter (mL)
	Weight	
pound (lb)	453.59	gram (g)
	Flow rate	
cubic foot per second (ft^3/s)	0.02832	cubic meter per second (m^3/s)

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

°F= (1.8×°C)+32

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

°C=(°F-32)/1.8

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

Specific conductance is given in microsiemens per centimeter at 25 degrees Celsius (µS/cm).

Concentrations of chemical constituents in water are given in milligrams per liter (mg/L), micrograms per liter (µg/L), or nanograms per microliter (ng/µL).

Abbreviations

alum	aluminum sulfate
BV	Biovolume
col/100 mL	colonies per 100 milliliters
CT	threshold cycles
CWTP	Celina Water Treatment Plant
DO	dissolved oxygen
DNA	deoxyribonucleic acid
E. coli	*Escherichia coli*
ELISA	enzyme-linked immunosorbent assay
EPA	U.S. Environmental Protection Agency
GLSM	Grand Lake St. Marys
HAB	Harmful Algal Bloom
mM	millimolar
mRNA	messenger ribonucleic acid
N:P	nitrogen to phosphorus ratio
NWQL	USGS National Water Quality Laboratory
ODNR	Ohio Department of Natural Resources
OEPA	Ohio Environmental Protection Agency
PVC	polyvinyl chloride
qPCR	quantitative polymerase chain reaction
qRT-PCR	quantitative reverse-transcription polymerase chain reaction
R2	coefficient of determination
rho	Spearman's rank correlation coefficient
RNA	ribonucleic acid
TN:TP	total nitrogen to total phosphorus ratio
USGS	U.S. Geological Survey
WHO	World Health Organization
µL	microliters

Acknowledgments

The authors acknowledge W. Ringo for assisting with some early operations on the lake. The authors also acknowledge K. Waits and Yellow Springs Instruments Inc., for the loan of equipment during both field seasons. Lastly, the authors acknowledge the Celina Fire Department Dive Team for the retrieval of the lower monitor at site B1, which became tangled in the buoy chains during spring 2011.

Chemical and Biological Quality of Water in Grand Lake St. Marys, Ohio, 2011–12, with Emphasis on Cyanobacteria

By D.H. Dumouchelle and E.A. Stelzer

Abstract

Grand Lake St. Marys (GLSM) is a shallow lake in northwest Ohio, which is about 9 miles long and 3 miles wide with depths averaging less than 8 feet. Cyanobacteria blooms are common in GLSM, and high concentrations of microcystins—toxins produced by cyanobacteria—have been documented therein. During 2011–12, the U.S. Geological Survey collected 11 sets of water samples at 6 locations in the lake. The water samples were analyzed for concentrations of nutrients, chlorophyll, and microcystin and to determine plankton community structure and abundance. Analysis by quantitative polymerase chain reaction (qPCR) and quantitative reverse-transcription polymerase chain reaction (qRT-PCR) was used to identify the relations between microcystin concentrations and *Planktothrix* and *Microcystis* genotypes (toxic versus non-toxic). The qPCR analysis targets deoxyribonucleic acid (DNA) genes and quantifies the potential for toxin production, whereas the qRT-PCR analysis targets ribonucleic acid (RNA) transcripts and quantifies the expression of the toxin gene. Water samples were collected six times at one site for analyses of major ions and trace elements. In addition, field measurements were made to determine transparency, temperature, dissolved oxygen, pH, and specific conductance of the water.

GLSM is shallow with a long fetch, which contributes to the warm and turbid water conditions. Secchi-disk measurements generally ranged from 0.2 to 0.3 meters, and summer water temperatures in GLSM frequently exceed 25 degrees Celsius (°C), with peak temperatures greater than 30 °C. Dissolved oxygen readings below 0.5 milligrams per liter (mg/L) occurred at the lake bottom, which can lead to the internal recycling of phosphorus in the lake.

Phytoplankton analyses indicated that GLSM is dominated by cyanobacteria with *Planktothrix*, the dominant genera during 2011–12. Nitrate ranged from 0.19 to 3.23 mg/L, although concentrations in most samples were less than 1 mg/L. Total nitrogen concentrations ranged from 1.86 to 5.42 mg/L. Orthophosphate (as P) concentrations ranged from less than 0.004 to 0.067 mg/L, although concentrations of most samples were less than 0.004 mg/L. Total phosphorus (as P) concentrations ranged from 0.12 to 0.43 mg/L. Microcystin concentrations ranged from 7.3 to 83 micrograms per liter.

Microcystin concentrations were correlated to cyanobacteria biovolumes, and to concentrations of one ion (sodium) and three trace elements (molybdenum, antimony, and lithium). Concentrations of toxin genes (*mcyE*) determined by qPCR were consistently low for *Microcystis* and consistently high for *Planktothrix* throughout both sampling years. Concentrations of cyanobacteria found by qPCR were correlated to microcystin concentrations, cyanobacteria biovolumes, selected nutrient concentrations, and other parameters. Results from qRT-PCR assays showed that toxin gene expression was predominantly from the genus *Planktothrix*, and concentrations of the RNA transcript varied throughout the two sampling years. A number of conditions that may play a role in the dominance of *Planktothrix* and the production of microcystin were identified including water temperature; low-light transmission; low concentrations of silica and manganese; and relatively high concentrations of sodium, sulfate, and the trace elements of strontium, vanadium, and boron.

Introduction

Grand Lake St. Marys (GLSM), the largest man-made lake in Ohio, is located in northwest Ohio and spans the border between Auglaize and Mercer Counties. The city of Celina is located on the western end of the lake, and the city of St. Marys is located on the eastern end. The lake, completed in 1845, was constructed to store and supply water for the Miami-Erie canal. GLSM is now a State park, managed by the Ohio Department of Natural Resources (ODNR). In addition to being used for recreation, GLSM also is the drinking-water supply for the city of Celina.

Over time, water quality in GLSM has been impacted by excessive nutrient contributions from multiple sources in the watershed, which likely have contributed to algal blooms, including blooms of cyanobacteria (also called blue-green algae because the bacteria are photosynthetic and contain chlorophyll). Cyanobacteria can produce a variety of compounds that can affect water quality, ranging from compounds that cause taste-and-odor problems to toxic compounds that can

affect human and animal health. Several species of cyanobacteria can produce microcystins, a class of peptides known for their liver toxicity. The World Health Organization (WHO) provisional guidelines for microcystin-LR (a common form of microcystin) in recreational contact waters is 20 micrograms per liter (µg/L) (World Health Organization, 1999). The WHO guideline is based upon a moderate probability of adverse health effects in adults owing to accidental ingestion of 100 milliliters (mL) of water while swimming.

In 2009, the Ohio Environmental Protection Agency (OEPA), the ODNR, and the Celina Water Treatment Plant (CWTP) sampled for microcystins in GLSM throughout the recreational season as part of OEPA's Harmful Algal Bloom (HAB) sampling program (*http://epa.ohio.gov/habalgae.aspx*). Concentrations of microsystins ranged from 6 to 82 parts per billion (µg/L) in GLSM and exceeded the WHO recreational-contact guideline of 20 µg/L for the majority of the sampling season. As a result, the OEPA issued a water-quality advisory for the lake in May 2009, which remained in place all summer.

The dominant genus of cyanobacteria at GLSM was identified as *Planktothrix* (Ohio Environmental Protection Agency, 2010a, b). *Planktothrix* (also known as *Oscillatoria*) is very common in Ohio's inland lakes; it is a filamentous cyanobacterium that fixes nitrogen from the atmosphere and is known to produce the microcystin toxin (Ohio Sea Grant Fact Sheets, 2010). Along with *Microcystis aeruginosa,* certain species of the genus *Planktothrix* are commonly cited as microcystin producers (U.S. Environmental Protection Agency, 2008). These species also include strains that lack the cluster of genes required to produce a toxin. The toxin-producing strains cannot be differentiated from the non-toxin-producing strains by traditional microscopy because the differences occur at the sub-species level. Fortunately, quantitative polymerase chain reaction (qPCR) assays have been developed that can determine concentrations of deoxyribonucleic acid (DNA) toxin genes present from both *Microcystis* and *Planktothrix* genera. These assays are used to measure the potential for toxin production. In order to produce a toxin, a cyanobacteria cell must transcribe its DNA-encoded toxin gene into messenger ribonucleic acid (mRNA) to initiate the biosynthetic process. New quantitative reverse-transcription polymerase chain reaction (qRT-PCR) assays provide relative quantification of ribonucleic acid (RNA) transcripts from cells involved in or about to be involved in active toxin production.

There have been a number of studies that investigated cyanobacteria and HABs. The role of nutrients has been investigated, and some studies have seen a relation between cyanobacteria, microcystin concentrations, and water-quality conditions or concentrations of nutrients (Jacoby and others, 2000; Graham and others, 2004; J. Graham, U.S. Geological Survey, oral commun., 2009). A recent study on Lake Erie found that the proportion of *Microcystis* in the algal population was linked with microcystin concentrations but that the percentage of toxin-producing genotypes was not linked (Rinta-Kanto and others, 2009); this may indicate that another toxin-producing genus, such as *Planktothrix,* was an important contributor of microcystin. The U.S. Geological Survey (USGS) is working to gain a better understanding of factors that promote and sustain HABs. To complement the OEPA's Inland Lakes Program data and to help achieve recommendations set forth in the Total Maximum Daily Loads report for the Beaver Creek and GLSM Watershed (Ohio Environmental Protection Agency, 2007), the USGS, in cooperation with the Ohio Water Development Authority, the Ohio Department of Natural Resources, Division of Ohio State Parks and the Celina Water Treatment Plant, investigated the chemical and biological quality of water in GLSM.

Purpose and Scope

The purpose of this report is to describe the data and findings from water-quality samples collected by the USGS at six sites in GLSM from May to October 2011 and March to October 2012. Samples were collected five times in 2011 and six times in 2012. Selected additional data from other sites and sources also are presented. Water-quality data included field parameters (temperature, water transparency, dissolved oxygen (DO), pH, and specific conductance); phytoplankton and zooplankton identification; and concentrations of chlorophyll, nutrients, total microcystins, major ions, and trace elements. Molecular methods were used to quantify the cyanobacteria found in GLSM on three levels (all toxin gene assays used the *mcyE* fragment of the microcystin synthetase toxin gene):

1. Total cyanobacteria and total *Microcystis* DNA genes by qPCR
2. Genus-specific (*Microcystis* and *Planktothrix*) DNA toxin genes by qPCR
3. Genus-specific (*Microcystis* and *Planktothrix*) RNA transcripts by qRT-PCR

Description and Conditions of Study Area

The study area, in rural northwestern Ohio, is in the north temperate climate zone, where the average summer temperature is 72.1 degrees Fahrenheit (°F), and the average spring and summer precipitation is 22.54 inches (in.) (National Oceanic and Atmospheric Administration-National Climatic Data Center, 2014). The watershed lies in the glaciated Eastern Corn Belt Plains ecoregion and the principal aquifer in the region is the limestone and dolomite of the Bass Island and Lockport groups (Tetra Tech, Inc., 2010; Kostelnick, 1983). GLSM is approximately 9 miles (mi) long (west to east) and approximately 3 mi wide (north to south) with 56 mi of shoreline (fig. 1). The lake is generally less than 8 feet (ft) deep at the summer pool elevation. The drainage area for the lake is about 112 square miles (mi^2) (Ohio Environmental Protection Agency, 2007). Inflow to the lake is primarily from six small

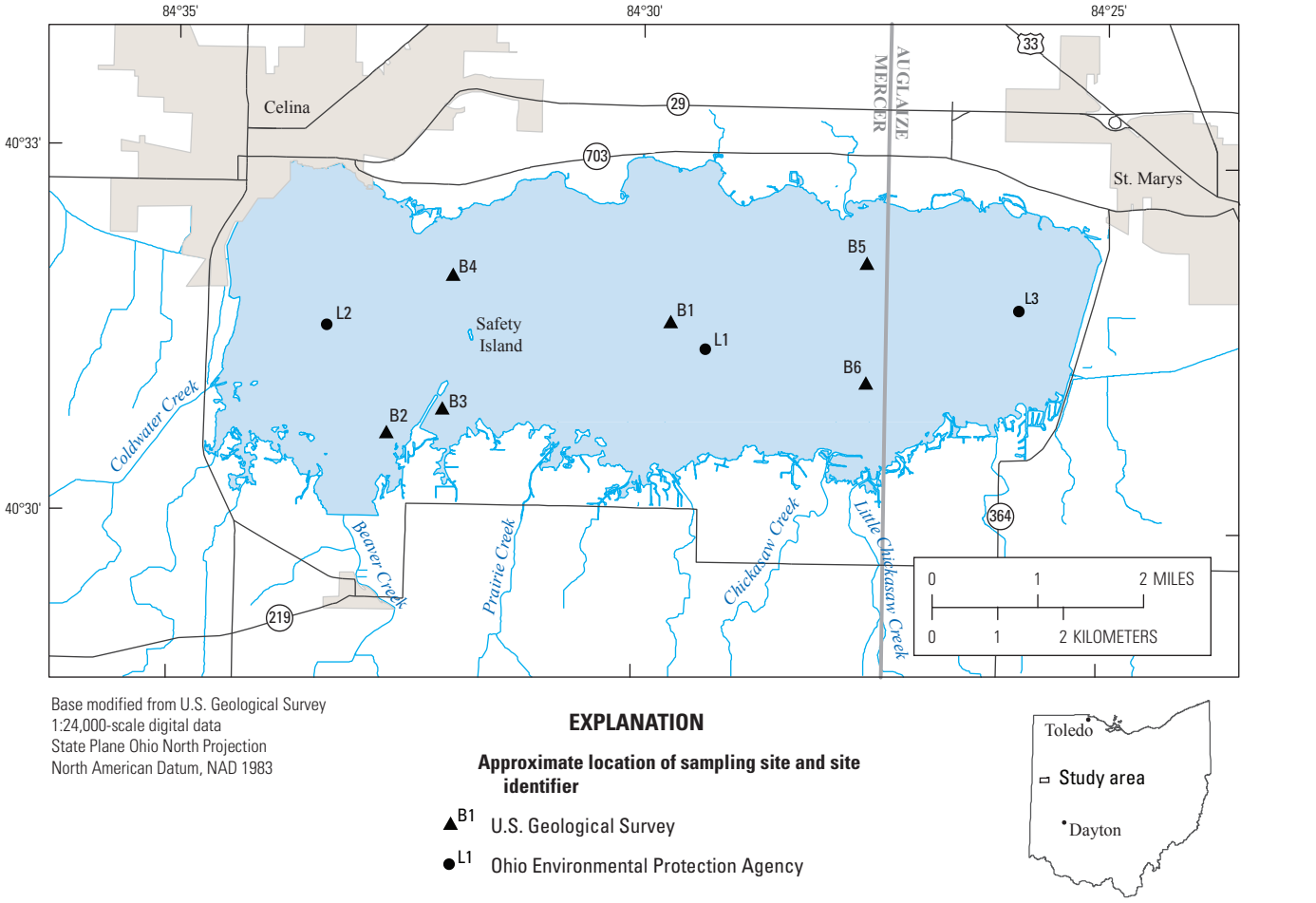

Figure 1. Location of Grand Lake St. Marys, Ohio, and sampling sites.

streams on the south side of the lake. The largest tributaries, Coldwater and Beaver Creeks, each have drainage areas of about 20.3 and 19.4 mi^2, respectively. Chickasaw Creek has a drainage area of about 18.7 mi^2, and the remaining three tributaries have combined drainage areas of about 15.7 mi^2 (Ohio Environmental Protection Agency, 2007; Tetra Tech, Inc., 2010). The lake discharges from two outflows—a spillway on the west end of the lake and a little-used control structure on the east end of the lake. Numerous channels extend inland from the shore and are dredged and maintained for recreational boat access.

Agriculture is the largest land use in the area, in addition to livestock-feeding operations, and more than 80 percent of the land use is either row crops or hay and pasture (Ohio Environmental Protection Agency, 2007; Ohio Department of Natural Resources, 2013a, b; U.S. Army Corps of Engineers, 2013). Runoff from livestock-feeding operations, manure spreading, and fertilizer applications are known nutrient sources, as are failing septic systems in residential areas (Ohio Environmental Protection Agency, 2007).

In 2011, heavy spring rains resulted in lake levels above the normal-pool elevation throughout most of the recreation season; in contrast, less than average spring and summer rains during 2012 resulted in low lake levels (table 1). Stream-discharge hydrographs from Chickasaw Creek (fig. 2) show the difference in spring-runoff events between the wet year (2011) and the drier year (2012).

From June 2–29, 2011, and April 2–30, 2012, about 40 percent of GLSM was treated with aluminum sulfate (alum). The alum was applied to the central area of the lake. The purpose of the alum application was to remove phosphorus from the water column and bind mobile phosphorus in the sediments (Tetra Tech, Inc., 2013).

Table 1. Average monthly lake-level data for Grand Lake St. Marys, Ohio, and precipitation data for Toledo and Dayton, Ohio, March–October 2011 and March–October 2012.

[Lake-level data from Brian Miller, Grand Lake St. Marys State Park, written commun., 2013. Normal pool datum, 870.6 feet above mean sea level. Toledo and Dayton precipitation data from National Oceanic and Atmospheric Administration, 2012a, b]

	March	April	May	June	July	August	September	October
				Lake level (inches, relative to normal pool datum)				
2011	+17.5	+14.5	+14.5	+10	+5.5	+1	−0.5	+1.5
2012	+5	0	−4	−6	−12.5	−14	−13	−11
				Toledo precipitation (inches)				
2011	3.09	6.30	5.88	0.51	3.34	3.19	6.51	3.16
2012	3.84	1.74	1.50	2.92	3.45	4.91	2.58	2.06
58-year average	2.52	3.15	3.27	3.50	3.28	3.22	2.77	2.26
				Dayton precipitation (inches)				
2011	4.17	8.72	6.06	2.56	2.22	2.02	10.84	3.00
2012	2.66	2.17	2.04	1.57	2.86	1.65	5.27	3.81
93-year average	3.41	3.67	3.99	3.88	3.50	3.09	2.91	2.49

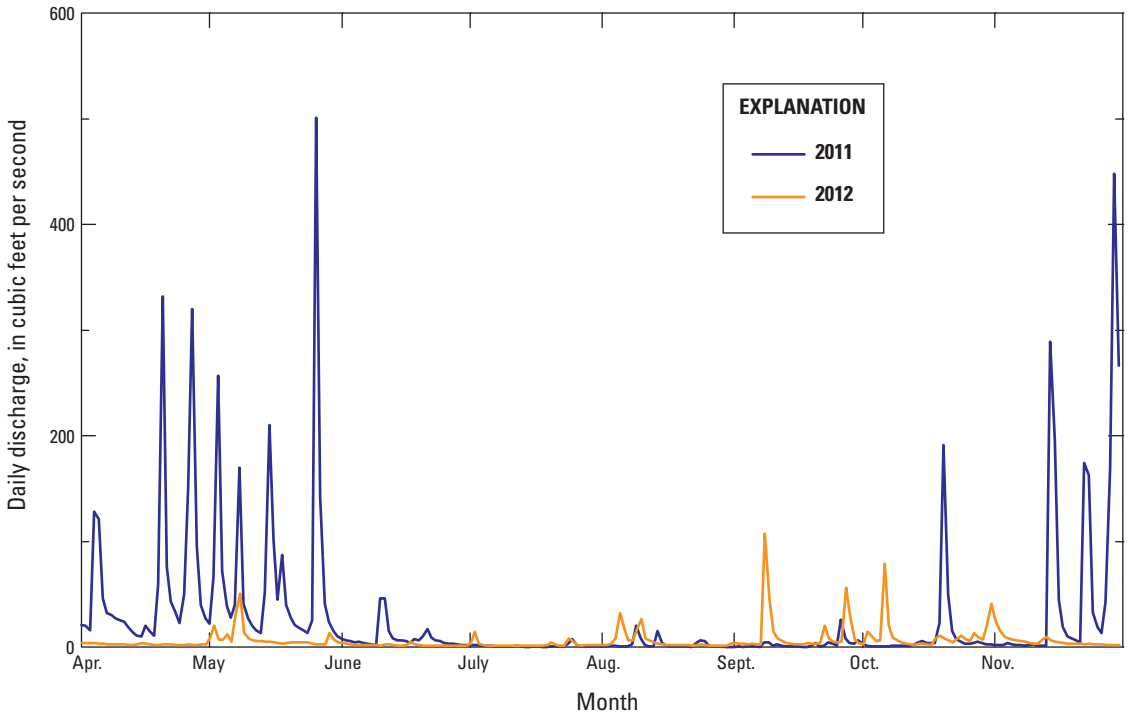

Figure 2. Stream discharge hydrographs from Chickasaw Creek at St. Marys, Ohio, April–November 2011 and April–November 2012.

Methods

Site Selection

Six locations (fig. 1) were selected to provide water-quality data representative of the open areas of the lake. Spatial coverage of the lake, as well as areas that had the potential to show variation in water quality, were the criteria considered for selecting sampling locations. Site B1 was in the center of the lake near a site previously sampled by the USGS and near the OEPA site L1. Sites B2 and B6 were on the southern side of the lake and were expected to reflect partial mixing of lake water and inflows from the tributaries. Site B3 was in a slightly protected, nearshore area. Site B4 was north of Safety Island, in an area mixed by water movement between the western and central areas of the lake. Site B5 was on the northern side at the eastern end of the lake, which often is the downwind region of the lake.

Field Data Collection

In this report, field data refers to water-quality data collected on-site either during a site visit or recorded by continuous data loggers. The parameters measured were temperature, transparency, DO, pH, and specific conductance. Equipment for continuous recording of water-quality parameters was installed in a buoy placed at site B1; the other five sites had continuous data loggers recording only temperature and changes in light.

Continuous Sensors (Site B1)

Site B1 was equipped with a buoy outfitted with two continuous data loggers (YSI sondes, Yellow Springs Instrument Co., Inc., Yellow Springs, Ohio), which were equipped to measure and record pH, water temperature, DO, specific conductance, and chlorophyll. The accuracy of the measurements depends upon the sensors installed—accuracies for sensors used in this study are pH, ± 0.2 units; temperature, ± 0.15 degrees Celsius (°C); conductivity, ± 5 percent of the reading; and DO, ± 0.1 mg/L.

The upper sonde was suspended beneath the buoy, at a depth of about 2 ft, within a polyvinyl chloride (PVC) pipe. In 2011, biofouling within the pipe affected the data; for 2012, the PVC pipe was removed and the sonde was suspended in open water. The lower sonde was secured to a metal stand that was placed on the lake bottom with the sensors located about 1 ft above the lake bottom. Records were discontinued at the lower sonde after July 29, 2012, owing to storm damage and scouring problems that caused the sonde-stand to tip over. Field data were measured every 30 minutes by the sondes. Specific information on the equipment and daily mean or median values from these continuous measurements is available in the annual data reports for Ohio, 2011–12 (U.S. Geological Survey, 2011, 2012).

