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Estimating microcystin levels at recreational sites in western Lake Erie and Ohio

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ABSTRACT

Cyanobacterial harmful algal blooms (cyanoHABs) and associated toxins, such as microcystin, are a major global water-quality issue. Water-resource managers need tools to quickly predict when and where toxin-producing cyanoHABs will occur. This could be done by using site-specific models that estimate the potential for elevated toxin concentrations that cause public health concerns. With this study, samples were collected at three Ohio lakes to identify environmental and water-quality factors to develop linear-regression models to estimate microcystin levels. Measures of the algal community (phycocyanin, cyanobacterial biovolume, and cyanobacterial gene concentrations) and pH were most strongly correlated with microcystin concentrations. Cyanobacterial genes were quantified for general cyanobacteria, general *Microcystis* and *Dolichospermum*, and for microcystin synthetase (*mcyE*) for *Microcystis*, *Dolichospermum*, and *Planktothrix*. For phycocyanin, the relations were different between sites and were different between hand-held measurements on-site and nearby continuous monitor measurements for the same site. Continuous measurements of parameters such as phycocyanin, pH, and temperature over multiple days showed the highest correlations to microcystin concentrations. The development of models with high R^2 values (0.81–0.90), sensitivities (92%), and specificities (100%) for estimating microcystin concentrations above or below the Ohio Recreational Public Health Advisory level of 6 $\mu g\,L^{-1}$ was demonstrated for one site; these statistics may change as more data are collected in subsequent years. This study showed that models could be developed for estimates of exceeding a microcystin threshold concentration at a recreational freshwater lake site, with potential to expand their use to provide relevant public health information to water resource managers and the public for both recreational and drinking waters.

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1. Introduction

Cyanobacterial harmful algal blooms (cyanoHABs) cause a multitude of water-quality concerns, including the potential to produce potent toxins in rivers and lakes used for recreation and source-water supplies. These toxins have been implicated in human and animal illness and death in over fifty countries and in at least 36 states in the United States (Graham et al., 2009). The human health risk from cyanoHABs commonly is associated with ingestion or inhalation of toxins during recreational activities (Chorus and Bartram, 1999). Because the incidence of cyanoHABs has been increasing in frequency and severity worldwide (Paerl et al., 2011), water-resource managers need tools to predict when and where toxin-producing cyanoHABs will occur (He et al., 2016). Satellites that collect multispectral data have been used to predict and detect cyanoHABs in lakes and estuaries, including western Lake Erie (Wynne et al., 2013). Satellite data often include estimates of phycocyanin, chlorophyll-a, and cell counts; however, this technology has several challenges because cyanobacterial toxins cannot be directly detected by remote sensing (Lunetta et al., 2015; Stumpf et al., 2016). Although satellite data currently are useful on a broad scale and as an initial warning system for cyanoHAB occurrence, site-specific models are needed to estimate the potential for elevated toxin concentrations that cause public health concerns.

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Microcystins are one of the most frequently detected hepatotoxins in freshwaters and are commonly produced by cyanobacteria in the genera *Microcystis*, *Planktothrix* and *Dolichospermum* (formerly *Anabaena)* (Rantala et al., 2006). Not all strains of these cyanobacterial genera produce toxins, and not all toxin-producing strains continuously produce toxins. Microscopy has been used traditionally to identify and quantify cyanobacteria genera that may produce toxins; however, microscopy cannot identify whether a strain has the ability to produce toxins. Molecular methods, such as quantitative polymerase chain reaction (qPCR), can quantify specific toxin-producing genes from known microcystin-producing cyanobacterial genera. In particular, qPCR can be used to quantify cyanobacteria that contain the microcystin synthetase (*mcy*) gene cluster that is required for microcystin production in the cell.

Currently, toxin measurements require that a sample be collected and analyzed in a laboratory with varying amounts of sample processing and analysis time before results are available. In the interim between sample collection and obtaining results, potential exposures to the public from using or consuming a water source may have already occurred. Models provide the opportunity for public health protection prior to exposure and allow users to be proactive rather than reactive; however, predictions are complicated and likely site specific because of the many factors affecting toxin production.

Many studies have been done worldwide to identify factors that affect cyanoHAB occurrence in freshwater lakes. For those who manage and use a water body for recreation or source-water supply, however, it is important to identify the factors that are related to human and animal health risk—that is, the factors related specifically to toxin concentrations. In a few previous studies, investigators identified factors related to microcystin concentrations on a site-specific level (Joung et al., 2011; Lee et al., 2015; Otten et al., 2012). Other investigators found significant relations between qPCR results for cyanobacteria or cyanobacterial toxin genes and microcystin concentrations (Fortin et al., 2010; Otten et al., 2012; Rinta-Kanto et al., 2009; Conradie and Barnard, 2012; Davis et al., 2009). Optical sensors that provide an indicator of algal pigments (chlorophyll and phycocyanin) have been shown to be promising for providing early warnings of cyanobacterial abundance or elevated microcystin concentrations in recreational waters (Marion et al., 2012) and source-water supplies (Brient et al., 2008; Izydorczyk et al., 2005; McQuaid et al., 2011). Lacking among these previous studies, however, were comprehensive examinations of all types of environmental factors affecting toxin concentrations that could potentially be used in site-specific models.