Buoys, provided by the GLSM State Park, were equipped with sensors to record temperature and relative light intensity at multiple depths at sites B2–B6. The sensors were placed on the buoy chains at 1-ft intervals with the uppermost sensor located just beneath the buoy at about 2 ft in depth. The temperature sensors had a resolution of 0.10 °C with an accuracy of 0.47 °C. Temperature and relative light readings were recorded hourly. The equipment at site B4 was lost sometime after mid-July 2011 and was not replaced for 2012.

Depth Profiles

During sampling trips, vertical profiles of temperature, DO, pH, and specific conductance were measured prior to sample collection. Readings were taken at the surface, 1 ft, and 2 ft of depth, and then in 2-ft intervals to the lake bottom. Measurement depths were approximate owing to variations in the lengths of the probes and wave action. Data from Secchi-disk readings also were collected. Secchi-disk measurements are generally accepted measurements for the transparency of water; however, these measurements are not an exact measure of transparency because factors, such as sun glare, weather, and the visual acuity of the observer, affect the repeatability of the measurement.

Sampling

Water samples were collected every 4 to 6 weeks at the six sites (fig. 1); five times from May to October 2011, and six times from March to October 2012. During each sampling round, samples were collected at all six sites on 1 day. Secchi-disk readings, field parameters, and general observations of the environment, such as wave heights and weather, were recorded prior to sample collection.

Water samples were collected off the side of a boat using a peristaltic pump equipped with weighted polymeric silicone tubing. The sample tubing was cleaned and sterilized prior to use by washing in soap and water, rinsing with deionized water, soaking in 0.1 percent bleach for 1 hour, dechlorinating in 0.05 percent sodium thiosulfate for 30 minutes, and lastly rinsing with copious amounts of sterile deionized water. Water samples were collected from the lake surface to the photic depth, 2.5 times the Secchi-disk depth, by manually raising and lowering the tubing during collection. The tubing was rinsed before sample collection by pumping lake water through it for a few minutes. The bottles were rinsed three times with lake water prior to sample collection, except for sample bottles pre-filled with preservative. Samples were placed on ice immediately after collection; chlorophyll samples were stored on ice in opaque black bags.

Samples from all sites were analyzed for phytoplankton abundance and biovolume, cyanobacteria gene concentrations (samples were analyzed for RNA transcripts at only two sites), alkalinity and concentrations of nutrients, total microcystins (hereafter referred to as microcystin), and chlorophyll. During

every trip, zooplankton samples were collected at four sites using a Wisconsin Sampler with 53 micron net. Samples from sites B2 and B4 were composited together as were samples from sites B5 and B6. At site B1, samples were collected three times each year for major ion and trace ion concentrations.

Nutrient and ion concentrations were determined by the USGS National Water Quality Laboratory (NWQL) in Denver, Colorado, using alkaline persulfate digestion, Kjeldahl digestion, and mass spectrometery methods. Samples were shipped on ice, within 3 days of collection. Total microcystin concentrations were determined by the CWTP laboratory using an enzyme-linked immunosorbent assay (ELISA) and an Abraxis kit (Abraxis LLC, Warminster, Pennsylvania). Samples were delivered to the laboratory the day of collection and either analyzed the next day or frozen for later analysis. Chlorophyll samples were analyzed by the USGS Kansas Water Science Center. Samples were filtered within 24 hours, and the filters were frozen. After the field season, the frozen filters were shipped on dry ice. Total chlorophyll, uncorrected for degradation products, was extracted in heated ethanol (Sartory and Grobbelar, 1986) and analyzed fluorometrically using U.S. Environmental Protection Agency (EPA) method 445.0 (Knowlton, 1984; Arar and Collins, 1997). Samples were analyzed in duplicate, and the results were reported as an average. Samples for cyanobacteria and toxin genes were processed within 24 hours of collection; processing and analyses were done at the USGS Ohio Water Science Center microbiology laboratory. The phytoplankton and zooplankton samples were analyzed using microscopy by BSA Environmental Services, Inc., in Beachwood, Ohio, following methods described in Beaver and others (2013). The plankton samples were collected in provided bottles, containing Lugol's solution to preserve the sample. Duplicate samples were collected for quality assurance/quality control purposes; data are shown in appendix 5.

Molecular Methods

Water samples to be analyzed by qPCR (for DNA) and by qRT-PCR (for RNA) were filtered through Nucleopore polycarbonate filters (Whatman/GE Healthcare, Piscataway, New Jersey) within 30 hours of sample collection at the USGS Ohio Water Science Center microbiology laboratory. Filters were preserved at −70 °C in screw-cap vials with 0.3 grams of acid-washed glass beads (Sigma-Aldrich, St. Louis, Missouri) until batch analysis was done after the fall of each year. One filter blank using buffered water also was filtered every day that samples were filtered. All of the molecular methods used in this study are described in Stelzer and others (2013).

DNA Extraction and qPCR Analyses

Samples to be analyzed for DNA using qPCR were extracted by use of a DNA-EZ extraction kit (GeneRite, North Brunswick, N.J.) according to the manufacturer's instructions, except no prefilter was used and the final elution volume was 100 or 150 microliters (µL) for samples collected in 2011 and 2012, respectively. The larger elution volume in 2012 was needed to run extra test assays not described in this report. An extraction blank was included with each batch of sample extractions.

Five µL of the extraction eluate was analyzed in duplicate by qPCR for total cyanobacteria DNA genes (Rinta-Kanto and others, 2005), total *Microcystis* DNA genes (Rinta-Kanto and others, 2005), *Microcystis mcyE* DNA toxin genes (Sipari and others, 2010), and *Planktothrix mcyE* DNA toxin genes (Rantala and others, 2006) using primer and probe sets as well as run conditions described in Stelzer and others (2013). A no-template control was added to each plate in duplicate. All assays were run on either an Applied Biosystems 7500 or a StepOne Plus (Foster City, California) thermal cycler. Depending upon the assay, either TaqMan Universal PCR Master Mix or SYBR Green PCR Master Mix (Applied Biosystems, Foster City, Calif.) was used.

Sample inhibition was determined using matrix spikes by seeding the sample with an extracted positive control target in a duplicate qPCR reaction. The concentration of target in the sample was then compared to the concentration of target in the clean matrix control that was seeded with the same extracted positive control target. Sample extracts were considered inhibited and were diluted if the seeded test sample was greater than 2 threshold cycles (C_T) higher than the seeded clean matrix control.

RNA Extraction and qRT-PCR Analyses

Samples to be analyzed for RNA using qRT-PCR were extracted using an Ultraclean Plant RNA extraction kit (MO BIO Laboratories, Inc., Carlsbad, Calif.) according to manufacturer's instructions. An extraction blank was included with each batch of sample extractions. A DNase treatment was included during extraction, and a DNA *Microcystis mcyE* qPCR was run to verify that the RNA samples were completely DNA free.

RNA was reverse transcribed using a two-step process. In brief, 6.7 µL of RNA extract was mixed with 10 nanograms per microliter (ng/µL) random primers (Promega Corporation, Madison, Wisconsin) and nuclease-free water, heated for 4 minutes at 99 °C, placed on ice, and then supplemented with 16.8 µL of an RT reaction mixture. The mixture components and their final concentrations were as follows: 10 millimolar (mM) Tris-HCl (pH 8.3) (Applied Biosystems, Foster City, Calif.), 50 mM KCl (Applied Biosystems), 3 mM MgCl2 (Applied Biosystems), 10 mM dithiothreitol (Promega Corporation, Madison, Wis.), 0.8 mM deoxynucleotide triphosphates (Promega Corporation), 20 units of RNase Inhibitor (Promega Corporation), and 64 units of SuperScript II reverse transcriptase (Invitrogen Corporation, Carlsbad, Calif.). Reaction tubes were inserted into a thermal cycler (Applied Biosystems), and the following thermal profile was run: 25 °C for 15 minutes,

42 °C for 60 minutes, and 99 °C for 5 minutes and then held at 4 °C until qPCR amplification. There is a higher potential for contamination during the two-step RT method; therefore, a no-template control was added after every four samples.

After the RT reaction, 6 µL of sample was analyzed by qPCR for *Microcystis mcyE* RNA transcripts and *Planktothrix mcyE* RNA transcripts under the same conditions as the qPCR DNA toxin gene assays (Stelzer and others, 2013).

Inhibition of the RT reaction was checked for every sample by seeding the first step of the RT reaction with 1 µL of armored RNA Hepatitis G virus (Asuragen, Inc., Austin, Texas) as described by Lambertini and others (2008). RNA extracts were considered inhibited, were diluted, and the RT reaction rerun with the diluted extracts if the seeded test sample was >2 C_T higher than the seeded clean matrix control.

Quantifying Cyanobacteria by qPCR and qRT-PCR

Plasmid standards for each assay were used to establish standard curves for quantification. Plasmids were constructed by insertion of PCR-amplified target sequences into a pCR4 TOPO *Escherichia coli* (*E. coli*) plasmid vector (Invitrogen, Carlsbad, Calif.). The plasmid DNA was extracted and purified from *E. coli* cells using the QuickLyse Miniprep Kit (Qiagen, Inc., Valencia, Calif.). Plasmid sequences were verified by DNA sequencing at The Ohio State University Plant-Microbe Genomics Facility. The copy number of the target was calculated using the DNA concentration measured by the PicoGreen assay (Invitrogen, Carlsbad, Calif.) and the molecular weight of the plasmid. 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). Standard-curve characteristics are listed in table 2. The amplification efficiency of the qPCR should be 90–110 percent; an efficiency of 100 percent means an exact doubling of the target DNA sequence at each cycle. The dynamic range describes the lowest and highest standards analyzed by the laboratory for each assay in copies per qPCR reaction. The coefficient of determination (R^2) is used to assess the fit of a standard curve to the plotted data points. The closer the R^2 value is to 1, the better the fit. The assay limit of quantification is the lowest concentration that can be reliably measured and is the lowest standard indicated in the dynamic range. The limit of detection was determined by taking the 95th percentile of any blank detections. If there were no detections in the blanks, the limit of detection was set to 3 copies per qPCR or qRT-PCR reaction (Bustin and others, 2009). Samples with results lower than the detection limit were reported as less than the sample reporting limit (described in the next paragraph). All sample results lower than the limit of quantification but above the limit of detection were reported as estimated values. Standard-curve characteristics for all molecular assays used in this study are listed in table 2.

Sample reporting limits are reported as "less-than values" for each sample and assay. They were sample specific because original sample volumes were sometimes different; also, a sample may have been diluted before being analyzed if it was found to be inhibited. To determine sample reporting limits, the assay's limit of detection was divided by the actual amount of sample that was analyzed.

Table 2. Standard-curve characteristics for molecular methods.

[DNA, deoxyribonucleic acid; qPCR, quantitative polymerase chain reaction; R^2, coefficient of determination; RNA, ribonucleic acid; qRT-PCR, quantitative reverse-transcription polymerase chain reaction; dynamic range and limit of detection are reported in copies per reaction]

DNA qPCR assay	Year of analysis	Dynamic range	Amplification efficiency (percent)	R^2 value	Limit of detection
Total cyanobacteria DNA gene	2011	13.9–1.39E+07	90	0.998	41
Total cyanobacteria DNA gene	2012	13.2–1.32E+06	85	0.997	34
Total *Microcystis* DNA gene	2011	16.3–1.63E+07	88	0.999	3
Total *Microcystis* DNA gene	2012	94.5–9.45E+06	91	0.999	3
Microcystis mcyE DNA toxin gene	2011	11.0–1.10E+06	98	0.998	3
Microcystis mcyE DNA toxin gene	2012	6.85–6.85E+06	93	0.999	3
Planktothrix mcyE DNA toxin gene	2011	11.8–1.18E+06	98	0.999	3
Planktothrix mcyE DNA toxin gene	2012	90.4–9.04E+06	98	0.998	3
RNA qRT-PCR assay	**Year of analysis**	**Dynamic range**	**Amplification efficiency (percent)**	**R^2 value**	**Limit of detection**
Microcystis mcyE RNA transcript	2012	2.2–2.2 x105	95	0.996	3
Planktothrix mcyE RNA transcript	2012	1.8–1.8 x105	98	0.991	3

Molecular Methods Statistical Analysis

Spearman's rank correlation coefficient analysis was used to compare molecular methods results with the results from other constituents analyzed during this study. Spearman's correlation is a nonparametric measure of the strength of the associations between two variables. Spearman's correlation is typically used instead of Pearson's correlation for results from molecular methods due to the non-normal distribution of the results. Spearman's rank correlation coefficient (rho) evaluates the correlation of the ranks of the microbe concentrations, rather than the concentrations themselves. The closer Spearman's rho is to 1 or −1, the stronger the correlation. Correlation coefficients with p-values less than or equal to 0.05 are considered statistically significant.

Water-Quality Data and Interpretations

Water-quality data include results of laboratory analyses for chemical constituents and molecular assays and data collected in the field. Field data included temperature, water transparency, DO, pH, and specific conductance. Laboratory analyses included concentrations of the nutrients, chlorophyll, major ions, and trace elements and the cyanobacteria toxin, microcystin. Other analyses included the identification and enumeration of plankton samples and molecular methods on three levels:

1. Total cyanobacteria and total *Microcystis* DNA genes by qPCR
2. Genus-specific (*Microcystis* and *Planktothrix*) DNA toxin genes by qPCR
3. Genus-specific (*Microcystis* and *Planktothrix*) RNA transcripts by qRT-PCR

Field Data

Field data consisted of data collected on-site either during a site visit or recorded by continuous data loggers. The parameters measured were temperature, transparency, DO, pH, and specific conductance of water.

Water Temperature

Water temperatures can affect the phytoplankton population because temperature affects the rate of photosynthesis; however, the minimum temperature for photosynthesis to occur varies amongst phytoplankton. The minimum temperature for photosynthesis is 5 °C for some diatoms and 15 °C for others. For many green algae and cyanobacteria, water temperatures greater than 15 °C are needed for photosynthesis to begin. As a general rule, cyanobacteria are more tolerant of high temperatures than other phytoplankton (Wetzel, 2001). Figure 3 shows the range and fluctuations of water temperatures measured during 2011–12 at about 2 ft of depth at four sites in GLSM. Throughout the study period, temperatures varied by no more than a few degrees between sites at a given depth. Water-column variations in temperature between top and bottom at a given site often were less than the measurement accuracy of the sensors. Temperatures peaked at above 30 °C around July 22 (July 30 at site B5) in 2011, around July 7 in 2012, and began dropping below 20 °C in early September in both years.

Table 3 summarizes the 7 A.M. raw-water temperatures for May–September at the CWTP intake for the years 2001–6 and 2009–12 (data for 2007–8 were unavailable). The CWTP data indicate that it is not uncommon for the morning water temperatures, which are generally the coolest of the day, to exceed 25 °C; temperatures above 30 °C can occur. In each of the years 2002, 2005, and 2010–12, the 5-day running average of water temperatures exceeded 25 °C for more than 75 days (table 3). These temperature conditions at GLSM may help select for the cyanobacteria *Planktothrix,* which tolerates a wider range of temperatures than other cyanobacteria, and may grow best in the 20–30 °C range (Halstvedt and others, 2007 citing previous research).

Water Transparency

In general, Secchi-disk readings can range from a few centimeters in turbid water to over 40 meters (m) in clear water (Wetzel, 2001). Secchi-disk data from this study and other sources are presented in table 4 for GLSM. The largest average Secchi-disk measurement, 0.93 m, was made by OEPA in April 2010; more typical values were in the range of 0.2–0.3 m. For comparison, 21 out of 27 lakes had average Secchi-disk measurements of greater than 0.5 m in a 2011 study of Ohio lakes (Oleskiewicz, 2011); lakes with measurements less than 0.5 m were classified as hypereutropic. Secchi-disk data from the 1970s and 1990s (table 4) show that the transparency of water in GLSM has been less than 0.5 m for decades indicating that hypereutrophic conditions at GLSM are not a recent occurrence.

Like true algae, cyanobacteria use photosynthesis for energy production; therefore, the available light can play a role in the community composition and in the formation of blooms. Secchi-disk measurements are indicative of the photic depth, which is the maximum depth at which photosynthesis is possible. The photic depth is around two to two-and-half times the Secchi-disk depth (*http://www.eoearth.org/article/Secchi_disk*, accessed May 1, 2013). Many cyanobacteria can thrive under low-light conditions, and *Planktothrix*, in particular, is adapted to grow at low-light levels (Halstvedt and others, 2007, citing previous research; Wetzel, 2001).

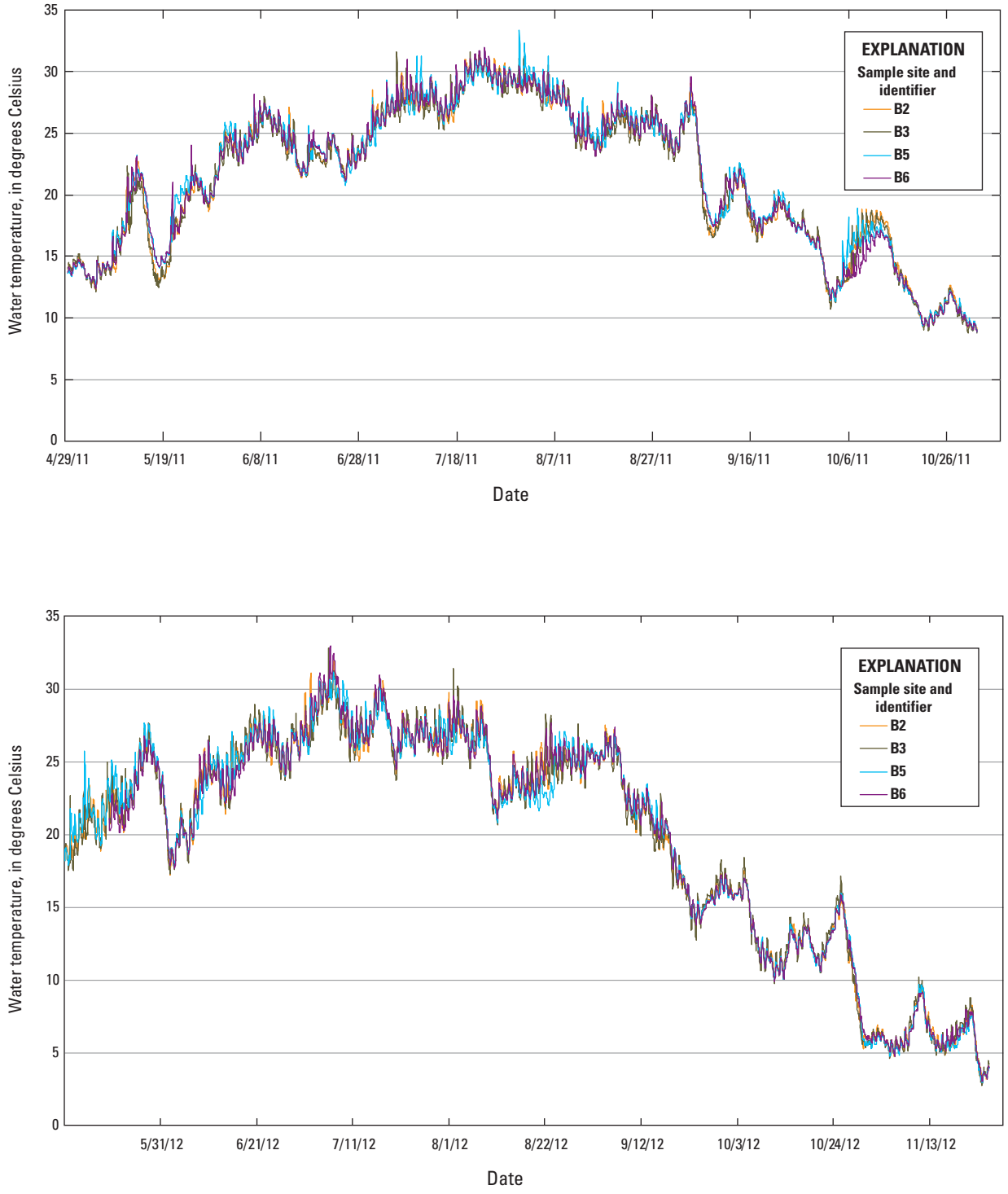

Figure 3. Water temperatures, at about 2 feet in depth in four locations, Grand Lake St. Marys, Ohio, 2011–12. [See figure 1 for location of sites B2, B3, B5, and B6.]

Table 3. Summary of 7:00 a.m. raw water temperatures during April–September at the Celina Water Treatment Plant intake, Grand Lake St. Marys, Ohio, 2001–12.

[Based on data from M. Sudman, Celina Water Treatment Plant, written commun., 2012; data for 2007−8 were unavailable]

Year	Number of days with a 5-day running average above 25 degrees Celsius	Number of days at or above 29 degrees Celsius	Peak temperature (degrees Celsius)
2001	49	8	29
2002	77	0	28
2003	38	1	29
2004	19	0	27
2005	79	7	29
2006	51	3	29
2009	24	1	29
2010	90	11	31
2011	76	25	32
2012	89	3	30

Table 4. Water-transparency depth, in meters, based on average Secchi-disk measurements in Grand Lake St. Marys, Ohio, 1973–12.

[OEPA, Ohio Environmental Protection Agency; USGS, U.S. Geological Survey; —, no data; number of measurements in parentheses]

	1973 OEPA[1]	1975 USGS[2]	1992 OEPA[1]	1999 OEPA[1]	2010 OEPA[3]	2011 OEPA[3]	2011 USGS	2012 OEPA[3]	2012 USGS
March	—	—	—	—	—	0.37 (3)	—	0.27 (3)	0.20 (6)
April	—	—	—	—	0.93 (3)	0.31 (3)	—	0.20(12)	—
May	—	0.46 (1)	—	—	0.53 (3)	0.31 (6)	0.40 (6)	0.23 (3)	0.20 (6)
June	—	—	—	—	0.40 (3)	0.18 (8)	0.26 (6)	0.30 (3)	0.20 (6)
July	—	—	—	—	0.29 (3)	0.16 (6)	—	0.19 (3)	—
Aug.	0.45 (4)	0.31 (1)	0.27 (3)	0.20 (3)	0.20 (3)	0.17 (6)	0.22 (6)	0.18 (3)	0.18 (6)
Sept.	—	—	0.28 (3)	0.16 (3)	0.18 (3)	0.18 (3)	0.20 (6)	0.16 (3)	0.17 (6)
Oct.	0.65 (4)	—	—	—	0.19 (3)	—	0.21 (6)	0.23 (3)	0.15 (6)

[1] Ohio Environmental Protection Agency (2010b).

[2] Tobin and Youger (1977).

[3] D. Glomski, Ohio Environmental Protection Agency, written commun., 2012.

Dissolved Oxygen

Several factors affect DO concentration in water including temperature, air pressure, organic matter, salinity, and rates of photosynthesis. Oxygen is more soluble in colder water. For example, in pure water at standard pressure at 0 °C, the DO will be 14.62 mg/L at 100 percent saturation. At 30 °C, the DO will be 7.56 mg/L at 100 percent saturation. Diurnal variations in DO concentrations occur due to biological activities and fluctuations in temperature. For example, oxygen is consumed overnight by respiration and DO concentrations drop, whereas during the day, photosynthesis produces oxygen and DO concentrations rise. In eutrophic lakes, diurnal variations in DO concentrations tend to be larger than in non-eutrophic lakes, with diurnal variations as large as 10 mg/L or more compared to around 2 mg/L for non-eutrophic lakes. The lowest DO concentration generally occurs just before sunrise and the highest concentration in late afternoon. High DO concentrations are indicative of oxygen production by rapid algal growth; *Planktothrix* is often a significant contributor to this scenario (Wetzel, 2001).

The DO profile measurements determined during the sampling trips are shown in figures 4*A* and *B*. On a number of occasions, the DO concentrations were alike at any given site, throughout the profile, (e.g., October 2011); however, there were noticeable decreases in DO concentrations with depth, particularly in the warmer months (e.g., August 2011). On a few sampling trips, there were noticeably wider ranges of surficial DO concentrations among the sites such as in May, August, and September 2012. These differences in surface DO concentrations on the same day can be explained by increasing DO concentrations in the afternoons owing to photosynthetic activity. For example, in September 2012, sites B3, B5, and B6—with the higher DO concentrations—were measured after noon. June 2011 is another example: site B2—with the lowest DO concentration—was measured at 0927; site B6—with the highest DO concentration—was measured at 1550. An exception to this pattern of increasing DO concentrations after noon occurred at site B2 during the May 2012 sample, in which the highest surface DO concentration that day was measured at 1018.

Data from the continuous DO measurements at site B1 provide additional examples of the low DO concentrations at the bottom and diurnal variations in DO concentrations (table 5, fig. 5). The occurrence of low DO readings at the bottom of the lake is important to note because oxidation-reduction conditions at the sediment-water interface affect mineral solubility as well as sorption and biologic activity. Low DO conditions (DO less than 1 mg/L) near the sediment-water interface (like those that occurred in July 2011) can result in the release of phosphorus and iron from the sediments back into the water column (Wetzel, 2001).

pH

Generally, unpolluted river water will have a pH ranging from 6.5 to 8.5. The pH of an aqueous solution is affected by numerous interrelated chemical reactions; in natural waters, one of the most important of these reactions is that of dissolved carbon dioxide and water. During photosynthesis, the consumption of dissolved carbon dioxide lowers the concentration of carbonic acid in the water, which can cause pH levels to increase to 9.0 or greater (Hem, 1985). The pH levels measured at GLSM were usually above 8.0 and frequently equal to or greater than 9.0 (figs. 6*A* and *B*).

Table 5 shows the extremes and median values for the continuous pH measurements in the upper and lower sondes at site B1. The maximum pH values on the upper and lower sondes were 9.9 and 9.8, respectively; the minimum values were 6.8 and 7.2, respectively. In general, pH data collected during the sampling trips were within the same range as that recorded by the sondes. The pH data measured in the depth profiles during sample collection showed little variation with depth at any given site, with 51 of the 66 profiles having pH ranges of less than 0.5 units (fig. 6; appendix 1).

Specific Conductance

Specific conductance is a measure of the ability of a solution to conduct electrical current and is indicative of the concentrations of ions and therefore is an indirect measure of the concentration of dissolved solids. In lakes and rivers, the specific conductance is closely linked to concentrations of eight major ions: chloride, sulfate, carbonate, bicarbonate, potassium, sodium, calcium, and magnesium.

Table 5 shows the extremes and median values for the continuous specific conductance measurements in the upper and lower sondes at site B1 for 2011–12. The maximum value of specific conductance on the upper sonde was 570 microsiemens per centimeter (µS/cm) and 508 µS/cm on the lower sonde; the minimum values were 225 and 346 µS/cm, respectively. Specific conductance measurements recorded during the sampling trips ranged from around 370 to 485 µS/cm and typically showed little variation with depth at a given site (figs. 7*A* and *B*).

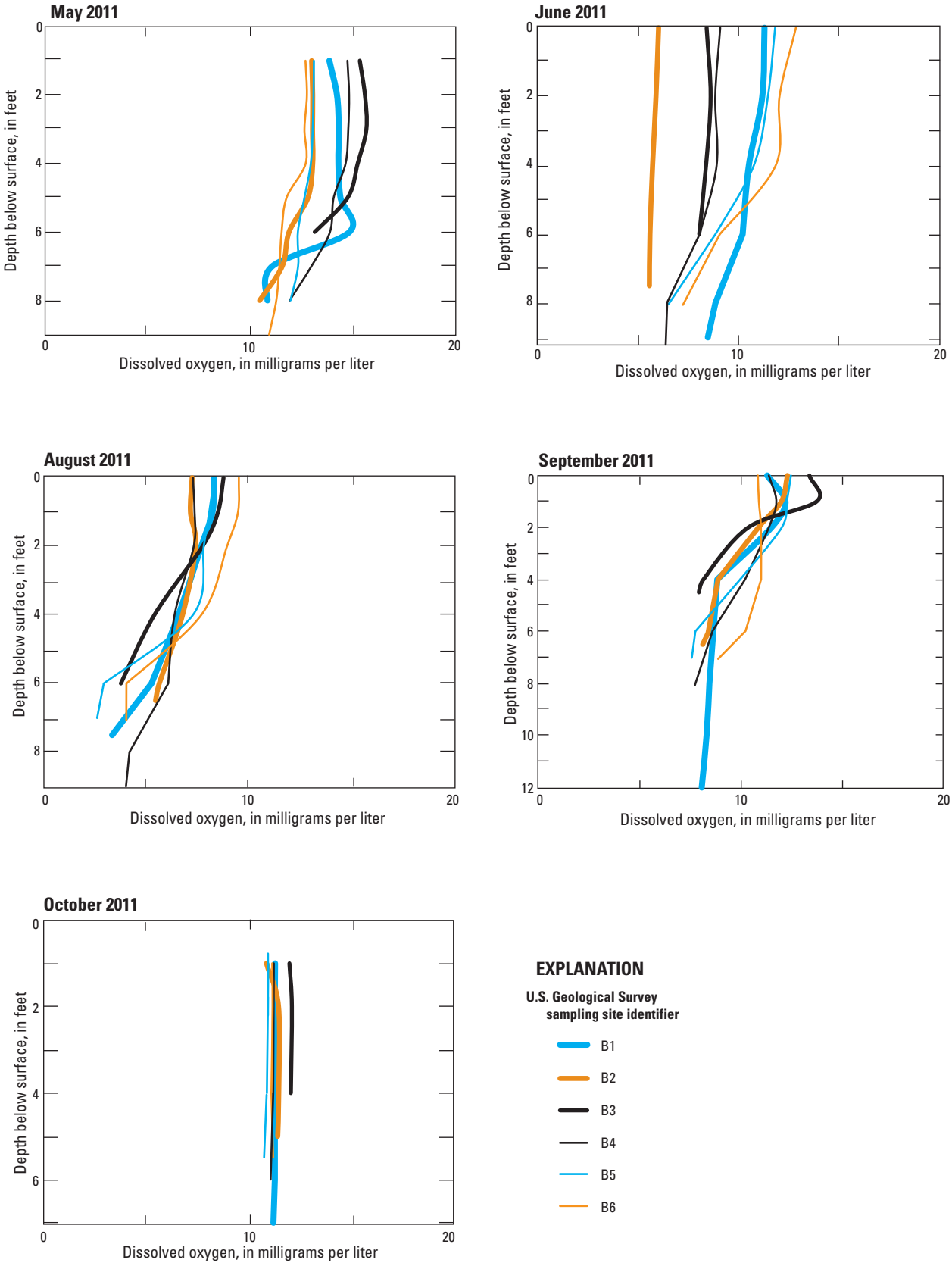

Figure 4*A*. Depth profiles of dissolved oxygen concentrations at sites B1–B6 in Grand Lake St. Marys, Ohio, 2011. [Scales of the y-axes differ between graphs because of differing depths between sampling dates; scales of the x-axes differ between 2011 and 2012 because of the wider range of concentrations in 2011.]

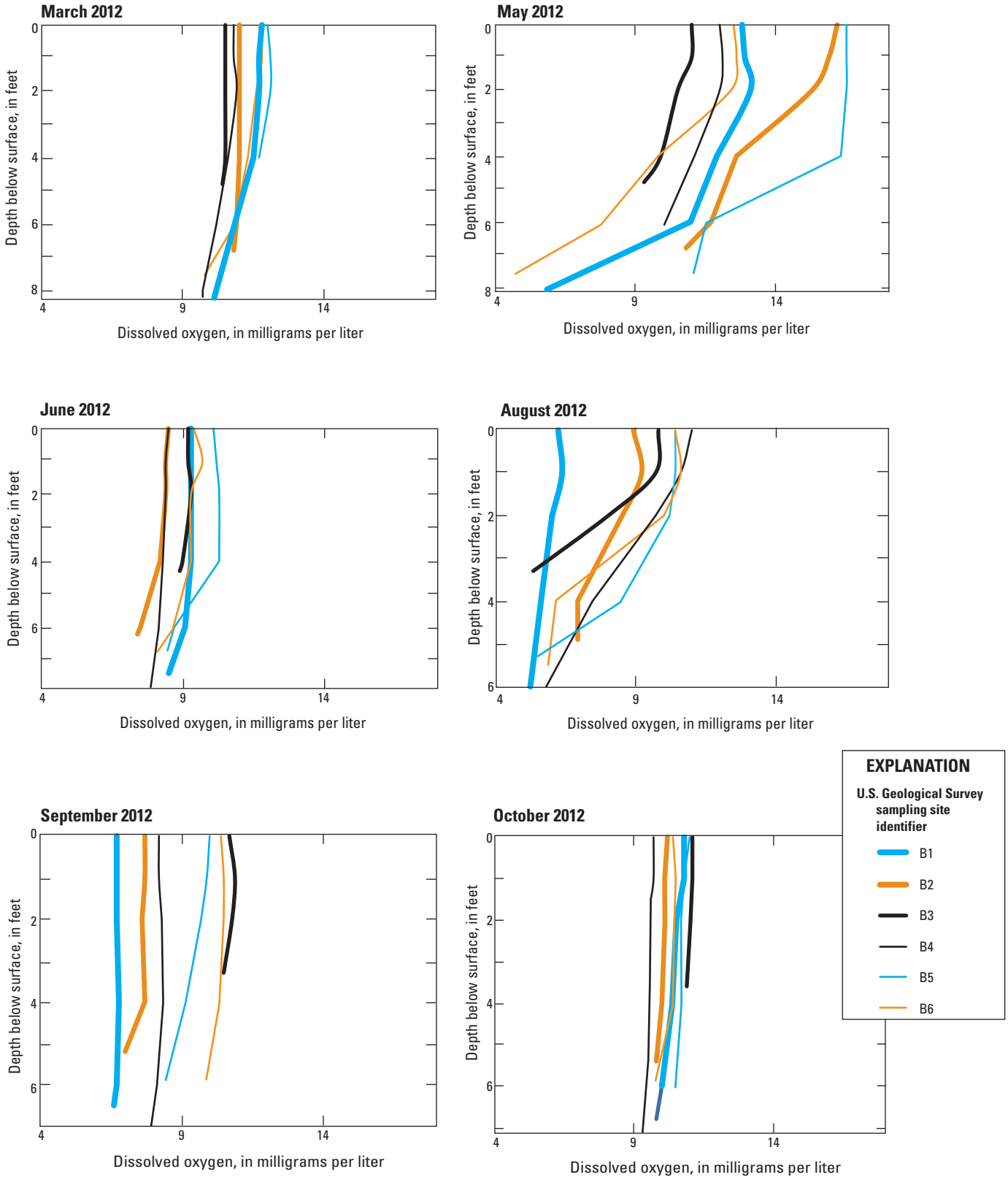

Figure 4*B*. Depth profiles of dissolved oxygen concentrations at sites B1–B6 in Grand Lake St. Marys, Ohio, 2012. [Scales of the y-axes differ between graphs because of differing depths between sampling dates; scales of the x-axes differ between 2011 and 2012 because of the wider range of concentrations in 2011.]

Table 5. Maximum, minimum, and mean values for continuous measurements of selected water-quality parameters at site B1 in Grand Lake St. Marys, Ohio, 2011–12.

[Max, maximum; Min, minimum; µS/cm at 25°C, microsiemens per centimeter at 25 degrees Celsius; mg/L, milligrams per liter; Upper monitor location was about 2 feet below lake surface; lower monitor location generally was 0.75 feet above lake bottom]

	Specific conductance (µs/cm at 25°C)			pH (standard units)		Dissolved oxygen (mg/L)		
	Max	Min	Mean	Max	Min	Max	Min	Mean
Upper	570	225	400	9.9	6.8	20.0	0.5	8.9
Lower	459	346	399	9.8	7.6	14.1	0.1	5.9

[1]Data are from April 29–September 30, 2011.

	Specific conductance (µs/cm at 25°C)			pH (standard units)		Dissolved oxygen (mg/L)		
	Max	Min	Mean	Max	Min	Max	Min	Mean
Upper	527	382	433	9.8	7.4	20.0	0.6	9.7
Lower	508	385	435	9.4	7.2	13.6	0.1	8.1

[1]Upper unit data for October 1–November 1, 2011, and March 29–September 30, 2012.

[2]Lower unit data for October 1–November 1, 2011, and March 29–June 29, 2012.

Grand Lake St. Marys, Ohio, 2011.

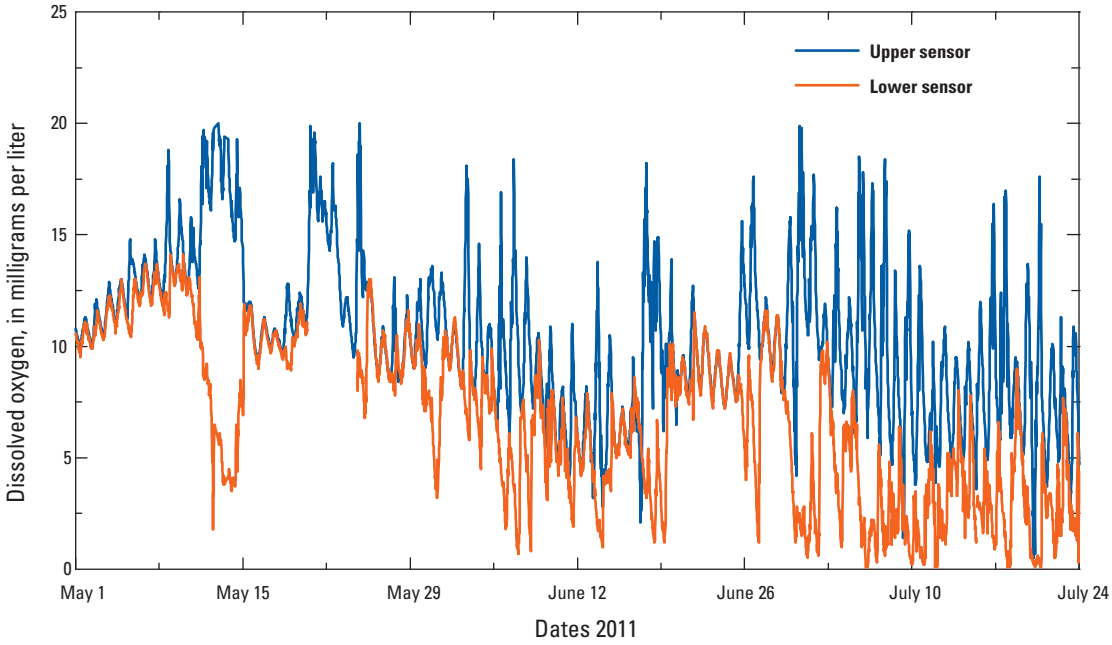

Figure 5. Examples of dissolved oxygen readings from continuous monitors at site B1, Grand Lake St. Marys, Ohio, 2011. [See figure 1 for location of site B1.]

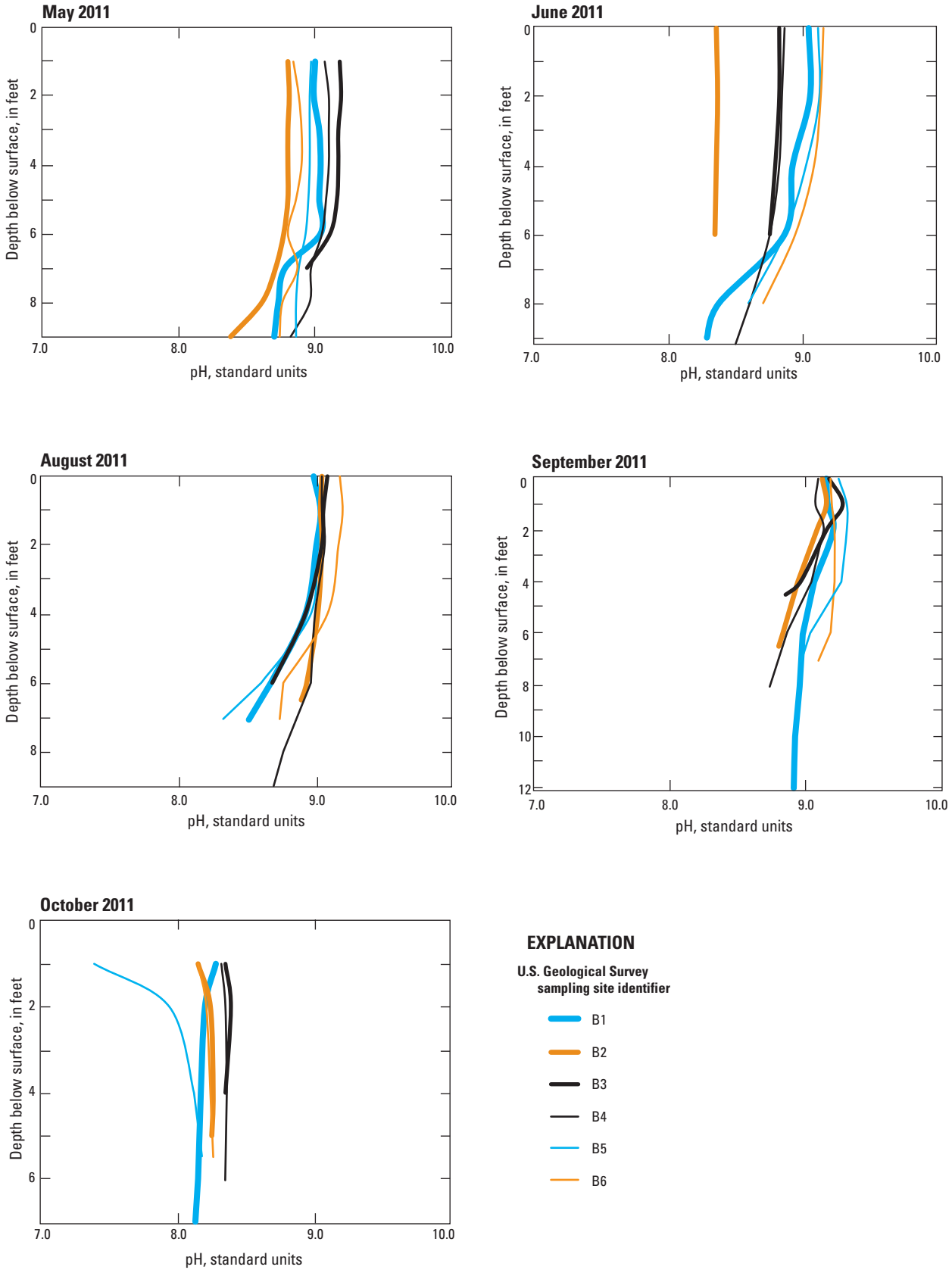

Figure 6*A*. Profiles of pH measurements at sites B1–B6 in Grand Lake St. Marys, Ohio, 2011. [Scales of the y-axes differ between graphs because of differing depths between sampling dates. See figure 1 for location of sites B1–B6.]

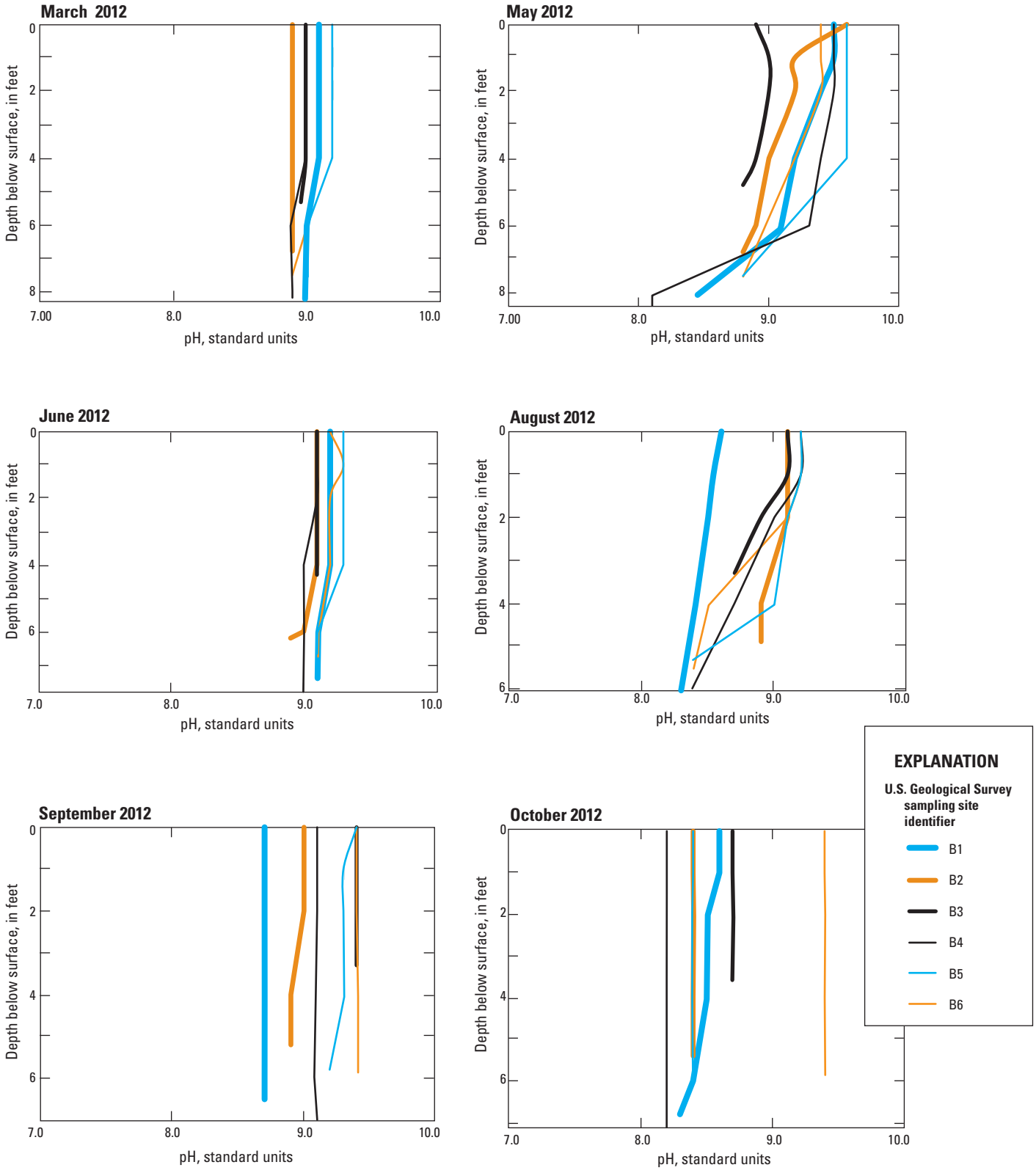

Figure 6*B*. Profiles of pH measurements at sites B1–B6 in Grand Lake St. Marys, Ohio, 2012. [Scales of the y-axes differ between graphs because of differing depths between sampling dates. See figure 1 for location of sites B1–B6.]