This study was done to work towards providing water resource managers with models to estimate as quickly as possible the human and animal health risk associated with cyanoHABs. We identified factors that could be used to develop two types of models to estimate microcystin concentrations at freshwater recreational sites. Real-time models include easily- or continuously-measured factors and available environmental data that do not require a sample be collected. Comprehensive models include results from samples collected and analyzed in a laboratory along with real-time factors. The objectives were to (1) identify the environmental and cyanobacterial community composition factors (as measured by microscopy and molecular methods) that were significantly correlated with microcystin concentrations at lake sites and (2) test the feasibility of developing models for estimates of toxin levels. Samples and data were collected at three Ohio lakes, where cyanobacterial proliferation and elevated microcystin concentrations have caused water-resource managers to issue water-quality advisories (Ohio Environmental Protection Agency, 2014).

2. Materials and methods

2.1. Study lakes and sampling frequency

Water-quality and environmental data are presented for samples collected at one site each on three lakes in Ohio, USA (Fig. 1, Buckeye Lake, Harsha Lake, and Lake Erie), that were part of a larger study. Data from the larger study are presented in a companion report that describes weekly to monthly sampling at 11 sites on 8 lakes in Ohio before, during and after the cyanoHAB season during 2013–14 (Francy et al., 2015). The three sites were selected for this article because they were included in the more frequent weekly to semiweekly sampling during 2014.

Buckeye Lake is a man-made lake located approximately 30 miles east of Columbus, Ohio. The Buckeye Lake Onion Island site (39N54′31″, 82W31′00″) was added to the sampling network in 2014 because it is a popular offshore boater swim area. During June–October, 2014, 10 samples were collected at Buckeye Onion Island. William H. Harsha Lake (Harsha Lake) is a reservoir located about 25 miles east of Cincinnati, Ohio. During May–October 2014, 17 samples were collected at an official bathing beach (Harsha Main, 39N01′11″, 84W08′03″) that is part of East Fork State Park. Samples collected in 2013 from Harsha Main were not included in this article because different sondes were used in 2013 and 2014 and the phycocyanin and chlorophyll data could not be verified to be sufficiently homogeneous to combine both years. Maumee Bay is located in the western basin of Lake Erie, east of Toledo, Ohio. For the purpose of this article, Maumee Bay is considered a discrete entity, although it is part of Lake Erie. During May–November, 2013–14, 24 samples were collected at the Maumee Bay State Park (MBSP) Lake Erie beach (41N41′11″, 83W22′32″).

Morphometric, land-use, chemical, physical, and cyanobacterial community composition characteristics of the three study lakes are shown in Table 1. Buckeye Lake and Maumee Bay are both shallow (mean depth <3 m) and Maumee Bay has the largest area among the three lakes. The study lakes are predominantly located in agricultural watersheds (>60% agriculture), with small percentages of impervious surfaces (<4%). Among the physical measurements, pH and average phycocyanin measurements were highest at Buckeye Onion Island and average chlorophyll and turbidity measurements were highest at MBSP Lake Erie. Microcystin concentrations consistently were elevated at Buckeye Onion Island, were lowest at Harsha Main, and ranged most widely at MBSP Lake Erie. At Buckeye Onion Island, cyanobacterial concentrations by qPCR and microscopy were highest (9.1 log copies.100 mL^{-1} and 6.3 log $\mu m^3 mL^{-1}$) among the three sites and *Planktothrix* dominated. Harsha was characterized by a mixed cyanobacterial assemblage and *Dolichospermum*, *Microcystis* and *Planktothrix mcyE* DNA were present in significant quantities. *Microcystis* dominated the cyanobacterial community at MBSP Lake Erie.

2.2. Sample collection and field measurements

At each site, three 1-L subsamples were collected within the designated swimming area from the same depths at Harsha Main and MBSP Lake Erie (1-m depths) or the same locations at Buckeye Onion Island (1–1.5-m depths) throughout the season and composited into a 5-L bottle (Graham et al., 2008). The 1-L bottle was lowered approximately 0.3 m below the water's surface, the lid was removed, and the bottle was filled while bringing it up to the water's surface. Water temperature, pH, dissolved oxygen, specific conductance, chlorophyll, and phycocyanin were measured at each subsample location using a hand-held sensor calibrated and operated by use of standard USGS methods (Wilde, variously dated) or per the manufacturer's instructions (YSI Incorporated,