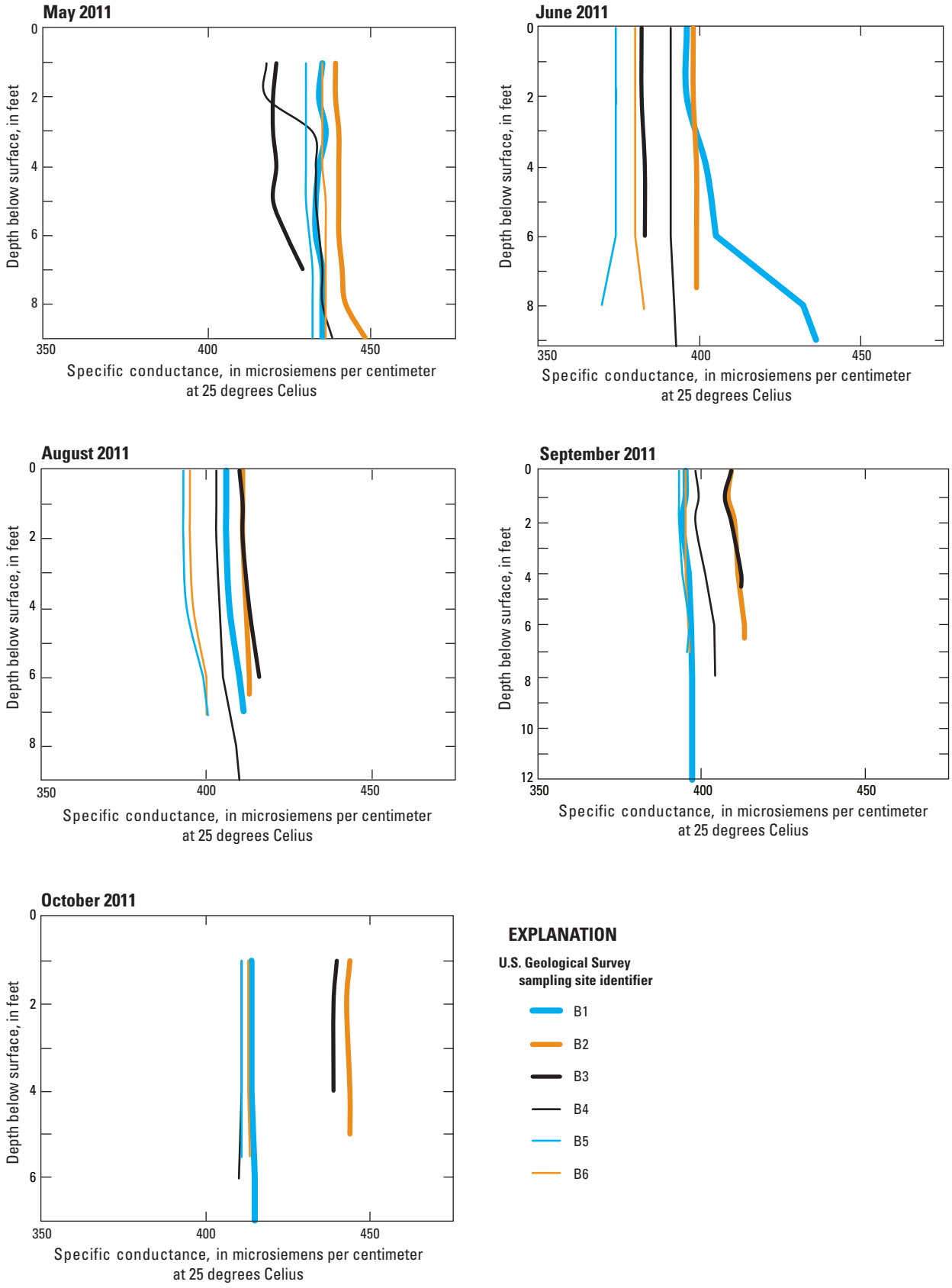

Figure 7*A*. Profiles of specific conductance measurements at sites B1–B6 in Grand Lake St. Marys, Ohio, 2011. [Scales of the y-axes differ between graphs because of differing depths between sampling dates. See figure 1 for location of sites B1–B6.]

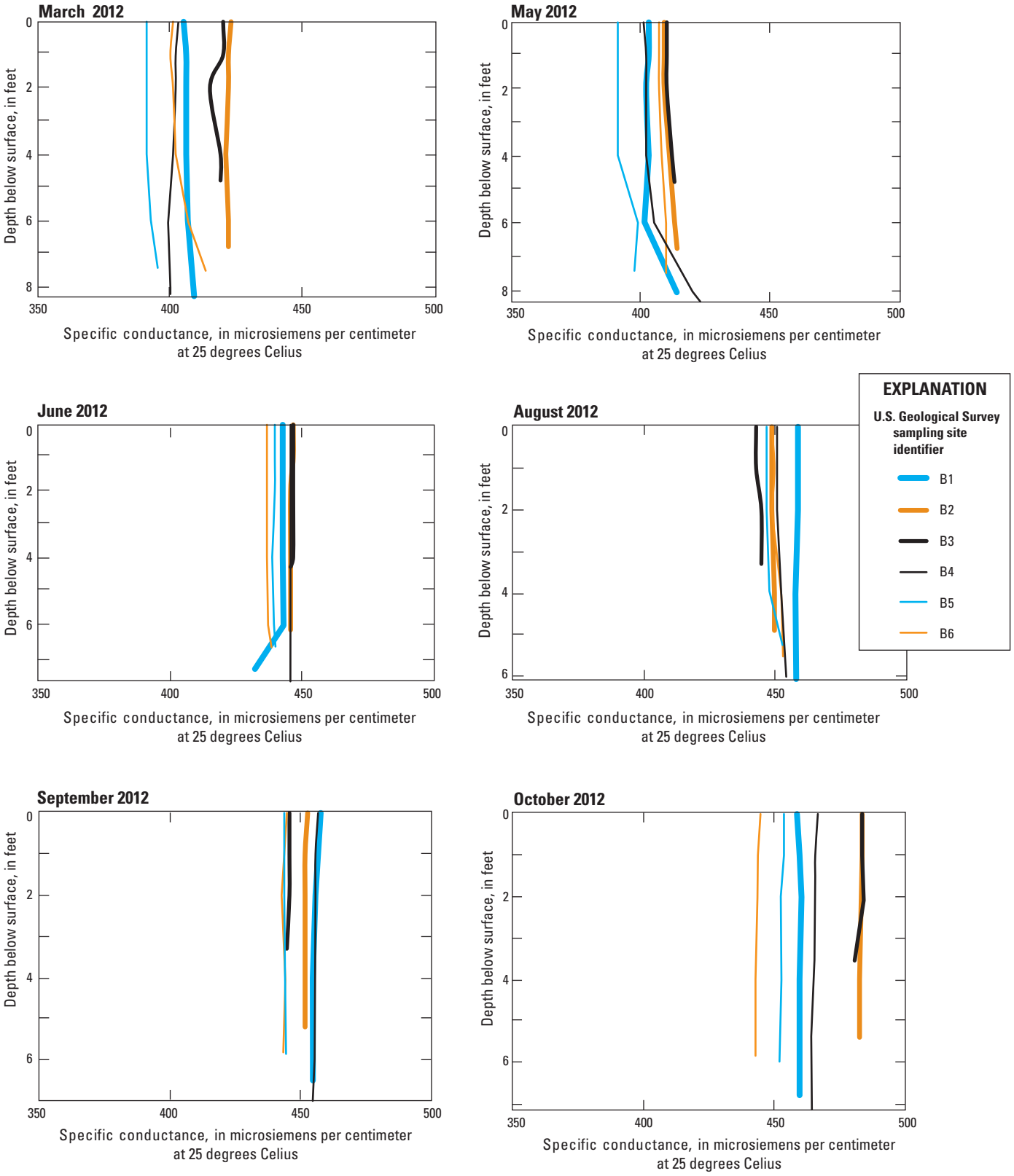

Figure 7*B*. Profiles of specific conductance measurements at sites B1–B6 in Grand Lake St. Marys, Ohio, 2012. [Scales of the y-axes differ between graphs because of differing depths between sampling dates. See figure 1 for location of sites B1–B6.]

Chemical and Biological Water Quality

Nutrients

Nitrogen and phosphorus are nutrients affecting the growth of organisms. However, phosphorus, particularly important to metabolism, is the least abundant nutrient and as such, is usually the limiting nutrient in a biological system. Wetzel (2001) has written chapters on the importance and cycles of nutrients in freshwater and is the source for much of the background material in the next few paragraphs.

The dominant forms of nitrogen in water include dissolved molecular nitrogen, ammonia (NH_4^+), nitrite (NO_2^-), and nitrate (NO_3^-). Dissolved molecular nitrogen is often in equilibrium with the atmosphere. Ammonia concentrations are generally low as it is readily assimilated by plants and oxidized by bacteria to nitrite and nitrate. Nitrite concentrations are generally low (less than 100 µg/L) as well, as it is readily oxidized. Nitrate is the common form of inorganic nitrogen in freshwaters (Wetzel, 2001).

Phosphorus can be found in several forms in water but orthophosphate (PO_4^{-3}), also called soluble reactive phosphorus and inorganic phosphorus, is the only form that is bioavailable. Orthophosphate is reactive with many cations and, particularly in oxidizing conditions will form relatively insoluble compounds that will precipitate out of solution. Sorption onto clays and inorganic colloids also can remove phosphorus from solution. In reducing (low oxygen; less than 1 mg/L) conditions, phosphorus that is bound to particles can be released back into water; turbulence of the sediments can accelerate the release of phosphorus (Wetzel, 2001). The movement of phosphorus from the sediments to the water column in a lake setting is often referred to as "internal loading" or recycling of phosphorus.

Table 6 is a chronological summary of nutrient analyses at GLSM from 1973 to 2012, including the six USGS sampling sites in GLSM for 2011–12; the full USGS dataset is shown in appendix 1. Dissolved nitrate concentrations ranged from less than 0.19 to 3.23 mg/L. The highest dissolved nitrate concentrations were observed in the 6 samples from May 2011; concentrations in the other 60 samples for 2011–12 were less than 1 mg/L. Total nitrogen concentrations ranged from 1.87 to 5.42 mg/L. The range of total nitrogen concentrations at GLSM is within the range (0.39 to 6.1 mg/L) that has been used to classify lakes as eutrophic (*http://www.nalms.org/media.acux/e176c3e1-6e07-4439-96ce-3cf7905a33e2*, accessed July 21, 2014). After the sampling period of this study, the NWQL identified a possible negative bias in the total nitrogen analyses when sediment is present in the sample (D. Myers, U.S. Geological Survey, written commun., 2012).

Table 6. Chronological summary of selected nutrient analyses in Grand Lake St. Marys, Ohio, for sites B1–B6 during 2011–12, and other sites during 1973–2012.

[N, nitrogen; mg/L, milligrams per liter; P, phosphorus; —, no data or data not presented; n, number of samples; <, less than]

Summary statistics	Ammonia, dissolved, as N (mg/L	Nitrite, dissolved as N (mg/L)	Nitrate, dissolved as N (mg/L)	Total nitrogen, dissolved (mg/L)	Total nitrogen[1] (mg/L)	Orthophosphate, dissolved, as P (mg/L)	Total phosphorus, dissolved, as P (mg/L)	Total phosphorus, as P (mg/L)
				[2]1973				
Maximum	—	—	—	—	2.36	—	—	0.48
Minimum	—	—	—	—	1.25	—	—	0.08
Median	—	—	—	—	1.95	—	—	0.13
n	—	—	—	—	8	—	—	8
				[3]1975				
Maximum	—	0.01	0.07	—	—	0.01	—	0.19
Minimum	—	0.01	0.00	—	—	0.01	—	0.13
Median	—	0.01	0.00	—	—	0.01	—	0.16
n	—	4	4	—	—	4	—	4

Table 6. Chronological summary of selected nutrient analyses in Grand Lake St. Marys, Ohio, for sites B1–B6 during 2011–12, and other sites during 1973–2012.—Continued

[N, nitrogen; mg/L, milligrams per liter; P, phosphorus; —, no data or data not presented; n, number of samples; <, less than]

Summary statistics	Ammonia, dissolved, as N (mg/L	Nitrite, dissolved as N (mg/L)	Nitrate, dissolved as N (mg/L)	Total nitrogen, dissolved (mg/L)	Total nitrogen[1] (mg/L)	Orthophosphate, dissolved, as P (mg/L)	Total phosphorus, dissolved, as P (mg/L)	Total phosphorus, as P (mg/L)
[4]1992								
Maximum	—	—	—	—	1.86	—	—	0.2
Minimum	—	—	—	—	1.05	—	—	0.06
Median	—	—	—	—	1.65	—	—	0.12
n	—	—	—	—	6	—	—	6
[4]1999								
Maximum	—	—	—	—	4.15	—	—	0.25
Minimum	—	—	—	—	2.44	—	—	0.19
Median	—	—	—	—	2.61	—	—	0.20
n	—	—	—	—	6	—	—	6
U.S. Geological Survey sites, 2011								
Maximum	0.395	0.108	3.23	1.48	3.97	0.067	0.1	0.43
Minimum	<0.010	<0.001	<0.019	0.72	2.29	<0.004	<0.02	0.12
Median	<0.010	<0.001	<0.020	0.815	3.38	<0.004	0.02	0.28
n	30	30	30	24	24	30	30	30
[5]Ohio Environmental Protection Agency sites, 2011								
Maximum	—	—	—	—	3.76	0.080	—	0.31
Minimum	—	—	—	—	1.53	<0.010	—	<0.01
Median	—	—	—	—	3.07	<0.010	—	0.12
n	—	—	—	—	5	50	—	50
U.S. Geological Survey sites, 2012								
Maximum	0.014	0.04	0.58	1.25	5.42	0.008	0.03	0.36
Minimum	< 0.010	< 0.001	< 0.040	0.48	1.87	<0.004	<0.02	0.13
Median	< 0.010	< 0.001	< 0.040	0.78	4.04	<0.004	<0.02	0.28
n	36	36	36	36	36	36	36	36
[5]Ohio Environmental Protection Agency sites, 2012								
Maximum	—	—	—	—	—	<0.010	—	0.26
Minimum	—	—	—	—	—	<0.010	—	0.08
Median	—	—	—	—	—	<0.010	—	0.16
n	—	—	—	—	—	22	—	33

[1] Total nitrogen analyses could be negatively biased owing to sediment in the sample (D. Myers, U.S. Geological Survey, written commun., 2012).

[2] Ohio Environmental Protection Agency (2010b).

[3] Tobin and Youger (1977).

[4] Ohio Environmental Protection Agency (2010).

[5] Data in appendix 3 (D. Glomski, Ohio Environmental Protection Agency, written commun., 2011, 2012).

Unfortunately, the degree of the bias in total nitrogen analyses could not be determined; however, this bias could mean that the total nitrogen concentrations reported in this study were lower than actual concentrations. Orthophosphate (as P) concentrations ranged from less than 0.004 to 0.067 mg/L; however, only 13 of the 66 samples had concentrations greater than 0.004 and 12 of those were in the May, June, and August 2011 samples. Total phosphorus (as P) concentrations ranged from 0.12 to .43 mg/L and were similar in both years. The higher orthophosphate concentrations in 2011 may be due to the higher runoff in 2011 as compared with the drought conditions in 2012 (table 1, fig. 2).

Some studies have shown that a low total nitrogen to total phosphorus ratio (TN:TP) is associated with cyanobacterial dominance, with a commonly listed threshold for cyanobacterial dominance as TN:TP less than 29 (Schindler, 1977; Smith, 1983; Dokulil and Teubner, 2000; Havens and others, 2003). In this study, the ratios of TN:TP ranged from 7 to 23, with a median of 14, which is below the threshold associated with cyanobacterial dominance.

Plots of orthophosphate, total phosphorus, and total nitrogen concentrations by sampling location (figs. 8, 9, and 10) show that sites B2 and B3 frequently had the highest concentrations on any sampling trip. The location of site B2 (fig. 1), in the southwestern portion of the lake, may be more affected by the discharge of the two tributaries into this bay-like area of the lake than sites B1 and B4–B6, which are in more open-water areas. The higher concentration at site B3 may be owing to the flow of water from the western portion of the lake through the cut south of Safety Island. Total phosphorus concentrations had very similar patterns over time among the sampling sites (fig. 9*B*), whereas total nitrogen concentrations had somewhat less consistent patterns (fig. 10*B*).

Table 6 summarizes data from OEPA at three sites on GLSM for orthophosphate, total nitrogen, and total phosphorus concentrations during 2011–12. The total nitrogen and phosphorus concentrations for the USGS data are comparable to the OEPA data for both years. Selected nutrient data for 1973–99 from several other sources also is summarized in table 6.

Sampling sites and cleaning.

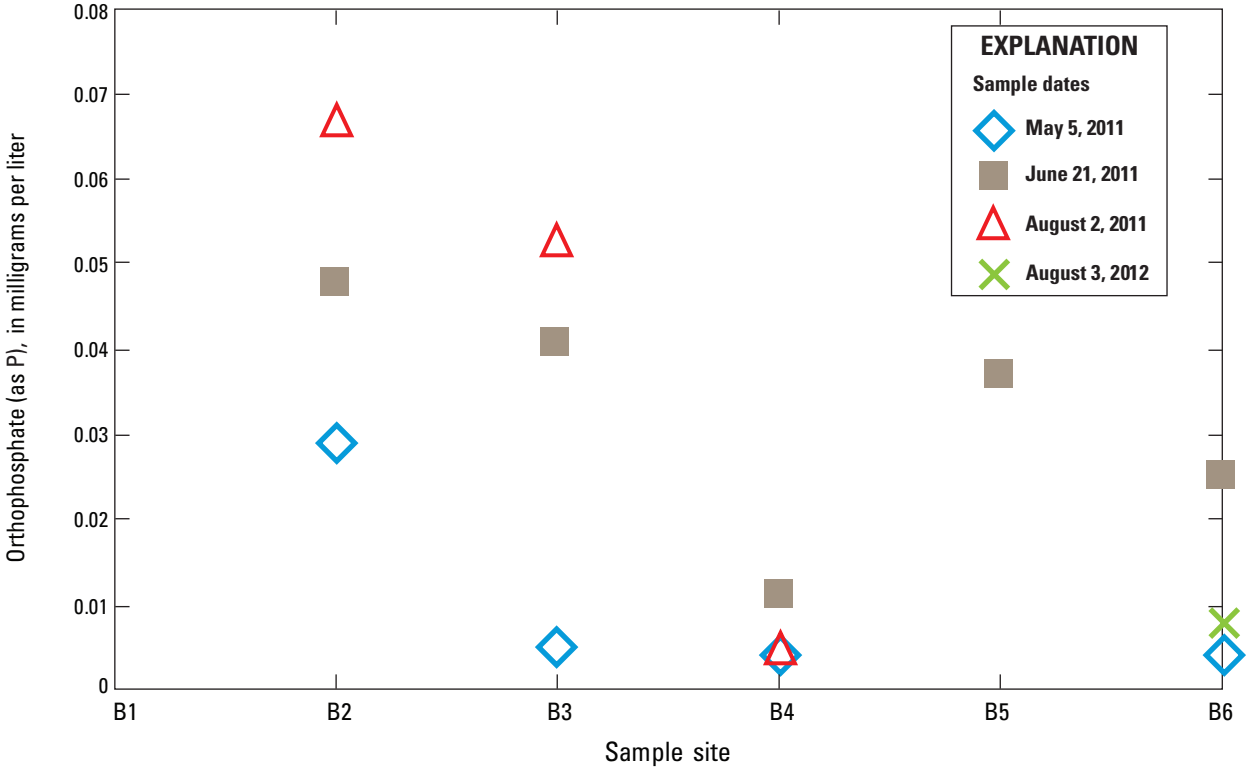

Figure 8. Orthophosphate concentrations above the reporting limit in Grand Lake St. Marys, Ohio, by sampling location and date. [See figure 1 for location of sites B1–B6.]