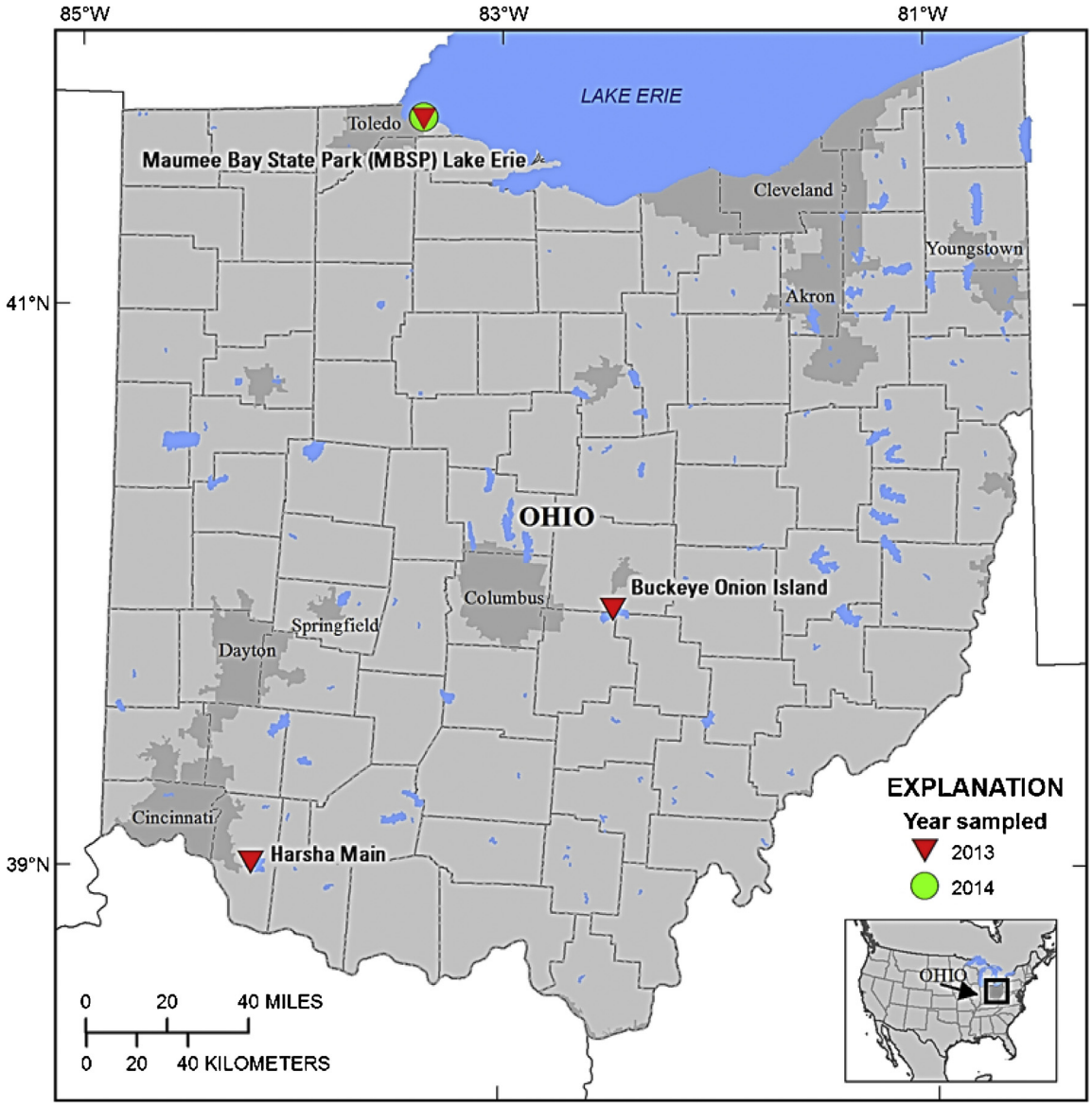

Fig. 1. Locations of recreational sampling sites in Ohio.

Yellow Springs, Ohio). An aliquot from the 5-L composite bottle was removed to measure turbidity by use of a turbidimeter (Hach Company, Loveland, Colo.). Secchi depth was measured using a standard Secchi disk, wave height was measured using a measuring rod, and the observed qualitative algae category was recorded. Algae categories were 0, none; 1, mild; 2, moderate; 3, serious; and 4, extreme.

2.3. Measurement of toxin and nutrient concentrations, phytoplankton community composition, and cyanobacterial genes

Composited samples were processed on-site and preserved for subsequent analysis of toxin and nutrient concentrations, phytoplankton abundance and community composition, and cyanobacterial genes. Details of sample processing and analytical methods are described in Francy et al. (2015) and Wilde et al. (2004–09).