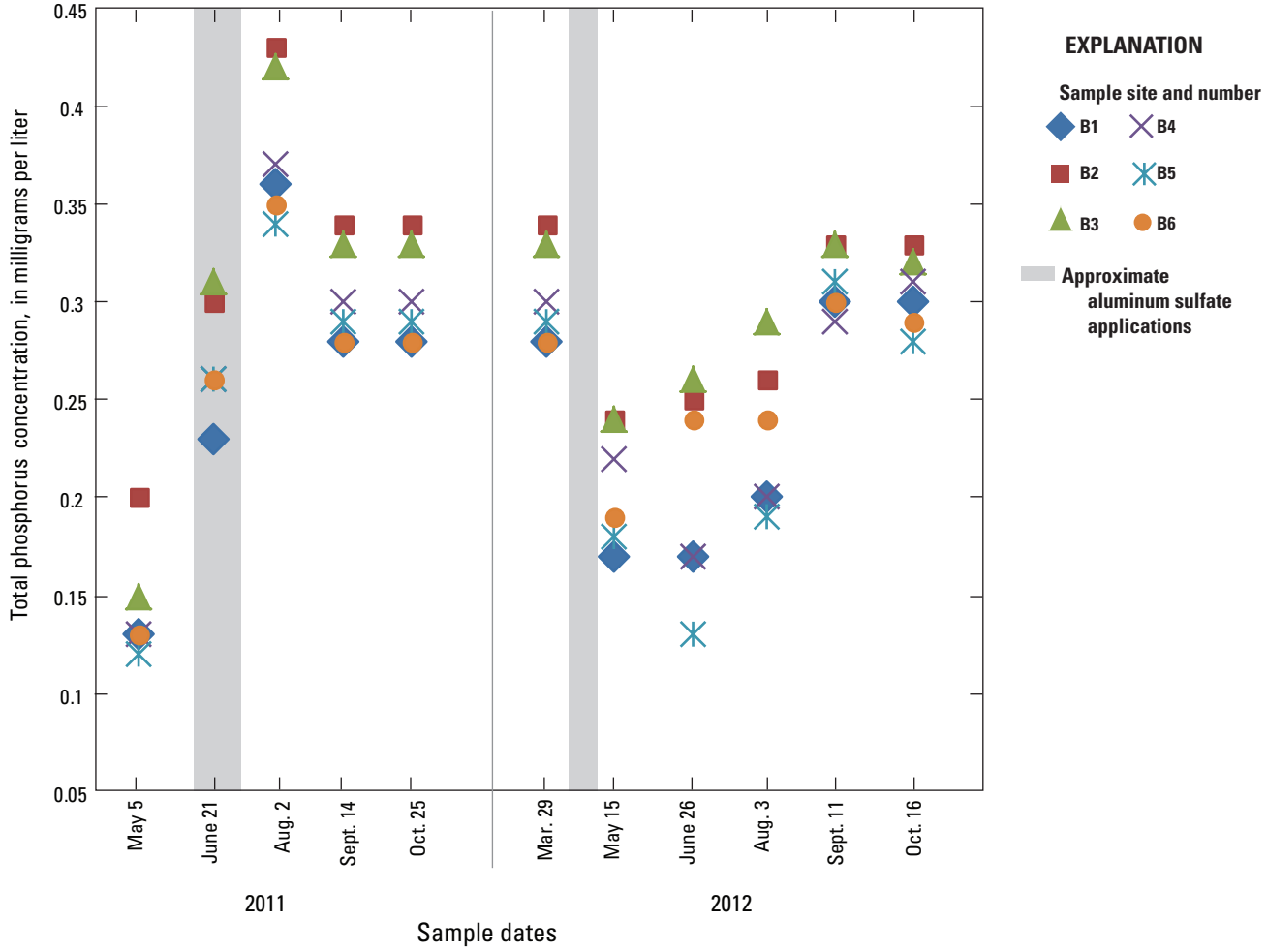

Figure 9*A*. Total phosphorus concentrations in Grand Lake St. Marys, Ohio, by sampling location and date.
[See figure 1 for location of sites B1–B6.]

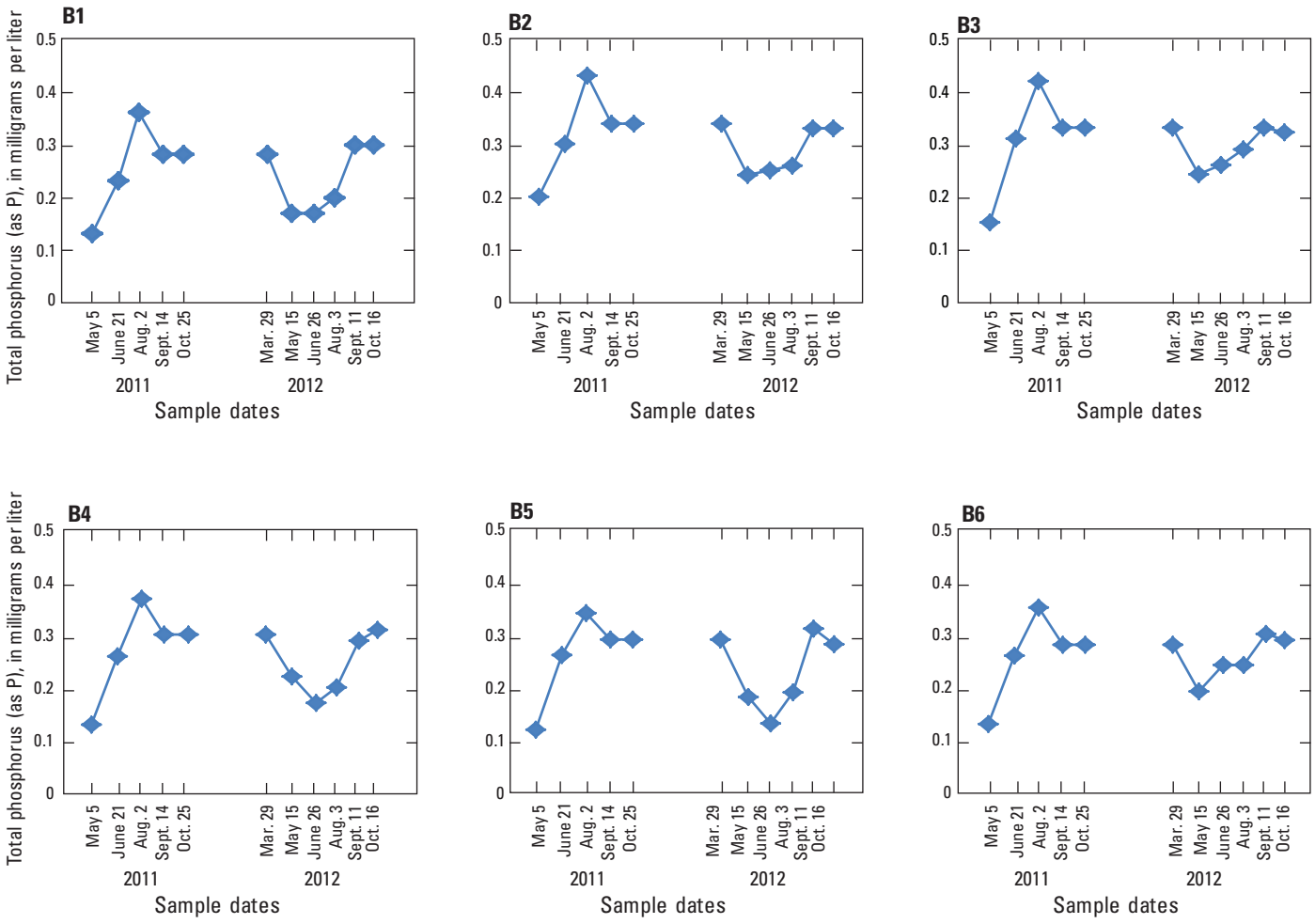

Figure 9*B*. Total phosphorus (as P) in Grand Lake St. Marys, Ohio, by sampling location and date. [See figure 1 for location of sites B1–B6.]

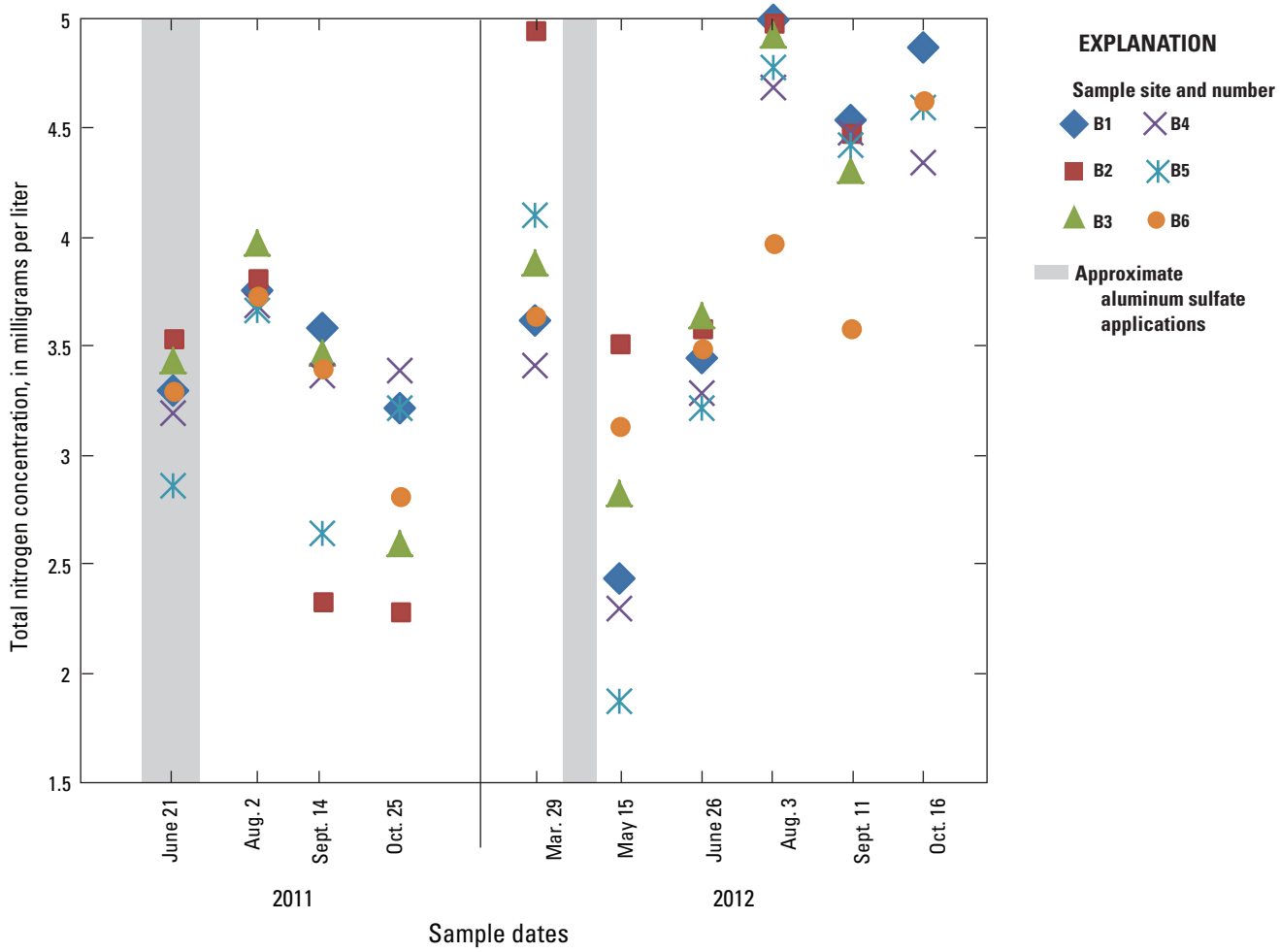

Figure 10*A*. Total nitrogen concentrations in Grand Lake St. Marys, Ohio, by sampling location and date. [See figure 1 for location of sites B1–B6.]

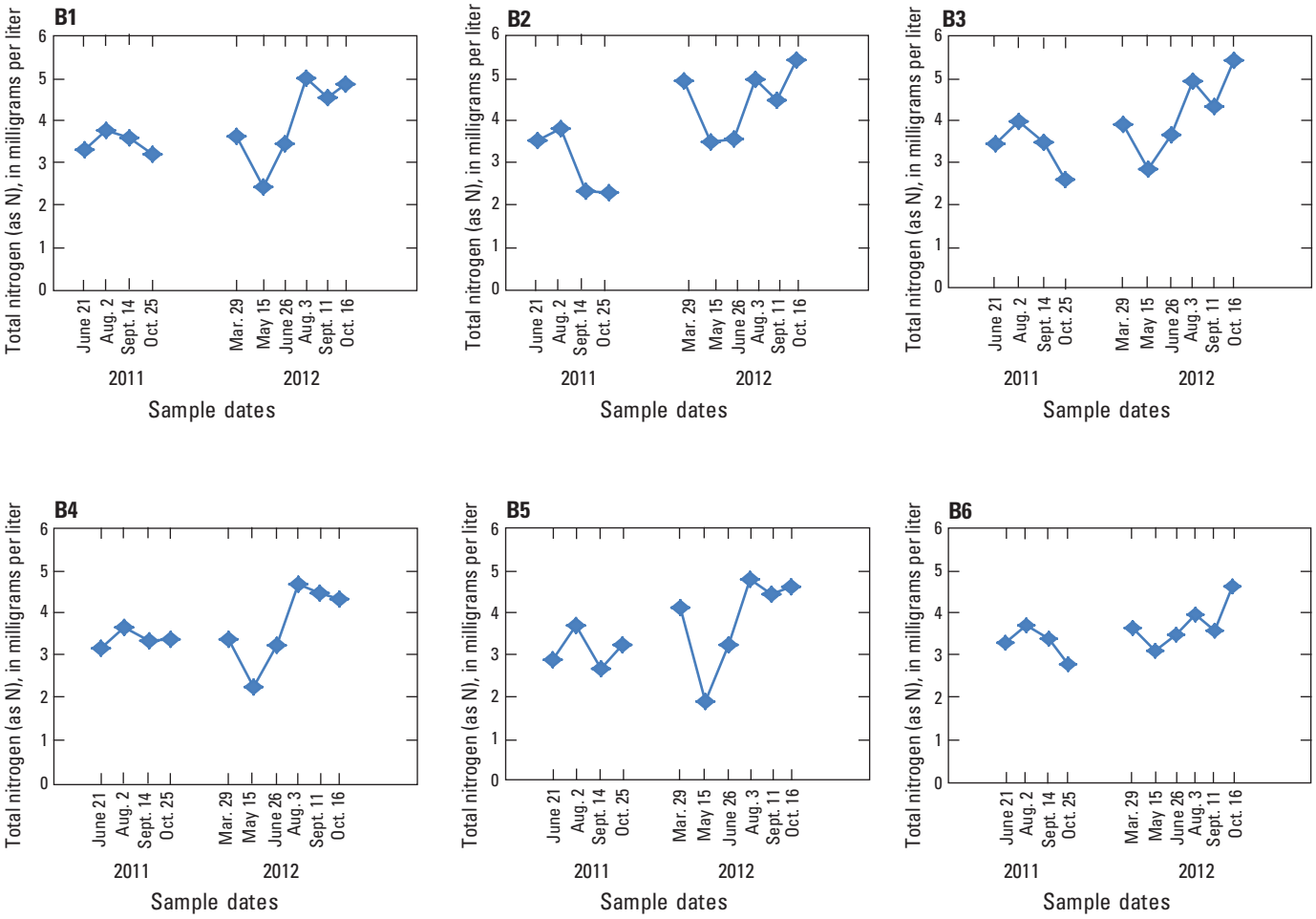

Figure 10*B*. Total nitrogen in Grand Lake St. Marys, Ohio, by sampling location and date. [See figure 1 for location of sites B1–B6.]

Chlorophyll

Chlorophyll is a green pigment used in the photosynthesis process to help convert the energy in sunlight into chemical energy (sugars) for growth. Concentrations of chlorophyll are often used as an indicator of the algal abundance in a lake. When chlorophyll concentrations are greater than 20 µg/L in a north temperate lake, a possible cause may be phytoplanktonic communities dominated by cyanobacteria; concentrations greater than 56 µg/L are indicative of a hypereutrophic condition (Carlson and Simpson, 1996).

All of the 176 water samples collected by the USGS and the OEPA during 2011–12 had chlorophyll concentrations greater than 56 µg/L; the monthly median concentrations in 13 of the 15 months sampled were greater than 200 µg/L (table 7). Samples collected by the OEPA during July–October 2010 also had high chlorophyll concentrations; however, the concentrations earlier in 2010 were considerably lower,

Table 7. Monthly maximum, minimum, and median concentrations of chlorophyll from samples collected by the U.S. Geological Survey at sites B1–B6 during 2011–12, and by the Ohio Environmental Protection Agency from multiple locations during 1973–2012, in Grand Lake St. Marys, Ohio.

[Concentrations in micrograms per liter;—, no data; n, number of samples]

	March	April	May	June	July	August	September	October
				[1]1973				
Maximum	—	88.4	—	—	—	—	—	63.2
Minimum	—	69.7	—	—	—	—	—	45.3
Median	—	85.2	—	—	—	—	—	54.6
n	—	4	—	—	—	—	—	4
				[1]1992				
Maximum	—	—	—	—	—	126.2	127.8	—
Minimum	—	—	—	—	—	115.4	119.1	—
Median	—	—	—	—	—	119.6	124.1	—
n	—	—	—	—	—	3	3	—
				[1]1999				
Maximum	—	—	—	—	—	277.3	266.6	—
Minimum	—	—	—	—	—	238.3	241.1	—
Median	—	—	—	—	—	246.5	—	—
n	—	—	—	—	—	3	2	—
				[1]2010				
Maximum	—	41.3	42.3	77.7	323	263	314	293
Minimum	—	2.7	5.6	53.4	140	241	252	258
Median	—	4.4	17.7	67.5	206	243	276	260
n	—	3	3	3	3	3	3	3
				[2]2011				
Maximum	—	126	330	370	290	323	333	276
Minimum	—	114	95	165	137	201	248	170
Median	—	115	241	247	235	271	285	227
n	—	3	8	30	14	17	6	10
				[2]2012				
Maximum	270	332	302	283	392	413	380	368
Minimum	79	117	98	93	260	236	234	159
Median	219	212	156	260	297	331	336	289
n	11	20	11	10	5	11	11	9

[1] Ohio Environmental Protection Agency (2010b).

[2] Ohio Environmental Protection Agency data are shown in appendix 3 (D. Glomski, Ohio Environmental Protection Agency, written commun., 2011, 2012).

less than 80 µg/L (table 7). The chlorophyll concentrations in samples from 1973 were lower than the samples from 2011–12 (table 7); however, it should be noted that these samples were collected in the spring and fall and might not be representative of the concentrations in the summer of 1973. It is interesting to note that the lowest chlorophyll concentrations were in the spring of 2010 (table 7). During that spring, there were anecdotal reports that the water in the lake was the clearest in memory; this was followed (in June) by a bloom of *Aphanizomenon gracile*. Overall, the chlorophyll data indicate that the hypereutrophic condition of GLSM is not a recent occurrence.

Major Ions and Trace Elements

During the May, August, and October sampling trips in both years, samples were collected at site B1 and analyzed for major ions and trace elements. The analytical results are presented in appendix 1; table 8 presents results for selected parameters. Although many of the constituents that were sampled for are nutrients or micronutrients, the absolute requirements of metabolic processes for each are not always clearly known. In addition, some elements are chemically similar and can potentially substitute for each other. Wetzel (2001) discusses these topics in much greater detail and was the source for much of the discussion that follows.

In most cases, it can be expected that the concentrations of major cations in surface waters will be in the following proportions (Wetzel, 2001):

Calcium > Magnesium ≥ Sodium > Potassium

Data from GLSM and other Ohio lakes from the 1970s were consistent with this relation (table 8). The 2011–12 data from GLSM follows this relation with respect to calcium, magnesium, and potassium. However, sodium concentrations are greater than magnesium in all six samples and greater than calcium in the three samples from 2012 (table 8).

Calcium is the dominate cation in natural waters. Calcium concentrations in surface waters are commonly around 15–20 mg/L (Hem, 1985; Wetzel, 2001). In GLSM, the calcium concentrations were somewhat higher than typical surface-water averages, though comparable or less than other northwestern Ohio lakes (table 8). These higher-than-typical calcium concentrations could be due to the contribution of groundwater from the carbonate aquifer as well as the high levels of photosynthesis in the lake. As photosynthesis increases pH, calcium concentrations can become supersaturated (Hem, 1985). Calcium is a nutrient for plants and while its requirement for algae has not been proven, it is likely a necessary micronutrient (Wetzel, 2001).

Sodium is required for photosynthesis, bicarbonate transport, cellular pH regulation, nitrogen fixation, and phosphate uptake (Wetzel, 2001). Some studies have found that the sodium requirements for some species of cyanobacteria are significant, with the best growth for several species occurring at 40 mg/L (Wetzel, 2001; Provasoli, 1958). In GLSM, the sodium concentrations from the samples collected during 2011–12 ranged from 23.5 to 37.0 mg/L. These concentrations are greater than groundwater in the area, North American rivers, and other northwestern Ohio lakes (as measured in the 1970s) (table 8). Typically, there are not large temporal variations of sodium concentrations in lakes (Wetzel, 2001), so the increase in sodium concentrations in GLSM during 2011–12 may reflect the concentrating of sodium as a function of the lower water volume in the lake during the drier year (2012) when compared to 2011 (table 1).

Sulfur appears to have a role in the production of microcystin in *Microcystis aeruginosa* (Long, 2010; Jahnichen and others, 2011). In laboratory tests, sulfate concentrations of 49 and 0.49 mg/L were compared with microcystin production, and the lower concentration of sulfate may inhibit the production of the toxin (Jahnichen and others, 2011). Water samples from GLSM show sulfate concentrations from 35.6 to 69.6 mg/L (table 8). Sulfate concentrations in GLSM during 2011–12 were comparable to those measured in groundwater, GLSM, and other Ohio lakes in the 1970s.

Silica is a moderately abundant compound that is chemically unreactive but is important to diatoms. Commonly, silica concentrations in water peak in the fall and winter and decrease in the spring with the growth of diatoms. As silica concentrations drop and the growth of diatoms slows, green algae and cyanobacteria will begin to outcompete the diatoms. With diatom death, the silica can be transferred to the sediments. In systems with phosphorus and nitrogen enrichment, diatom production may initially increase but, over time, the silica in the photosynthetic zone is depleted and the dominance of green algae and cyanobacteria can become permanent. In nutrient-enriched lakes with silica concentrations less than 5 mg/L, diatoms cannot compete effectively (Wetzel, 2001). Silica concentrations in GLSM were generally less than 5 mg/L (table 8).

Many trace elements are required for the nutrition of plants and animals; however, most studies on such micronutrients investigate deficiencies or toxicity. Some studies have observed that faunal succession may be influenced by micronutrient concentrations and that the relative concentrations of trace elements can impact the competition in algal communities. For example, low concentrations of manganese are correlated with the growth of cyanobacteria, whereas diatoms dominate at concentrations greater than 40 µg/L (Wetzel, 2001). In GLSM, where the phytoplankton is dominated by cyanobacteria, manganese concentrations were less than 3.3 µg/L (table 8). In another example, growth of certain algae and cyanobacteria are enhanced by vanadium (Wetzel, 2001). Concentrations of vanadium in GLSM were around 5 to 20 times greater than the average reported for rivers worldwide (table 8).

Strontium concentrations in GLSM ranged from five to seven times greater than the median value for North American rivers (table 8) (Hem, 1989); however, high strontium concentrations have been found in the groundwater from the carbonate aquifer of western Ohio (Hem, 1989; Dumouchelle, 1999), which likely accounts for the concentrations seen in GLSM.

Table 8. Selected water-quality data for dissolved major ions and trace elements from Grand Lake St. Marys, Ohio, May 1975 and at site B1, 2011–12.