Samples were analyzed at the USGS Organic Geochemistry Research Laboratory in Lawrence, KS, for cylindrospermopsins (2013 only), microcystins/nodularins, and saxitoxins by means of three separate enzyme-linked immunosorbent assays (ELISA, Abraxis LLC, Warminster, PA; Graham et al., 2010). Because cylindrospermopsin was not detected in any samples, and saxitoxin was detected in only 8% of samples from the larger study (Francy et al., 2015), only microcystin results were used for this paper. Samples were analyzed at the USGS National Water Quality Laboratory (NWQL) in Denver, CO, for concentrations of dissolved nitrite, dissolved nitrite plus nitrate, dissolved ammonia, dissolved orthophosphate, total nitrogen, and total phosphorus by standard USGS methods (Fishman, 1993; Patton and Kryskalla, 2003, 2011). Samples were analyzed for phytoplankton abundance and community composition by BSA Environmental Services, Inc., in Beachwood, OH, by standard membrane-filtration and microscopy techniques (Beaver et al., 2013). Samples were analyzed for cyanobacterial genes at the USGS Ohio Water Microbiology Laboratory (OWML) in Columbus, OH, by use of qPCR (DNA) and qRT-PCR (RNA) according to procedures in Stelzer et al. (2013). Molecular assays for cyanobacteria were done to enumerate (1) general cyanobacteria, (2) general *Microcystis* and *Dolichospermum*, (3) *mcyE* DNA toxin genes for *Microcystis*, *Dolichospermum*, and *Planktothrix*, and (4) *mcyE* RNA transcripts for *Microcystis*,

Table 1
Selected morphometric, land-use, chemical, physical, and cyanobacterial community structure characteristics of the three study lakes.

Lake (recreational site)	Buckeye Lake (Buckeye Onion Island)	Harsha Lake (Harsha Main)	Maumee Bay[a] (MBSP Lake Erie)
USGS site identification number	395431082310000	390111084080300	414111083223200
Years sampled (number of samples)	2014 (10)	2014 (17)	2013–14 (24)
Mean lake depth (m), estimated	1.8	>6	<3
Lake area (km^2)	11.6	8	70
Entire lake watershed area (km^2)[b]	127	886	1800
Percent agricultural and urban land use (upstream watershed)[c]	60 ag%; 15 urban%; 14 forest%	64 ag%; 7 urban %; 27% forest	76 ag%; 12 urban %; 7% forest
Percent impervious (upstream watershed)[d]	3.1	1.4	3.4
pH	9.1 ± 0.3	8.9 ± 0.9	8.7 ± 0.6
Phycocyanin (RFU)	23.4 ± 5.9	8.1 ± 7.5	12.5 ± 14.6
Chlorophyll (RFU)	3.8 ± 1.4	2.3 ± 1.5	26.6 ± 25.2
Turbidity (NTU)	53 ± 10	10 ± 6.4	59 ± 50
Microcystin ($\mu g\,L^{-1}$)	49 ± 12	1.8 ± 3.7 (88%)	24 ± 51 (96%)
Total nitrogen ($mg\,L^{-1}$-N)	3.2 ± 0.3	1.1 ± 0.3	2.8 ± 1.4
Total phosphorus ($\mu g\,L^{-1}$-P)	215 ± 70	104 ± 53	128 ± 75
Cyanobacteria by qPCR (log copies.100 mL^{-1})	9.1 ± 0.3	7.9 ± 0.7	6.9 ± 1.1
Cyanobacteria (log $\mu m^3\,mL^{-1}$)	6.3 ± 0.2	5.2 ± 0.5	4.3 ± 1.3 (96%)
Dolichospermum by qPCR (log copies.100 mL^{-1})	6.8 ± 0.5	7.9 ± 0.7	5.8 ± 1.3 (65%)
Dolichospermum (log $\mu m^3\,mL^{-1}$)	3.0 ± 2.6 (60%)	4.9 ± 2.0 (94%)	1.4 ± 1.9 (38%)
Microcystis mcyE DNA (log copies.100 mL^{-1})	4.1 ± 0.6 (90%)	5.1 ± 1.4 (88%)	6.2 ± 1.5 (96%)
Microcystis (log $\mu m^3\,mL^{-1}$)	1.1 ± 1.8 (30%)	4.1 ± 2.6 (76%)	5.4 ± 2.4 (88%)
Planktothrix mcyE DNA (log copies.100 mL^{-1})	8.3 ± 0.3	4.8 ± 0.7 (88%)	4.0 ± 0.4 (33%)
Planktothrix (log $\mu m^3\,mL^{-1}$)	7.4 ± 0.2	4.7 ± 1.4 (94%)	1.3 ± 1.8 (38%)

Chemical, physical, and cyanobacterial characteristics are averages and standard deviations from samples collected at recreational sites during the study years (2013–14); percentages of detections are indicated if <100%; the highest average values among the three lakes for each measured characteristic are shaded.

[a] Maumee Bay was defined by National Oceanic and Atmospheric Administration, Chart 7, at http://www.charts.noaa.gov/PDFs/14846.pdf. Maumee Bay is considered a discrete entity, although it is part of Lake Erie.

[b] From Ohio Department of Natural Resrouces at http://wildlife.ohiodnr.gov/eastforklake,http://wildlife.ohiodnr.gov/buckeyelake, and U.S. Geological Survey at http://water.usgs.gov/osw/streamstats/for Maumee Bay.

[c] From U.S. Geological Survey at http://water.usgs.gov/osw/streamstats/and by use of Geographic Information System.