[mg/L, milligrams per liter; µg/L, micrograms per liter; —, no data; ~, approximately]

	Calcium (mg/L)	Magnesium (mg/L)	Sodium (mg/L)	Potassium (mg/L)	Sulfate (mg/L)	Chloride (mg/L)	Silica (mg/L)	Manganese (µg/L)	Vanadium (µg/L)	Strontium (µg/L)	Boron (µg/L)
[1]May 1975	21	16	10	3.3	63	19	[2]0.5	—	—	—	—
						2011					
May	40.2	19.6	23.5	4.76	46.7	36.6	0.871	3.26	0.52	401	41
August	26.3	16.9	25.5	5.35	35.6	34.2	5.93	1.46	2.1	363	56
October	30.4	18.9	26.8	5.63	46.5	33.8	3.61	2.46	0.47	386	46
						2012					
May	23.9	18.1	30.0	4.43	69.6	30.7	0.286	1.48	1.6	341	50
August	25.6	20.1	35.8	5.80	67.3	38.4	1.90	0.70	1.2	379	60
October	28.1	19.4	37.0	5.84	69.3	40.3	1.36	1.72	0.96	396	61
Groundwater[3]	88	36.5	13.5	1.35	40	4.3	16	26.5	—	4,800	54.5
Selected Ohio lakes[4]	54	18	7.8	2.7	57	20	[2]6.2	—	—	—	—
Surface water (Hem, 1989)	13.5–15	—	—	—	—	—	1–30	—	—	60	—
Surface water[5] (Wetzel, 2001)	21/15	5.0/4.1	9.0/6.3	1.4/2.3	20.0/11.2	8.0/7.8	9.0/13.1	—/35	—/~0.1	—	[6]10

[1] From Tobin and Youger (1977).

[2] Maximum from four readings.

[3] Median concentrations reported for groundwater from the Lockport Dolomite in western Ohio (Dumouchelle, 1999).

[4] Median concentrations for nine selected lakes, excluding Grand Lake St. Marys, in northwest Ohio (Tobin and Youger, 1977, 1979; Angelo and Youger, 1985).

[5] The first value is a mean for North American rivers; second value is mean for worldwide rivers.

[6] Table 10–8 in Wetzel (2001) specifies “natural freshwaters” but not the source of the water.

These relatively high concentrations of strontium result in a ratio of calcium to strontium that is 30 to more than 60 times smaller than that of other freshwaters (Wetzel, 2001). While strontium is chemically similar to calcium and the substitution of strontium for calcium may favor some cyanobacteria over other algae (Wetzel, 2001), it is unknown if these concentrations of strontium have any role in the growth of cyanobacteria in GLSM.

Like strontium, the concentrations of boron in GLSM ranged from four to six times higher than average concentrations in freshwater and might be accounted for by water from the carbonate aquifer (table 8). Boron is a known micronutrient for cyanobacteria (Wetzel, 2001) and is added, in the form of boric acid, to cyanobacteria growth solutions in the laboratory (C. Ecker, U.S. Geological Survey, written commun., 2014); however, the role of these concentrations of boron on the growth of cyanobacteria is unknown.

Plankton

Plankton are free-floating small organisms with limited locomotion. Phytoplankton are small photosynthetic plankton, including cyanobacteria. Zooplankton are more animal-like than phytoplankton and feed on other plankton and detritus to obtain energy. Zooplankton grazing can alter the phytoplankton population distribution by selectively grazing on some populations. Cyanobacterial toxins may prevent or discourage zooplankton grazing (Wetzel, 2001). In addition, filamentous cyanobacteria, like *Planktothrix*, appear to be poorly grazed because it is physically too difficult for zooplankton to ingest (Halstvedt and others, citing previous research 2007). Plankton data from the 2011–12 samples collected in GLSM are shown in appendix 2—the following sections summarize those data.

Phytoplankton

Phytoplankton populations are affected by environmental conditions including water quality, weather, temperature, physiological needs, and predation; therefore, descriptions of typical patterns should be considered only a general guide for expectations. Seasonal succession in temperate (and eutrophic) lakes may consist of small flagellates (Chrysophyta, Cryptophyta, and others) in the winter with a spring jump in diatoms (Bacillariophyta), followed by a small increase in green algae (Chlorophyta), then a lull moving into summer with cyanobacteria increasing in late summer and early autumn (Wetzel, 2001). The phytoplankton communities of eutrophic lakes are commonly dominated by diatoms (Bacillariophyta) with green algae (Chlorophyta) and cyanobacteria or solely with cyanobacteria (Wetzel, 2001). In GLSM, cyanobacteria dominated the phytoplanktonic community (table 9) in both 2011 and 2012. In 2011, diatoms, green algae, and Cryptophyta were present in the spring, decreased during the summer, and were increasing by late October; however, this sequence was not seen in 2012.

The conditions in GLSM (as described earlier in this report) such as warm water temperatures, low-light transmission, and chemical constituents, favor the growth of cyanobacteria in general and *Planktothrix*, in particular. The cyanobacteria population in GLSM during 2011–12 was dominated by *Planktothrix* (table 9). Other genera, such as *Aphanizomenon*, *Anabaena*, *Aphanocapsa*, and *Cylindrospermopsis* were occasionally present (table 9). All of these cyanobacteria genera have strains that can produce a variety of toxins, and three of the five can produce microcystins; however, *Planktothrix* blooms have been shown to produce the highest levels of toxins per biovolume (Halstvedt and others, 2007, citing previous research).

In 2011, the greatest biovolumes of phytoplankton were generally seen in the August and September samples. In 2012, the greatest biovolume was generally seen in the months of May and June (figs. 11*A* and *B*). Unseasonably warm weather in March 2012 and the lack of ice cover during winter 2011–12 may have contributed to the early spring high biomass in 2012 (Tetra Tech, Inc., 2013).

The spatial distribution of biovolumes was examined. In 2011, on any given trip, samples with the lowest biovolumes were usually collected on the west end of the lake at sites B2 and B4; the highest were often at site B5 or B6, although site B3 (the sheltered site) also tended to have high biovolumes (fig. 11). This pattern in biovolume distribution was not as clear in 2012.

Phytoplankton samples collected from the center of GLSM on two dates in 1975 (Tobin and Youger, 1977) had densities of 2.4 and 2.8 x 10^9 cells per liter, which are comparable to densities measured during 2011–12 (appendix 2). As with the 2011–12 samples, cyanobacteria were dominant in 1975, consisting of 99 percent of the total cell count. However, unlike the 2011–12 samples, the dominate genera in the 1975 samples were *Lyngbya*, *Anacystis*, and *Cylindrospermum*. *Oscillatoria* (*Planktothrix*) were present in the 1975 samples but composed only 3 and 7 percent of the total cell counts. Toxin analyses were not conducted in the 1970s so there are no data on microcystin concentrations for comparison.

Table 9. Percentage of phytoplankton divisions and selected genus, by biovolume, in samples from sites B1–B6, Grand Lake St. Marys, Ohio, 2011–12.

[Bolded numbers are the division with the greatest percentage present; —, not present; <, less than]

Division	Genus	B1	B2	B3	B4	B5	B6
			5/5/2011				
Bacillariophyta		7.88	23.5	12.8	13.3	17.82	4.12
	Aulacoseira granulata				9.9	13.64	
	Aulacoseira ambigua		11.7				
Chlorophyta		**64.6**	2.95	11.5	3.84	35.10	**81.36**
	Pediastrum duplex	63.6		9.14		34.03	79.27
Chrysophyta		—	0.06	0.04	—	<0.01	0.01
Cryptophyta		1.81	4.3	2.39	3.3	2.00	1.71
Cyanobacteria		26.1	**69.2**	**73.2**	**79.6**	**44.16**	12.79
	Planktothrix agardhii					43.73	
	Planktothrix sp.	25.7	67.9	72.3	79.2		
	Aphanizomenon gracile						12.67
Euglenophyta		0.02	—	0.14	—	—	—
			6/21/2011				
Bacillariophyta		1.08	5.01	3.24	1.49	6.32	7.34
Chlorophyta		40.69	0.40	0.2	0.37	0.47	5.65
	Pediastrum duplex	39.94					
Cryptophyta		0.58	0.88	0.42	0.79	0.56	3.90
Cyanobacteria		**57.4**	**93.71**	**96.1**	**97.34**	**92.64**	**83.11**
	Planktothrix agardhii	57.44	91.60	96.1	97.34	92.50	82.69
Euglenophyta		0.22	—	—	—	—	—
Pyrrophyta		0.02	—	—	0.02	0.01	—
			8/2/2011				
Bacillariophyta		0.86	1.45	3.86	0.85	2.38	2.66
Chlorophyta		1.60	1.16	0.52	0.30	9.59	0.17
Chrysophyta		—	—	0.23	0.18	—	—
Cryptophyta		—	2.14		—	—	—
Cyanobacteria		**97.54**	**95.24**	**95.38**	**98.67**	**88.03**	**97.17**
	Anabaena circinalis	6.97	8.45		17.10	10.43	5.74
	Planktothrix agardhii	85.41	85.48	89.14	76.89	71.76	87.93
Euglenophyta		—	<0.01	—	—	—	<0.01

Table 9. Percentage of phytoplankton divisions and selected genus, by biovolume, in samples from sites B1–B6, Grand Lake St. Marys, Ohio, 2011–12.—Continued

[Bolded numbers are the division with the greatest percentage present; —, not present; <, less than]

Division	Genus	B1	B2	B3	B4	B5	B6
				9/14/2011			
Bacillariophyta		1.43	0.74	1.90	1.33	0.87	2.34
Chlorophyta		0.28	0.33	0.43	0.21	0.81	5.18
Chrysophyta		—	—	0.02	—	—	—
Cryptophyta		1.02	0.52	0.77	1.56	0.23	0.82
Cyanobacteria		**97.28**	**98.41**	**96.87**	**96.90**	**98.09**	**91.66**
	Anabaena spp.	6.77					
	Planktothrix agardhii	84.20	93.82	90.08	93.64	88.83	83.92
Euglenophyta		—	—	0.01	<0.01	—	—
				10/25/2011			
Bacillariophyta		2.18	0.27	29.83	9.78	0.33	0.52
	Stephanodiscus niagarae	27.51					
Chlorophyta		1.37	2.15	0.33	0.09	10.09	1.12
	Pediastrum duplex					7.81	
Cryptophyta		6.01	4.27	0.88	3.87	2.56	3.44
Cyanobacteria		**90.44**	**93.31**	**68.96**	**85.10**	**86.99**	**94.93**
	Planktothrix agardhii	86.15	85.56	67.75	84.99	84.34	93.34
Euglenophyta		—	—	—	0.01	—	—
Pyrrophyta		—	—	—	—	0.03	—
				3/29/2012			
Bacillariophyta		14.67	7.82	26.11	11.79	11.14	17.86
	cf. Cyclostephanos invisitatus				7.07		
	Stephanodiscus hantzschii	10.20		11.37			14.15
	Stephanodiscus niagarae			7.55			
Chlorophyta		3.68	16.17	11.18	11.41	17.37	10.73
	Actinastrum hantzschii		14.49	10.63		16.28	
	Dictyosphaerium pulchellum						6.01
	Pediastrum duplex				8.72		

Table 9. Percentage of phytoplankton divisions and selected genus, by biovolume, in samples from sites B1–B6, Grand Lake St. Marys, Ohio, 2011–12.—Continued

[Bolded numbers are the division with the greatest percentage present; —, not present; <, less than]

Division	Genus	B1	B2	B3	B4	B5	B6
				3/29/2012—Continued			
Chrysophyta					<0.01	<0.01	—
Cryptophyta		24.64	7.27	4.53	13.28	2.27	3.92
	Cryptomonas spp.	20.77	6.01				
	Rhodomonas sp.				11.77		
Cyanobacteria		**57.01**	**68.74**	**58.13**	**63.43**	**69.21**	**67.45**
	Planktothrix agardhii	50.92	67.72	49.64	61.14	66.87	66.12
	Pseudanabaena limnetica			8.49			
Euglenophyta		—	—	0.02	—	0.01	0.04
Pyrrophyta		—	—	0.02	0.09	—	—
				5/15/2012			
Bacillariophyta		0.27	1.31	3.36	0.92	0.89	0.85
Chlorophyta		0.91	0.82	1.53	0.98	3.64	29.81
	Pediastrum duplex						29.14
Cryptophyta		1.33	0.77	2.43	2.39	0.47	0.71
Cyanobacteria		**97.44**	**96.94**	**92.46**	**95.69**	**95.00**	**68.58**
	Planktothrix agardhii	97.44	96.58	92.46	91.54	94.20	68.22
Euglenophyta		—	0.05	0.20	—	—	—
Pyrrophyta		0.04	0.11	0.00	0.03	—	0.04
				6/26/2012			
Bacillariophyta		4.38	2.69	9.19	2.62	0.66	3.90
Chlorophyta		0.98	0.61	1.45	0.87	0.74	0.42
Cryptophyta		1.62	0.39	0.51	0.43	0.44	0.08
Cyanobacteria		**93.68**	**96.30**	**88.40**	**96.08**	**98.10**	**95.48**
	Aphanizomenon sp.	32.68		10.16			
	Planktothrix agardhii	59.63	90.41	70.87	88.32	89.77	86.57
Euglenophyta		—	—	0.45	—	0.07	0.12
Pyrrophyta		0.04	—	<0.01	—	—	—

Table 9. Percentage of phytoplankton divisions and selected genus, by biovolume, in samples from sites B1–B6, Grand Lake St. Marys, Ohio, 2011–12.—Continued

[Bolded numbers are the division with the greatest percentage present; —, not present; <, less than]

Division	Genus	B1	B2	B3	B4	B5	B6
				8/3/2012			
Bacillariophyta		0.87	3.51	7.52	0.11	2.45	5.93
	Aulacoseira granulata			5.29			
Chlorophyta		2.43	1.17	3.67	0.55	0.49	1.23
Cryptophyta		1.27	—	2.92	0.29	1.78	3.01
Cyanobacteria		**95.43**	**95.32**	**85.70**	**99.05**	**95.28**	**89.84**
	Aphanizomenon sp.	15.42					
	Aphanocapsa sp.					11.11	
	Cylindrospermopsis raciborskii	19.69	28.63	7.12	5.58	8.75	10.26
	Planktothrix agardhii	57.88	62.03	68.28	91.47	64.14	74.67
Euglenophyta		—	—	0.19	—	—	—
				9/11/2012			
Bacillariophyta		2.59	2.74	2.97	1.00	2.12	1.55
Chlorophyta		1.91	0.64	0.07	0.36	0.77	0.26
Cryptophyta		1.75	1.03	1.22	2.18	3.09	0.18
Cyanobacteria		**93.8**	**95.58**	**95.74**	**96.46**	**94.03**	**98.00**
	Aphanizomenon gracile				5.27		
	Planktothrix agardhii	91.04	94.2	93.2	90.06	91.98	97.70
				10/16/2012			
Bacillariophyta		3.58	2.50	1.63	0.55	1.03	2.66
Chlorophyta		0.07	0.01	2.83	0.68	0.25	1.34
Cryptophyta		0.00	0.00	0.18	0.57	0.54	0.95
Cyanobacteria		**96.35**	**97.49**	**95.36**	**98.20**	**98.18**	**95.05**
	Planktothrix agardhii	96.10	97.34	93.85	98.03	97.97	94.68

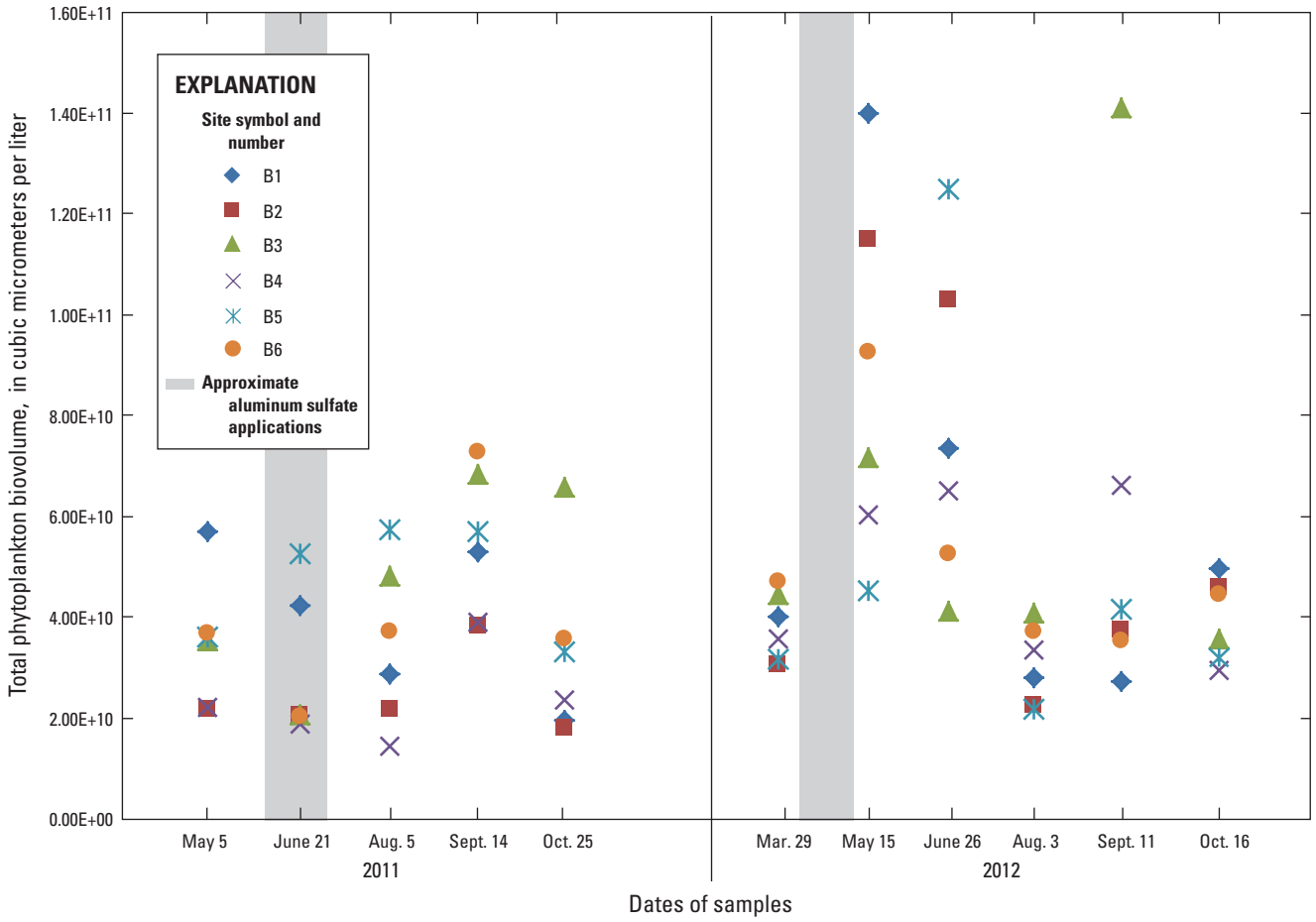

Figure 11*A*. Total phytoplankton biovolume at sites B1–B6 in Grand Lake St. Marys, Ohio, 2011.

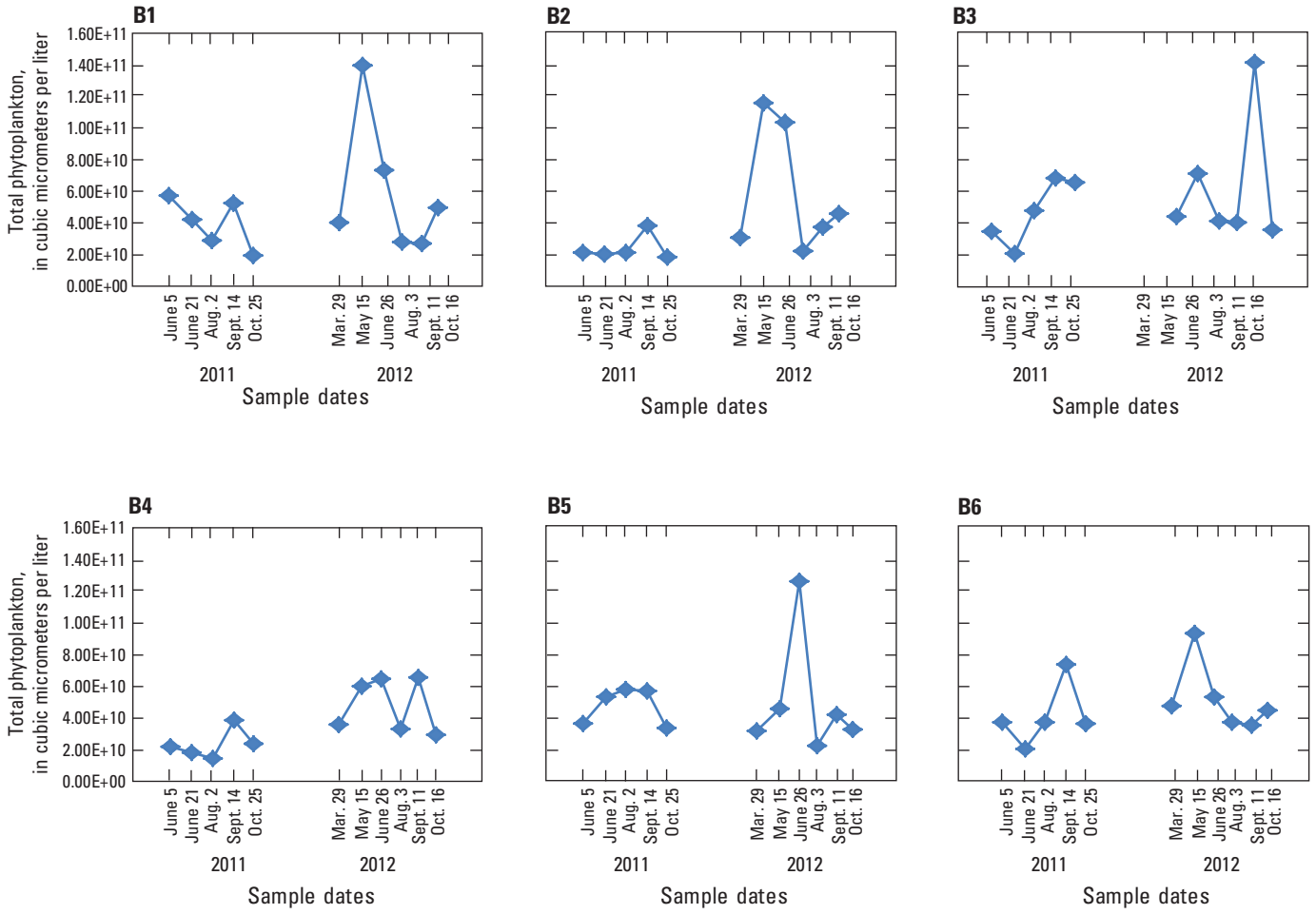

Figure 11*B*. Total phytoplankton biovolume at sites B1–B6 in Grand Lake St. Marys, Ohio, 2012.

Zooplankton

Samples composited from two sites each were collected from the east (sites B5 and B6) and west ends (sites B2 and B4) of GLSM for zooplankton analyses. Table 10 summarizes the zooplankton data, which can be found in its entirety in appendix 2. While rotifera were the most numerous taxa, copepods dominated the zooplankton biomass, with this group having the greatest mean biomass in 19 of the 22 samples and a copepod species contributing more than 50 percent biomass in 8 samples.

Olmstead (1936) presented zooplankton data from 52 lakes in Ohio, including GLSM. While there are differences in sample collection and processing, a basic comparison of the GLSM samples (table 11) indicates that there were more zooplankton present in 1936 than in 2011 or 2012, particularly cladocerans. Daphnia (cladoceran), generally a primary grazer of phytoplankton, were found to have reduced grazing rates in cyanobacteria blooms in western Lake Erie; whereas the grazing rates of microzooplankton (rotifera) did not decline (Davis and others, 2012). In the 2011–12 samples collected from GLSM, daphnia were present in 10 of the 22 samples but, despite their relatively large size, were the dominate biomass species in only 2 samples.

Faherty (1979) examined zooplankton in 4 lakes in northeastern Ohio and found 16 different species of cladocera and 8 species of copepod. In GLSM (assuming that immature individuals were not from an otherwise unidentified species), there were seven different species of copepods but only four species of cladocera. The ratio of cladocerans to copepods in the northeastern Ohio lakes ranged from 1.27 to 2.28 (Faherty, 1979), whereas the ratio in GLSM was appreciably less at 0.57. A greater copepod biomass compared to cladocerans is expected in hypereutropic lakes (J. Beaver, BSA Environmental Service Inc., written commun., 2012), and this is seen in the GLSM data (table 11).

Table 10. Summary of zooplankton analyses for composite samples collected at Grand Lake St. Marys, Ohio, 2011–12.

[Site locations are shown on fig. 1; µg d.w./L, micrograms dry weight per liter; taxa are listed as division, genus, and species or lifestage; N.P., not present; **, no Rotifera present, but Ostracoda present at 0.006 µg d.w./L; <, less than; complete zooplankton data are provided in appendix 2]

Division	Zooplankton analyses, by sampling date and site					
	March 29, 2012		May 5, 2011		May 15, 2012	
	East B5, B6	West B2, B4	East B5, B6	West B2, B4	East B5, B6	West B2, B4
	Mean biomass, µg d.w./L					
Cladocera	0.56	0.04	5.12	51.2	0.03	0.03
Copepoda	1.32	0.96	4.77	13.0	1.95	0.90
Rotifera	0.54	0.06	0.08	0.43	N.P. **	<0.01
	Number of species present					
Cladocera	1	1	3	4	1	1
Copepoda	5	4	6	6	5	3
Rotifera	5	3	4	4	**	3
Total	11	8	13	14	7	7
	Most numerous species (percent of individuals)					
	Rotifera, *Keratella cochlearis f. tecta* (53)	Rotifera, *Keratella cochlearis f. tecta* (65)	Rotifera, *Keratella Cochlearis* (27)	Rotifera, *Keratella cochlearis* (53)	Copepoda, *Acanthocyclops robustus* (47)	Copepoda, *nauplii* (39)
	Greatest biomass taxa (percent)					
	Rotifera, *Asplanchna priodonta* (26)	Copepoda, *Acanthocyclops robustus* (40)	Copepoda, *Leptodiaptomus siciloides* (40)	Cladocera, *Daphnia galeata* (58)	Copepoda, *Acanthocyclops robustus* (89)	Copepoda, *Acanthocyclops robustus* (81)

Table 10. Summary of zooplankton analyses for composite samples collected at Grand Lake St. Marys, Ohio, 2011–12.—Continued.

[Site locations are shown on fig. 1; µg d.w./L, micrograms dry weight per liter; taxa are listed as division, genus, and species or lifestage; N.P., not present; **, no Rotifera present, but Ostracoda present at 0.006 µg d.w./L; <, less than; complete zooplankton data are provided in appendix 2]

	Zooplankton analyses, by sampling date and site							
	June 21, 2011		June 26, 2012		August 2, 2011		August 3, 2012	
	East B5,B6	West B2,B3	East B5,B6	West B2,B4	East B5,B6	West B2,B4	East B5,B6	West B2,B4
	Mean biomass, µg d.w./L							
Cladocera	1.86	1.88	0.62	0.99	1.49	N.P.	N.P.	N.P.
Copepoda	3.26	11.78	1.13	2.12	3.74	3.73	2.16	2.35
Rotifera	0.34	0.30	0.11	0.10	0.19	0.09	0.64	0.32
	Number of species present							
Cladocera	2	3	2	2	1	N.P.	N.P.	N.P.
Copepoda	4	3	5	5	7	5	5	5
Rotifera	7	9	5	5	10	12	10	7
Total	13	15	12	12	18	17	15	12
	Most numerous species (percent of individuals)							
	Rotifera, *Pompholyx Sulcata* (35)	Rotifera, *Pompholyx Sulcata* (32)	Rotifera, *Polyarthra vulgaris* (49)	Rotifera, *Polyarthra vulgaris* (28)	Rotifera, *Brachionus caudatus* (30)	Rotifera, *Keratella cochlearis f. tecta* (34)	Rotifera, *Keratella cochlearis var. tecta* (23)	Rotifera, *Keratella cochlearis var. tecta* (29)
	Greatest biomass taxa (percent)							
	Copepoda, *Acanthocyclops robustus* (38)	Copepoda, *Acanthocyclops robustus* (61)	Copepoda, *Leptodiaptomus siciloides* (20)	Copepoda *cyclopoid copepodid* (45)	Copepoda, *Leptodiaptomus siciloides* (24)	Copepoda, *Paracyclops canadensis* (27)	Rotifera, *Asplanchna priodonta* (32)	Copepoda, *Leptodiaptomus siciloides* (40)

Table 10. Summary of zooplankton analyses for composite samples collected at Grand Lake St. Marys, Ohio, 2011–12.—Continued.

[Site locations are shown on fig. 1; µg d.w./L, micrograms dry weight per liter; taxa are listed as division, genus, and species or lifestage; N.P., not present; **, no Rotifera present, but Ostracoda present at 0.006 µg d.w./L; <, less than; complete zooplankton data are provided in appendix 2]

Division	Zooplankton analyses, by sampling date and site							
	September 14, 2011		September 11, 2012		October 25, 2011		October 16, 2012	
	East B5,B6	West B2,B3	East B5,B6	West B2,B4	East B5,B6	West B2,B4	East B5,B6	West B2,B4
	Mean biomass, µg d.w./L							
Cladocera	1.29	1.27	N.P.	0.41	2.34	0.16	N.P.	N.P.
Copepoda	2.64	1.89	1.27	0.71	1.23	0.76	0.78	1.27
Rotifera	0.16	0.41	0.12	0.12	0.05	0.32	0.03	0.07
	Number of species present							
Cladocera	1	1	N.P.	1	2	1	N.P.	N.P.
Copepoda	5	4	3	4	5	5	4	5
Rotifera	8	9	10	9	5	5	4	4
Total	14	14	13	14	12	11	8	9
	Most numerous species (percent of individuals)							
	Rotifera, *Pompholyx sulcata* (36)	Rotifera, *Polyarthra vulgaris* (50)	Rotifera, *Keratella cochlearis var. tecta* (34)	Rotifera, *Keratella cochlearis var. tecta* (39)	Rotifera, *Keratella cochlearis f. tecta* (60)	Rotifera, *Keratella cochlearis f. tecta* (54)	Rotifera, *Keratella cochlearis f. tecta* (74)	Rotifera, *Keratella cochlearis f. tecta* (58)
	Greatest biomass taxa (percent)							
	Copepoda, *Leptodiaptomus siciloides* (63)	Copepoda, *Leptodiaptomus siciloides* (28)	Copepoda, *Leptodiaptomus siciloides* (60)	Copepoda, *Leptodiaptomus siciloides* (51)	Cladocera, *Daphnia galeata* (43)	Copepoda, *Diacyclops thomasi* (34)	Copepoda, *calanoid copepodid* (52)	Copepoda, *Leptodiaptomus siciloides* (57)

Table 11. Comparison of zooplankton populations during August 1935 with populations during August 2011 and 2012 in Grand Lake St. Marys, Ohio.

[Data for 1935 are from Olmstead (1936)]

Date	Location	Number of individuals per liter		
		Cladocera	Copepoda	Rotifera
8-6-1935	Station 1, middle	5.4	3.6	61.2
8-6-1935	Station 2, top	0	125.4	53.2
8-6-1935	Station 2, bottom	10.0	160.0	114.0
8-7-1935	Station 3, middle	4.8	59.2	208.0
8-2-2011	Sites B2 and B4	0	27.4	98
8-2-2011	Sites B5 and B6	0.8	46.9	115.4
8-3-2012	Sites B2 and B4	0	12.4	78.7
8-3-2012	Sites B5 and B6	0	14.1	50.7

Microcystin Toxin

Microcystins are hepatoxins (poisons that damage the liver) produced by several types of cyanobacteria, including *Anabaena, Microcystis,* and *Planktothrix*. In Ohio, no-contact advisories are issued for microcystins in recreational waters if the concentrations equal or exceed 20 µg/L, and there are one or more probable cases of human illness or pet deaths (*http://wwwapp.epa.ohio.gov/gis/mapportal/hab.html*). Microcystin concentrations in the 66 USGS samples from GLSM during 2011–12 ranged from 7.3 to 83 µg/L (figs. 12*A* and *B*; appendix 1). Only one in four of the samples had microcystin concentrations less than 20 µg/L; all were from 2011, mostly from the May and August sampling trips. Overall, microcystin concentrations in 2011 were lower, 7.3 to 43 µg/L, with a median concentration of 19 µg/L; in 2012, concentrations ranged from 24 to 83 µg/L with a median concentration of 40 µg/L (table 12). Data from the CWTP also show microcystin concentrations were lower in 2011 (table 6).

In general, in both years, the microcystin concentrations decreased in August and increased in September and October (fig. 12). The lowest microcystin concentrations as a group, for any date, were from August 2011 samples; the highest concentrations were in the last round of samples, October 2012 (fig 12*A*). No consistent patterns were observed among the sites for microcystin concentrations (fig. 12*B*); for example, in 2011 the concentrations at sites B3 and B5 or B1 and B6 had similar patterns, but in 2012 the patterns among these sites were different. Microcystin concentrations were not consistently higher or lower at any given site; however, sites B5 and B6 (on the east side of the lake) had the lowest concentrations in 8 of the 11 sampling trips.

Some studies have noted correlations between microcystin and orthophosphate, total phosphorus, or total nitrogen concentrations (Jacoby and others, 2000; Rinta-Kanto and others, 2009; Graham and others, 2004). Unfortunately, there were no good correlations between those nutrients and microcystin concentrations observed at GLSM (fig. 13). In comparing individual samples, decreases in total nitrogen concentrations were frequently accompanied by increases in microcystin concentrations. For example, for August–September 2011 and August–September 2012, total nitrogen concentrations decreased (fig. 10*B*), and microcystin concentrations increased (fig. 12*B*). However, the correlation of all samples between the two constituents is poor (rho = 0.20, p = 0.1151) (fig. 13).

Statistically significant correlations were observed between microcystin concentrations and total cyanobacteria and *Planktothrix* biovolumes (rho = 0.41, p = 0.0007 and rho = 0.41, p = 0.0008, respectively). As discussed previously, cyanobacteria and *Planktothrix*, specifically, were the dominant phytoplankton in GLSM. Also, as discussed previously, *Planktothrix* has been shown to produce the highest levels of toxins per biovolume (Halstvedt and others, 2007, citing previous research). Therefore, it is not surprising that there would be a correlation between the biovolumes and microcystin concentrations.

Concentrations of the major ion sodium and the trace elements of antimony and lithium were found to be correlated with microcystin concentrations (rho = 0.83, p = 0.0416; rho = 0.83, p = 0.0416; and rho = −0.94, p = 0.0048, respectively). As discussed previously, sodium is required for numerous biological activities, and some studies have found that the sodium requirements for some species of cyanobacteria are significant (Wetzel, 2001; Provasoli, 1958). Thus, the correlation of sodium concentrations with microcystin concentrations may actually be related more to the biological activities of the phytoplankton than to microcystin concentrations. It is not clear what role antimony or lithium may have in phytoplankton growth or microcystin production.

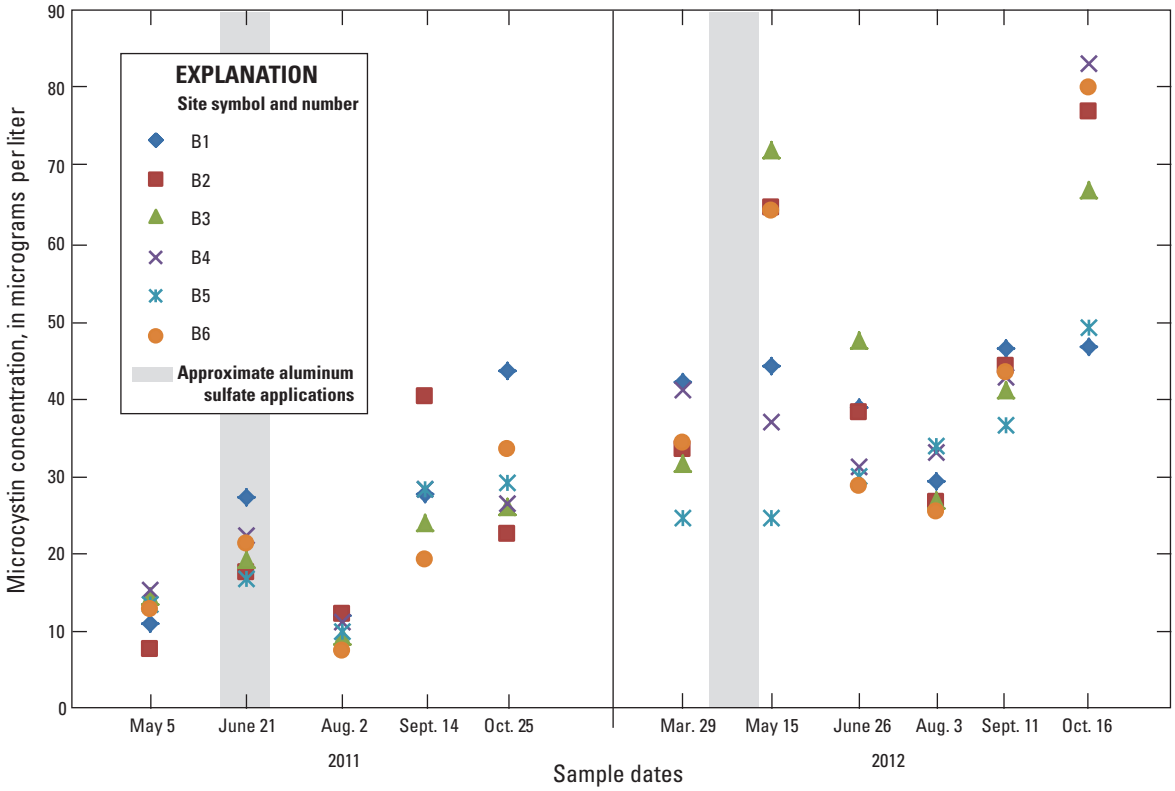

Figure 12*A*. Microcystin concentrations at sites B1–B6 in Grand Lake St. Marys, Ohio, 2011. [See figure 1 for location of sites B1–B6.]

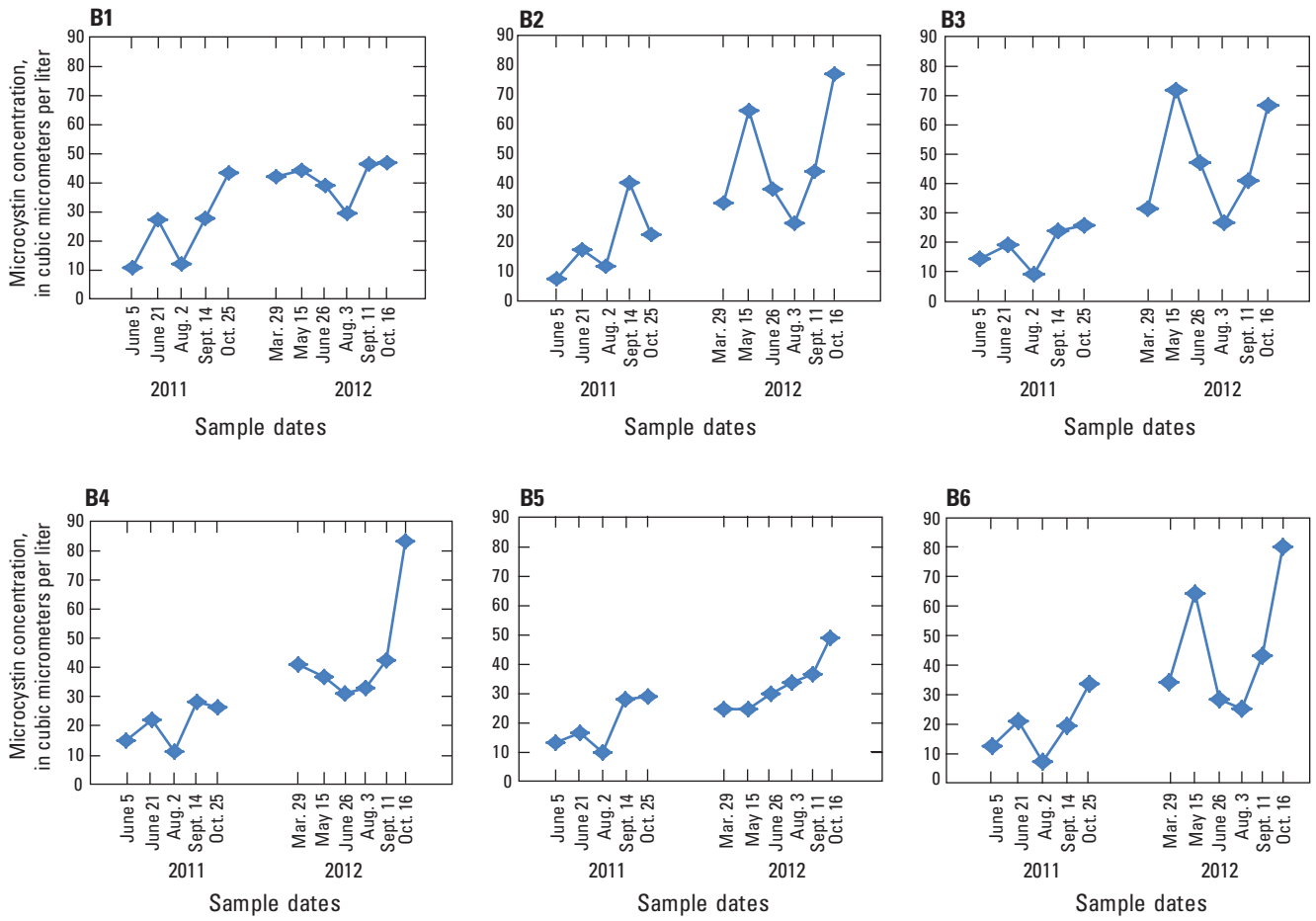

Figure 12*B*. Microcystin concentrations at sites B1–B6 in Grand Lake St. Marys, Ohio, 2012. [See figure 1 for location of sites B1–B6.]

Table 12. Median concentrations of microcystin, in micrograms per liter, from multiple sampling sites in Grand Lake St. Marys, Ohio, March–October 2011 and March–October 2012.

[USGS, U.S. Geological Survey, median value of six sites sampled once; ns, no sample; CWTP, Celina Water Treatment Plant, weekly sample from raw-water intake; CWTP data from Ohio Environmental Protection Agency Harmful Algal Bloom sampling program Web site as of May 13, 2013, reported as parts per billion]

	March	April	May	June	July	August	September	October
USGS–2011	ns	ns	13	20	ns	11	28	28
USGS–2012	33	ns	54	35	ns	28	43	72
CWTP–2011	0.4	1.7	17	23	30	15	23	24
CWTP–2012	19	46	34	29	47	48	54	56

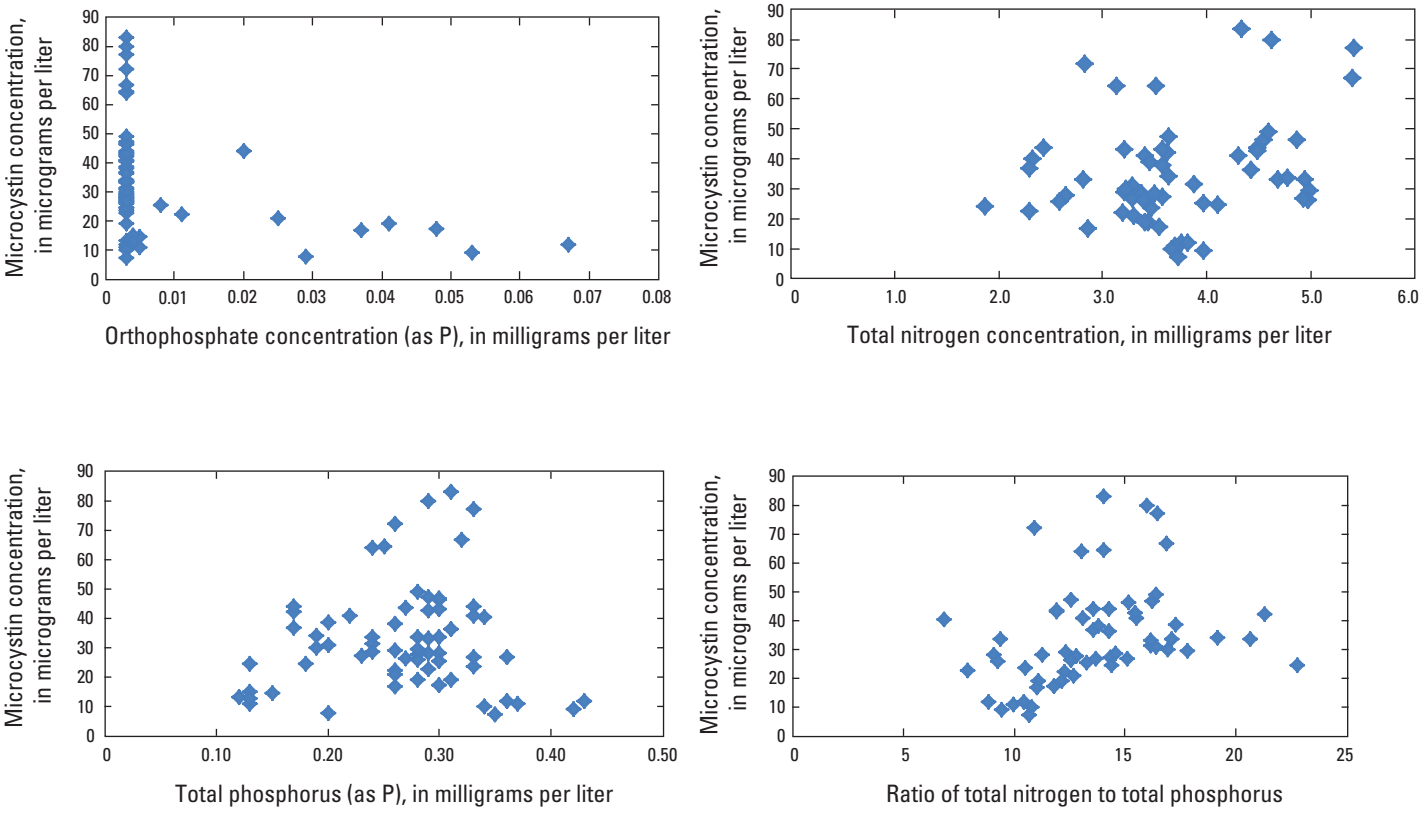

Figure 13. Microcystin concentrations with orthophosphate concentrations, total nitrogen concentrations, total phosphorus (as P), and microcystin concentrations with the ratio of total nitrogen to total phosphorus in samples from sites B1–B6, Grand Lake St. Marys, Ohio, 2011–12.