[d] From the National Land Cover Database (NLCD) at http://www.mrlc.gov/.

Dolichospermum, and *Planktothrix* (Rinta-Kanto et al., 2005; Doblin et al., 2007; Sipari et al., 2010; Vaitomaa et al., 2003; Rantala et al., 2006). Extraction procedures and standard curve data for qPCR and qRT-PCR assays are presented in Francy et al. (2015).

2.4. Environmental factors

Environmental data were compiled for locations in close proximity to each sampling site from a variety of sources, listed in Francy et al. (2015). These environmental data were collected at locations that were within 40 km from a study site, and most were within 16 km. The compiled data included measurements of rainfall and wind speed and direction (National Oceanic and Atmospheric Administration, 2014a), water levels (National Oceanic and Atmospheric Administration, 2014b; U.S. Geological Survey, 2014), daily mean streamflow (U.S. Geological Survey, 2014), and solar radiation (The Ohio State University, 2014). Change in lake level was calculated based on the differences between the 10 a.m. water-level value on the date of sampling relative to the previous day (24 h change) and 7-and 14-days prior. For some environmental factors, values were lagged and (or) averaged for various antecedent time periods, including 24-h, and 3-, 7- 14- and 30-days up to the date and time of sampling, to account for situations where the effect of environmental conditions on cyanobacteria may be delayed. For Harsha Lake, streamflow and water-level data were used to compute an indicator of lake residence times as described in Francy et al. (2015).

Continuous monitor data were collected with sondes suspended from buoys on Harsha Lake for May–October, 2014 (Joel Allen, U.S. Environmental Protection Agency, 2015) and for Lake Erie near Maumee Bay for August–October, 2014 (http://greatlakesbuoys.org/station_page.php?station=45165). The Harsha Lake continuous monitor was mounted 0.5 m below the water's surface on a buoy in open water at a site located about 1.5 km north of the Harsha Main site. The continuous monitor for MBSP Lake Erie was mounted 0.5 m below the water's surface on a structure approximately 10 km northeast of the MBSP Lake Erie site. Twenty-four hour averages (10 a.m.–10 a.m.) of measurements of phycocyanin, pH, temperature, chlorophyll, oxidative reductive potential (Maumee Bay only), and dissolved oxygen (Harsha Lake only) made at 10- or 15-min intervals by continuous monitors were computed; these averages subsequently were used to calculate 3-, 7-, and 14-day averages antecedent to the time of sampling.

2.5. Data management, statistical analysis and model development

Data on field parameters, wave heights, turbidity, water temperature, and nutrient, toxin, and cyanobacterial gene and transcript concentrations are available to the public through the USGS National Water Information System Web Interface (NWISWeb, U.S. Geological Survey, 2014). Data on phytoplankton abundance and community composition are available in Francy et al. (2015).

For data analysis and model development, the factors were segregated based on their potential use in real-time and (or) comprehensive models to estimate microcystin concentrations. Cyanobacterial gene abundance and biovolume data were $\log_{10}$-transformed before data analysis. Nonparametric correlation coefficients (Spearman's rho) were calculated to identify associations between microcystin concentrations and other factors. Spearman's rho measures the strength of the monotonic association between two variables (whether linear or nonlinear) and is resistant to effects of outliers. Multiple minimum reporting limits for molecular methods were accommodated in correlation analyses by assigning them a value lower than the lowest detection. Scatterplots for each variable and microcystin concentration were viewed to confirm that any positive or negative relation was not influenced by one or two data points.

Data exploration and linear-regression model development were done with Virtual Beach version 2.4 (U.S. Environmental Protection Agency, 2015). Virtual Beach is a software package designed for developing site-specific statistical models to estimate bacterial indicator concentrations or the probability of exceeding a threshold level at recreational beaches. Virtual Beach, however, can be used with any dependent variable; in this study, log microcystin concentration was the dependent variable. Virtual Beach was designed to help beach managers make decisions on beach closures and advisories, is user-friendly, and requires that the user have only a basic statistical knowledge.

Using Virtual Beach, independent variables were mathematically transformed as necessary to linearize the relations between the independent and dependent variables. The prediction error sum of square (PRESS) was used for model selection. Explanatory variables were limited to a maximum variance inflation factor (VIF) of 3 (to avoid harmful multi-collinearity among explanatory variables) and models were limited to no more than three explanatory variables. Model diagnostics and selection were done as described in Francy and Darner (2006) and U.S. Environmental Protection Agency (2015) and included tests for (1) statistical significance of explanatory variables, (2) influence and leverage of observations, (3) normality and homoscedasticity of residuals, and (4) development of scatterplots to ensure relations between explanatory variables and the dependent variable were linear. The Durbin–Watson test was used to test for autocorrelation of residuals. A final criterion for model selection was to be able to reasonably explain how each explanatory variable could potentially affect the observed variation in microcystin concentrations.

The output from each selected model was the probability of exceeding the Ohio Recreational Public Health Advisory level of 6 $\mu g\,L^{-1}$ (Ohio Environmental Protection Agency, 2014). This was done to obtain an estimate of the public health acceptability of the water's use. Model outputs were examined in terms of sensitivity, specificity, and accuracy in estimating microcystin concentrations above or below 6 $\mu g\,L^{-1}$. A threshold probability was set for each model by examining model-output sensitivities and specificities at different probability levels. The selection of the threshold is a compromise between false negative and false positive responses while maintaining a high number of overall correct responses (Francy and Darner, 2006).

As part of the Virtual Beach modeling process, daily averages of wind speed and direction vectors were used to calculate alongshore (wind A) and onshore/offshore (wind O) wind components in relation to the beach's orientation from geographical north (U.S. Environmental Protection Agency, 2015). The alongshore (wind A) components accounted for wind moving parallel to the beach whereas the onshore/offshore (wind O) components accounted for wind moving perpendicular to the beach. At MBSP Lake Erie, a positive wind A value represents winds moving generally from east to west whereas a negative wind A value represents winds moving from west to east. Similarly, winds moving onshore (north to south) had a positive wind O component. Wind components were summed for various time periods, including 3-, 5-, and 7-days.

3. Results

3.1. Relations between microcystin concentrations and environmental and water-quality factors

Factors for real-time models were grouped into those that were (a) measured with a hand-held meter or observed at the site, (b) measured with a nearby continuous monitor, and (c) compiled from other sources of available environmental data (Table 2). Factors for comprehensive models were grouped based on the type of analysis: (a) nutrients, (b) cyanobacterial genes, and (c) cyanobacterial biovolume or abundance (Table 3). Those factors that were significantly correlated with microcystin concentrations in samples collected from at least one of three sites are included in Tables 2 and 3.

Hand-held and continuous water-quality measurements are used to develop models that could be used in real-time. Several hand-held measurements were significantly correlated ($p < 0.05$) with microcystin concentrations at Harsha Main and MBSP Lake Erie, but only two were significant at Buckeye Onion Island (Table 2a). Hand-held measurements of phycocyanin and Secchi depth were significantly correlated with microcystin at all three sites. For continuous monitor data at Harsha Main (May–October), many antecedent time periods for phycocyanin, pH, temperature, and dissolved oxygen were significantly correlated with microcystin concentrations (Table 2b). In contrast, for continuous monitor data at MBSP Lake Erie (August–October), statistically significant correlations were found for only phycocyanin and oxidative reductive potential, likely due to the small dataset ($n = 6$–8). Although measurements of phycocyanin were significantly correlated with microcystin concentrations at all three sites, the relations were different between sites and between hand-held measurements at the site and nearby continuous monitor measurements at the same site (Fig. 2). Factors that were not significantly correlated at any site were specific conductance (hand-held and continuous monitor measurements), dissolved oxygen (on site measurements), and wave height.

Among environmental factors for real-time models, several timespans of streamflow and the wind O component (previous 5 days) were significantly correlated with microcystin concentrations at MBSP Lake Erie, lake level change over 24 h was significant at Buckeye Onion Island, and no factor was significant at Harsha Main (Table 2c). Environmental factors that were not significantly correlated at any site were rainfall, wind speed, barometric pressure, solar radiation (measured at MBSP Lake Erie), and residence times (calculated for Harsha Main). The wind O component at MBSP Lake Erie can be explained further by examining time–series plots (Fig. 3). Except for a period in early September 2014, strong offshore winds (negative wind O component values) were associated with periods of lower microcystin concentrations in 2013 and 2014. The highest microcystin concentration during 2014 occurred after a 10-day period of weak onshore winds (Fig. 3B) suggesting that the winds may have been keeping the bloom close to the beach during that time.

Among factors for comprehensive models, ammonia concentrations (negative correlation) and general cyanobacterial genes were the only two that were significantly correlated with microcystin concentrations at all three sites (Table 3). The most highly correlated molecular assays were *Planktothrix mcyE* DNA at Buckeye Onion Island and *Microcystis mcyE* DNA at Harsha Main and MBSP Lake Erie (Fig. 4, Table 3b). Several measures of cyanobacterial abundance and biovolume were significantly correlated with microcystin concentrations at Harsha Main and MBSP Lake Erie, but not at Buckeye Onion Island (Table 3c). Factors for comprehensive models that were not significantly correlated at any site were *Planktothrix mcyE* RNA, and abundance and biovolume for *Planktothrix* and other microcystin producers.

3.2. Models for estimating microcystin levels at an Ohio beach

As a demonstration of the feasibility of developing models for estimating microcystin levels at recreational lake sites, models were developed for MBSP Lake Erie, where there were 2 years of data and the largest data set ($n = 24$) among the three study sites. Factors based on continuous monitor measurements (10- or 15-min interval data from sondes suspended from buoys) were not

Table 2
Spearman Rank correlations (rho) between microcystin concentrations and real-time model factors that were statistically significant in samples collected from at least one of three Ohio recreational sites.