Concentrations of Cyanobacteria by Molecular Methods

Molecular Method Analyses

Results from the 2011–12 samples collected from six buoy sites are presented in Appendix 4. Total cyanobacteria DNA genes, total *Microcystis* DNA genes, and *Planktothrix mcyE* DNA toxin genes were detected in every sample. *Microcystis mcyE* DNA toxin genes were detected in 16 out of 30 samples in 2011, and 28 out of 36 samples in 2012. Median concentrations were higher for total cyanobacteria DNA genes and total *Microcystis* DNA genes in 2011 as compared to 2012, whereas median concentrations for both *Microcystis mcyE* DNA toxin genes and *Planktothrix mcyE* DNA toxin genes were higher in 2012 (table 13).

Due to the high cost of running qRT-PCR, this method was used to analyze samples from only two of the six sites. *Microcystis mcyE* RNA transcripts were not detected in any of the 22 samples analyzed. *Planktothrix mcyE* RNA transcripts were detected in all but two samples, and both of the samples without detections were from August 2, 2011. Median concentrations were higher for *Planktothrix mcyE* RNA transcripts in 2012 as compared to 2011 (table 13).

Concentrations of *Planktothrix mcyE* DNA toxin genes and *Planktothrix mcyE* RNA transcripts were not significantly correlated at the two buoy sites using Spearman's correlation analysis with the alpha level for significance set at equal to or less than 0.05; however, the significance was close to the alpha level at 0.0823. One reason why the DNA toxin gene and RNA transcript results were not significantly correlated could be that *Planktothrix mcyE* DNA toxin gene concentrations were more temporally consistent than the *Planktothrix mcyE* RNA transcript concentrations (fig. 14). In other words, the potential for toxin production remained at a relatively constant level, but toxin gene expression varied somewhat throughout the season. Correlations could not be done for *Microcystis mcyE* RNA transcript concentrations because there were no detections.

A study of cyanobacteria in GLSM in 2010 (Steffen and others, 2014) found the *Planktothrix* genus to be dominant over the *Microcystis* genus by use of non-quantitative PCR targeting the *mcyA* DNA gene. Similarly, our study also found higher concentrations of *Planktothrix* compared to *Microcystis* by use of qPCR and qRT-PCR.

Table 13. Minimum, maximum, and median concentrations of sample detections for DNA- and RNA-based molecular methods from multiple locations in Grand Lake St. Marys, Ohio during 2011–12.

[concentrations in copies per 100 milliliters; Detects n, number of samples with detections; Sample n, total number of samples; N/A, not applicable because all samples were undetected]

Assay	2011					2012				
	Min	Max	Median	Detects n	Sample n	Min	Max	Median	Detects n	Sample n
DNA-based qPCR assays										
Total cyanobacteria	$2.10x10^8$	$2.00x10^9$	$6.40x10^8$	30	30	$1.80x10^8$	$1.10x10^9$	$6.00x10^8$	36	36
Total Microcystis	$1.20x10^4$	$2.10x10^6$	$1.60x10^5$	30	30	330	$4.60x10^5$	$5.40x10^4$	36	36
Microcystis mcyE	310	$2.20x10^3$	740	16	30	190	$9.20x10^3$	$1.00x10^3$	28	36
Planktothrix mcyE	$6.20x10^5$	$4.90x10^8$	$6.10x10^6$	30	30	$1.10x10^7$	$6.90x10^7$	$2.80x10^7$	36	36
RNA-based qRT-PCR assays										
Microcystis mcyE	N/A	N/A	N/A	0	10	N/A	N/A	N/A	0	12
Planktothrix mcyE	$2.20x10^3$	$1.70x10^6$	$8.50x10^3$	8	10	$4.80x10^3$	$4.80x10^6$	$2.15x10^5$	12	12

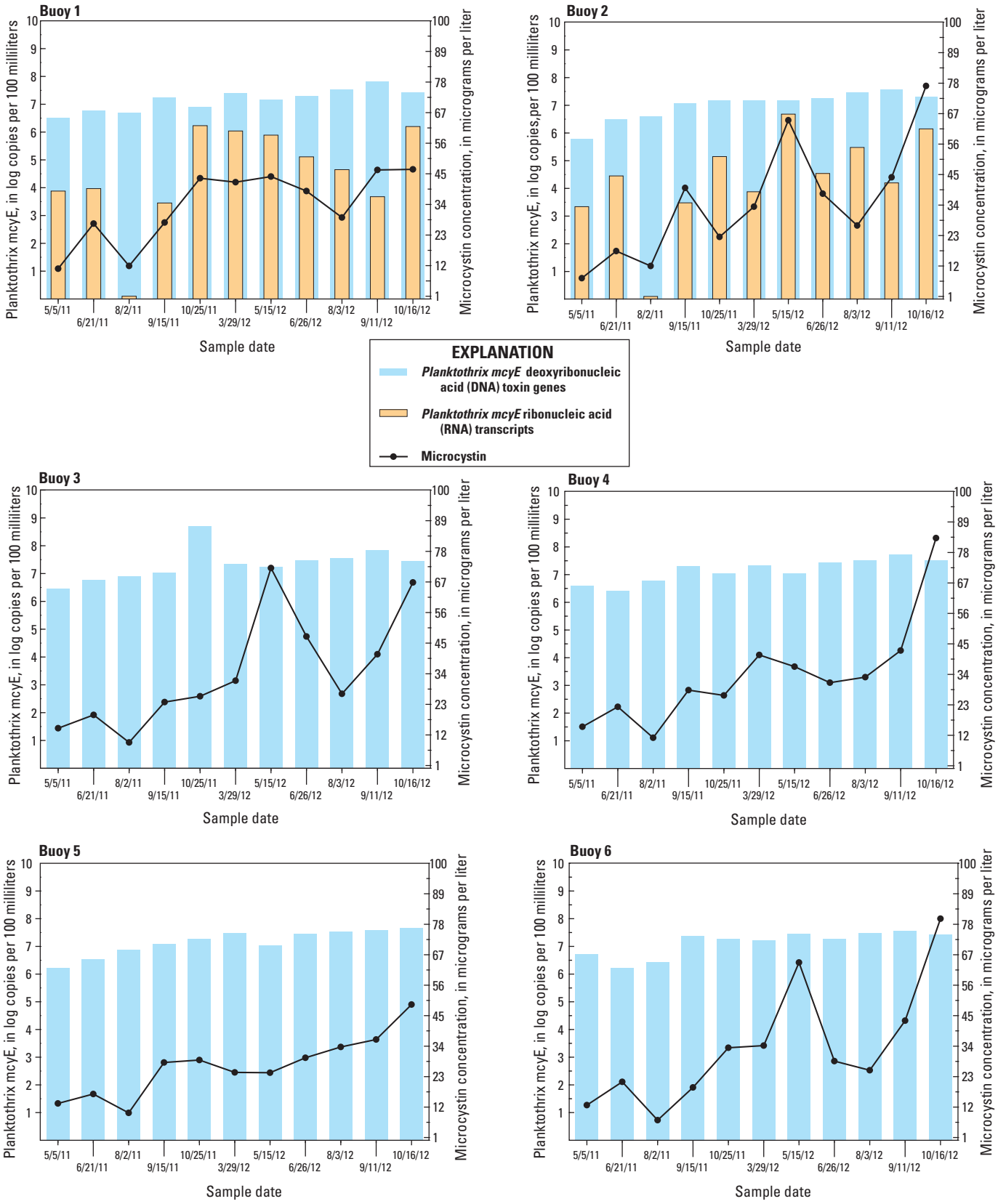

Figure 14. *Planktothrix* mcyE deoxyribonucleic acid (DNA) toxin gene and ribonucleic acid (RNA) transcript concentrations and microcystin concentrations, by date, for all six buoy sites at Grand Lake St. Marys, Ohio, 2011–12. [Aluminum sulfate was applied to Grand Lake St. Marys, Ohio, during the June 21, 2011, sampling event and between the March 29 and May 15, 2012, sampling events.]

Molecular Method Results Compared to Phytoplankton Identification

Total *Microcystis* DNA genes were detected in GLSM samples; however, in the previous discussions of phytoplankton by microscopy, only *Planktothrix* and a few other cyanobacteria were identified. Although *Microcystis* was not explicitly discussed previously, it was found in some microscopy samples in very small amounts; for example, less than 0.1 percent of the biovolume (appendix 2). Discrepant results between concentrations of cyanobacteria from molecular methods, such as qPCR and qRT-PCR, and biovolumes of cyanobacteria by microscopy may be owing to differences in sample processing and measurement procedures. Sample processing for molecular methods includes concentrating phytoplankton onto a filter to determine concentrations representative of a larger sample volume, whereas estimates of cyanobacteria abundance and biovolume by microscopy use only a small volume of water to extrapolate concentrations to a larger sample volume. Also, 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 analysis.

Correlations between cyanobacteria concentrations by qPCR or qRT-PCR and cyanobacteria biovolumes by microscopy are shown in table 14. Total cyanobacteria and *Planktothrix* biovolumes were significantly correlated to total *Microcystis* DNA toxin gene (negative) and *Planktothrix mcyE* DNA toxin gene (positive) concentrations.

Molecular Methods Results Compared to Toxin Concentrations

Microcystin concentrations by ELISA were significantly correlated to concentrations of total *Microcystis* DNA genes (negative), *Microcystis mcyE* DNA toxin genes, *Planktothrix mcyE* DNA toxin genes, and *Planktothrix mcyE* RNA transcripts (table 14). However, visually *Planktothrix mcyE* RNA transcript concentrations tended to follow microcystin concentrations, whereas the *Planktothrix mcyE* DNA toxin gene concentrations remained more temporally consistent for each individual site (fig. 14). Because *Microcystis mcyE* DNA toxin gene concentrations were on average four orders of magnitude lower than *Planktothrix mcyE* DNA toxin gene concentrations, there were no *Microcystis mcyE* RNA transcript detections, and total *Microcystis* DNA gene concentrations were significantly correlated (negative) with microcystin concentrations, it is hypothesized that the genus *Microcystis* was not a major contributor of microcystin occurrence in GLSM during the 2011–12 sampling periods.

Molecular Method Results Compared to Select Field Data, Nutrients, Major Ions, and Trace Elements

Total cyanobacteria DNA gene, *Microcystis mcyE* DNA toxin gene, and *Planktothrix mcyE* DNA toxin gene concentrations were significantly correlated to DO (negative), water transparency (negative), and total nitrogen (table 14). The negative correlation between three DNA assay concentrations and DO may be owing to degradation of the cyanobacteria, where cell lysis can cause decreasing oxygen levels (Ernst and others, 2001; Sivonen and others, 1990; Lindholm and Meriluoto, 1991). The negative correlation between water transparency and multiple molecular methods may be attributed to the fact that when cyanobacteria are blooming, water clarity diminishes. Nitrogen to phosphorus ratios (N:P) were significantly correlated to total *Microcystis* DNA gene concentrations (negative) and *Planktothrix mcyE* DNA toxin gene concentrations (table 14). A negative correlation is expected since low N:P ratios are associated with cyanobacterial dominance (Smith, 1983). The significantly positive correlation between N:P ratios and *Planktothrix mcyE* DNA toxin gene concentrations is likely owing to increased growth of *Planktothrix* (which was the dominant genus in GLSM during this study period) with higher nitrogen concentrations in a nutrient replete environment. In other studies, investigators suggest that total nitrogen and total phosphorus individually may be better predictors of cyanobacterial dominance than N:P ratios (Trimbee and Prepas, 1987; Downing and others, 2001). *Planktothrix mcyE* RNA transcript concentrations were significantly correlated only to water transparency (negative) (table 14). Nutrient concentrations may not relate to RNA transcript concentrations because multiple RNA transcripts could come from one cell if the *mcyE* gene was being expressed (Alberts and others, 2002).

The major ion and trace element concentrations that were correlated to microcystin concentrations also were correlated to *Planktothrix mcyE* DNA toxin gene concentrations with the addition of molybdenum: sodium (rho = 0.94, $p = 0.0048$), molybdenum (rho = 0.88, $p = 0.0188$), antimony (rho = 0.94, $p = 0.0048$), and lithium (rho = −0.83, $p = 0.0416$). More data are needed before drawing any conclusions about these correlations, because only six samples were collected for major ions and trace elements at one buoy site.

Table 14. Spearman correlation values for molecular methods against microcystin, cyanobacteria biovolumes, and select nutrients from six buoy sites in Grand Lake St. Marys, Ohio, 2011–12.

[ELISA, enzyme-linked immunosorbent assay; N:P ratio, ratio of total nitrogen to total phosphorus; qPCR, quantitative polymerase chain reaction; DNA, deoxyribonucleic acid; <, less than; p-values are given in parentheses; qRT-PCR, quantitative reverse-transcription polymerase chain reaction; RNA, ribonucleic acid; N/A, not applicable because all samples from one of the methods were undetected; significant correlations are shaded, with significance = $p < 0.5$; qRT-PCR assays were done at buoys 1 and 2 only]

Assay	Microcystin by ELISA	Total cyanobacteria by biovolume	*Microcystis* by biovolume	*Planktothrix* by biovolume	Dissolved oxygen	Water transparency	Total phosphorus	Total nitrogen	N:P ratio	Chlorophyll
qPCR assays										
Total cyanobacteria DNA genes	0.01 (0.9096)	0.19 (0.1193)	0.03 (0.8263)	0.15 (0.2183)	−0.26 (0.0319)	−0.29 (0.0192)	0.54 (<0.0001)	0.27 (0.0387)	−0.20 (0.1317)	0.44 (0.0068)
Total *Microcystis* DNA genes	−0.46 (0.0001)	−0.30 (0.0155)	0.12 (0.3340)	−0.26 (0.0330)	0.04 (0.7392)	0.22 (0.0782)	0.20 (0.1054)	0.17 (0.1993)	−0.35 (0.0062)	0.30 (0.0697)
Microcystis mcyE DNA toxin genes	0.51 (<0.0001)	0.16 (0.1973)	0.05 (0.6657)	0.15 (0.2196)	−0.29 (0.0193)	−0.57 (<0.0001)	0.34 (0.0059)	0.32 (0.0112)	0.07 (0.5698)	0.32 (0.0556)
Planktothrix mcyE DNA toxin genes	0.65 (<0.0001)	0.37 (0.0024)	0.01 (0.9546)	0.29 (0.0207)	−0.29 (0.0199)	−0.72 (<0.0001)	0.22 (0.0765)	0.47 (0.0002)	0.48 (<0.0001)	0.59 (0.0001)
qRT-PCR assays										
Microcystis mcyE RNA transcripts	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Planktothrix mcyE RNA transcripts	0.63 (0.0016)	0.27 (0.2203)	N/A	0.24 (0.2748)	0.17 (0.4384)	−0.51 (0.0155)	−0.25 (0.2621)	−0.04 (0.8550)	0.37 (0.1045)	−0.12 (0.6757)

Summary and Conclusions

Grand Lake St. Marys (GLSM), in northwest Ohio, is the largest man-made lake in Ohio. The shallow lake is roughly 9 by 3 miles and averages only about 8 feet deep. The water quality in GLSM has been impacted by nutrients from multiple sources in the watershed, which may have contributed to harmful algal blooms of cyanobacteria. Cyanobacteria can produce a variety of compounds that can affect water quality including toxic compounds that can affect human and animal health. In 2009, water samples collected from the GLSM by the Ohio Environmental Protection Agency (OEPA) showed concentrations of the cyanobacteria toxin—microcystin—above recommended guidelines for the majority of the sampling season.

From spring to fall in 2011 and 2012, a total of 66 water samples were collected from six sites in GLSM. These samples were analyzed for phytoplankton identification, concentrations of nutrients, and the toxin, microcystin. Analysis by quantitative polymerase chain reaction (qPCR) and quantitative reverse-transcription polymerase chain reaction (qRT-PCR) was used to help identify the relations between microcystin concentrations and *Planktothrix* and *Microcystis aeruginosa* (toxic versus non-toxic). Other analyses included zooplankton identification, and concentrations of chlorophyll, major ions, and trace elements. Other data collected included parameters such as water transparency, temperature, dissolved oxygen (DO), pH, and specific conductance.

Water temperatures peaked above 30 degrees Celsius (°C) in July during both years, and data from the Celina Water Treatment Plant showed that the 5-day running average of morning temperatures (generally the coolest of the day) in excess of 25 °C occurred for more than 75 days in some years from 2002 to 2012. Water transparency, as measured with Secchi disks, was generally less than 0.3 meters (m); data from earlier decades show this low water transparency is not unusual for GLSM. Water transparencies of less than 0.5 m are one indicator of hypereutrophic conditions. The chlorophyll concentrations were all greater than 56 micrograms per liter (µg/L), which also is indicative of hypereutrophic conditions. DO concentrations ranged from 0.1 milligram per liter (mg/L) near the lake bottom to 20 mg/L at the surface.

Microcystin concentrations ranged from 7.3 to 83 µg/L. Nitrate concentrations ranged from 0.19 to 3.23 mg/L for 66 samples collected from GLSM during 2011–12; however, concentrations in 60 of the samples were less than 1 mg/L. Total nitrogen concentrations ranged from 1.86 to 5.42 mg/L. Orthophosphate (as P) concentrations ranged from less than 0.004 to 0.067 mg/L, although concentrations in 53 of the samples were less than 0.004 mg/L. Total phosphorus (as P) concentrations ranged from 0.12 to 0.43 mg/L.

Cyanobacteria dominated the phytoplankton community in GLSM ranging from 50 to more than 90 percent of the biovolume, with *Planktothrix* the dominant species of cyanobacteria. Microcystin concentrations were correlated to total cyanobacteria and *Planktothrix* biovolumes, and to concentrations of the ions sodium, molybdenum, lithium, and antimony. Concentrations of cyanobacteria found by qPCR showed consistent low potential for toxic *Microcystis* and consistent high potential for toxic *Planktothrix* throughout both sampling years. Concentrations of ribonucleic acid (RNA) transcripts by qRT-PCR showed that toxin gene expression varied throughout the season. *Planktothrix mcyE* deoxyribonucleic acid (DNA) toxin gene concentrations were correlated to microcystin concentrations, total cyanobacteria and *Planktothrix* by biovolume, DO, water transparency, total nitrogen, chlorophyll, and same ions as microcystin. *Microcystis mcyE* DNA toxin gene concentrations were lower than *Planktothrix mcyE* DNA toxin gene concentrations, there were no *Microcystis mcyE* RNA transcript detections, and total *Microcystis* DNA gene concentrations had a significant negative correlation with microcystin; therefore, it is hypothesized that the genus *Microcystis* was not a major contributor of microcystin occurrence in GLSM during the study period.

Although there were no clear links between water quality and the production of microcystin, it appears that a number of conditions, in addition to nutrient loads, may be playing a role in the dominance of cyanobacteria and *Planktothrix* in GLSM and possibly in the production of microcystin.

- The lake is shallow with a long fetch, which contributes to turbid and warm water conditions. Secchi-disk data show that there is very low-light transmission in the lake, and *Planktothrix* is well adapted to low-light conditions.

- Summer water temperatures in GLSM frequently exceed 25 °C, and peak temperatures greater than 30 °C can occur. Temperatures greater than 15 °C favor cyanobacteria, and *Planktothrix* tolerates a wider range of temperatures than other cyanobacteria and may grow best in the 20–30 °C range.

- Low DO conditions at the lake bottom can release phosphorus that is bound to the sediments. This internal recycling of phosphorus can add to the nutrient load in the lake.

- Sodium concentrations appear to have increased since the 1970s, and some studies have found that the sodium requirements for some species of cyanobacteria are significant.

- Sulfur appears to have a role in the production of microcystin in *Microcystis aeruginosa* (Long, 2010; Jahnichen and others, 2011), with low concentrations (less than 1 mg/L) of sulfate possibly inhibiting the production of the toxin. Sulfate concentrations in GLSM ranged from 35.6 to 69.6 mg/L; therefore, microcystin production from *Microcystis aeruginosa* would not have been inhibited owing to low sulfate concentrations.

- The warm temperatures and low silica and manganese concentrations are not favorable to diatoms, which are a competitor to cyanobacteria. In five of six samples, concentrations of silica in GLSM were less than 3.7 mg/L, and manganese concentrations were less than 3.3 µg/L.
- Other trace elements found in the carbonate aquifer in the area such as strontium, vanadium, and boron were greater than the average for surface waters, and these elements may favor the growth of cyanobacteria.

Future Studies

In future studies, work is needed to determine if a lag time exists between high biovolume/molecular toxin gene concentrations and high microcystin toxin concentrations. Microcystin is typically released during cell lysis; therefore, biovolume and toxin gene concentrations may be on the decline when toxin concentrations are increasing. This may explain why relations with microcystin concentrations were not stronger in this study.

Micronutrients are another topic that could be studied further. Several micronutrients (vanadium, strontium, and boron) had concentrations in GLSM greater than most surface waters. These micronutrient concentrations may be contributing to the production of microcystin and plankton occurrence in GLSM. Additionally, with a dataset of only six samples, four micronutrients were found to be correlated to microcystin, with lithium having a very strong negative correlation. It would be interesting to see if these correlations held up with a more robust dataset.

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