Variable	Buckeye Onion Island $n = 10$	Harsha Main $n = 17$	MBSP Lake Erie $n = 24$[a]
(a) Hand-held measurements or observations at the site			
Phycocyanin sensor, RFU	**0.79**	**0.93**	**0.85**
Secchi depth, feet	**−0.76**	**−0.69**	**−0.67**
Turbidity, NTU	0.62	**0.73**	**0.80**
pH	0.26	**0.87**	**0.80**
Algae category	0.17	**0.72**	**0.62**
Chlorophyll sensor, RFU	−0.12	**0.63**	0.32
Water temperature, °C	0.01	**0.59**	0.10
(b) Nearby continuous monitor measurements			
Phycocyanin, 14-day ave	–	**0.90**	**1.00**
Phycocyanin, 7-day ave	–	**0.98**	**0.96**
Phycocyanin, 24-h ave	–	**0.94**	**0.71**
Phycocyanin, 3-day ave	–	**0.94**	0.57
pH, 14-day ave	–	**0.73**	0.77
pH, 7-day ave	–	**0.83**	0.57
pH, 24-h ave	–	**0.76**	0.17
pH, 3-day ave	–	**0.73**	0.17
Temperature, 7-day ave	–	**0.57**	0.64
Temperature, 3-day ave	–	**0.58**	0.60
Temperature, 24-h ave	–	**0.56**	0.60
Chlorophyll, 24-h ave	–	**0.53**	−0.12
Oxidative reductive potential, 14-day ave	–	–	**−0.83**
Oxidative reductive potential, 7-day ave	–	–	**−0.89**
Oxidative reductive potential, 3-day, 24-h ave	–	–	**−0.98**
Dissolved oxygen, 14-day ave	–	**0.88**	–
Dissolved oxygen, 7-day ave	–	**0.67**	–
Dissolved oxygen, 3-day ave	–	**0.55**	–
Dissolved oxygen, 24-h ave	–	**0.56**	–
(c) Environmental factors compiled from other sources			
Lake level change, 24-h	**−0.71**	−0.09	−0.36
Streamflow, daily mean, 3-day lagged	–	0.19	**−0.69**
Streamflow, 30-day ave daily means	–	0.17	**−0.53**
Streamflow, 14-day ave daily means	–	0.29	**−0.56**
Streamflow, 7-day ave daily means	–	0.29	**−0.47**
Wind O component, previous 5 days[b]	ND	ND	**0.42**

Correlations that were significant at $p < 0.05$ are in bold; shaded cells indicate the highest rho for each site in each factor category; –, the factor was not measured; ave, average; ND, not determined.
[a] Continuous monitor data for MBSP were only available from August 8–October 31 and included 6–8 samples for various factors.
[b] Onshore/offshore wind component calculated from wind speed and direction vectors for the previous 5 days, measured at Toledo Executive Airport (TDZ).

used in model development because of the limited timespan of data (August–September, 2014). Models were developed for MBSP Lake Erie based on (1) real-time variables (hand-held measurements at the site and data compiled from other sources), (2) all comprehensive variables, and (3) comprehensive variables excluding measurements of cyanobacterial abundance and biovolume. The third model category was created to include a model that relied on cyanobacterial gene concentrations, results of which can be obtained within 3 h, and excluded variables for the more subjective and laborious microscopy analysis. These models are not intended for use by beach managers, but are rather exploratory work to demonstrate the modeling steps that can be taken to aide in management decisions. Site-specific data collection and analysis over multiple seasons are required to develop and validate models before they can be practically applied.

For MBSP Lake Erie, adjusted R^2 values for selected models ranged from 0.81 to 0.90 (Table 4). Threshold probabilities established to maximize sensitivities and specificities in terms of correctly predicting exceedance or non-exceedance of the Ohio Recreational Public Health Advisory of 6 μg L^{-1} microcystin ranged from 24% to 35%. Sensitivities and specificities for all three models were 92% and 100%, respectively, and overall accuracy was 96% (data not shown). Phycocyanin and pH were included as explanatory variables in two out of three models. The model with all comprehensive variables included *Microcystis* biovolume, and the model with variables for cyanobacterial abundance and biovolume excluded included *Microcystis mcyE* DNA.

The parameters in each final model were statistically significant ($\alpha = 0.05$). The influence statistic, Cook's D, exceeded 0.1667 for two observations in the real-time model. Although influential, these observations were examined and could not be discredited, and so were used in the regression. For the real-time model and comprehensive model that excluded microscopy data, no significant autocorrelation of residuals was detected. However, for the comprehensive model with all data, second-order autocorrelation was indicated. An autocorrelation-corrected model was developed using the AUTOREG procedure in SAS (SAS Institute Inc., Cary, NC) and compared to the VB model. Differences between the mean squared errors and the parameter estimates of the two models were negligible. Consequently, we decided to use the VB model rather than switch to the more complex autocorrelation-corrected model.

4. Discussion

4.1. Relations between microcystin concentrations and environmental, water-quality, and cyanobacterial community composition factors

This study showed that there were many factors significantly correlated with microcystin concentrations that could potentially

Table 3
Spearman Rank correlations (rho) between microcystin concentrations and comprehensive model factors that were statistically significant in samples collected from at least one of three Ohio recreational sites.

Variable	Buckeye Onion Island $n = 10$	Harsha Main $n = 17$	MBSP Lake Erie $n = 24$
(a) Nutrients			
Ammonia, mg L^{-1}-N	**−0.58**	**−0.53**	**−0.78**
Nitrate + nitrite, mg L^{-1}-N	−0.35	**−0.53**	**−0.64**
Nitrite, mg L^{-1}-N	−0.35	−0.14	**−0.68**
Orthophosphate, mg L^{-1}-P	ND	**−0.48**	−0.30
Total phosphorus, mg L^{-1}-P	0.33	−0.09	**0.78**
Total nitrogen, mg L^{-1}-N	**0.72**	0.32	−0.06
N to P ratio (nitrogen to phosphorus, total)	−0.02	0.45	**−0.63**
(b) Cyanobacterial genes (log copies.100 mL^{-1})			
Cyanobacteria, general	**0.71**	**0.57**	**0.49**
Microcystis	0.12	**0.90**	**0.73**
Microcystis mcyE RNA	ND	0.40	**0.58**
Microcystis mcyE DNA	−0.30	**0.92**	**0.82**
Dolichospermum	−0.16	**0.90**	0.41
Planktothrix mcyE DNA	**0.73**	0.10	−0.09
(c) Cyanobacterial biovolume (μm^3 mL^{-1}) or abundance (cells mL^{-1})			
Cyanobacterial abundance	0.24	**0.63**	**0.84**
Cyanobacterial biovolume	−0.14	**0.90**	**0.86**
Microcystis biovolume	−0.05	**0.89**	**0.87**
Microcystis abundance	−0.05	**0.88**	**0.86**
Dolichospermum biovolume	−0.27	**0.89**	0.16
Dolichospermum abundance	−0.27	**0.89**	0.16
Non-microcystin cyanobacteria, biovolume	−0.45	**0.48**	0.26

Correlations that were significant at $p < 0.05$ are in bold; shaded cells indicate the highest rho for each site in each factor category; ND, not determined because the factor lacked any detected values.

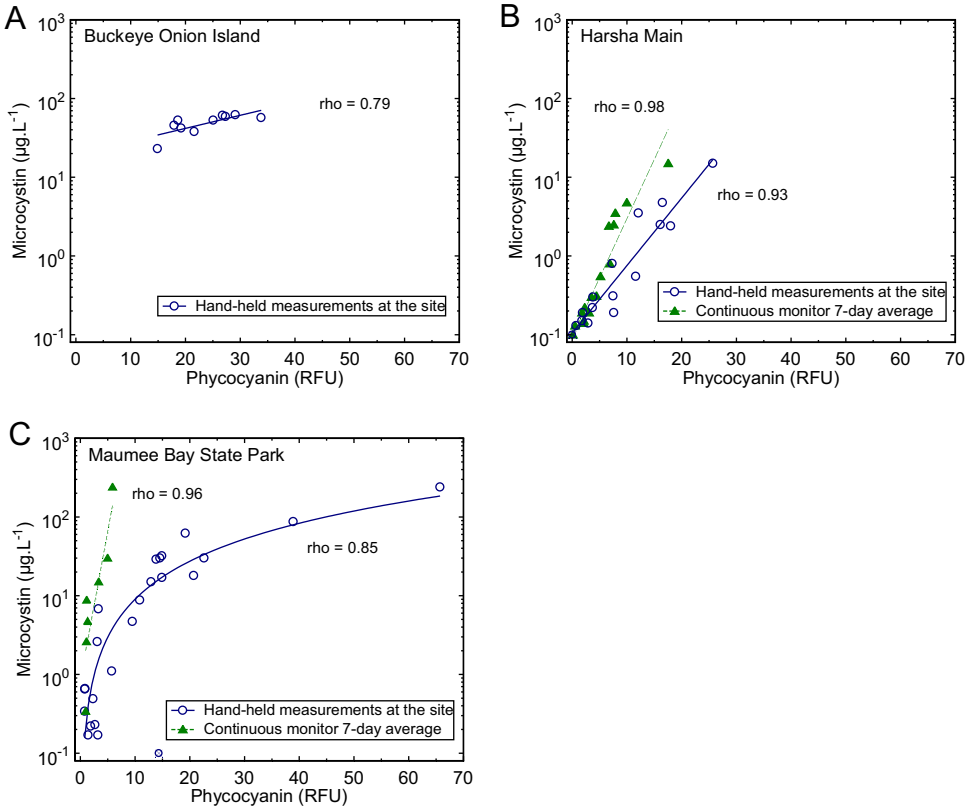

Fig. 2. Relations between hand-held measurements at the site and nearby continuous monitor measurements of phycocyanin and microcystin concentrations at three lake sites. Spearman's rank correlations were all statistically significant at $p < 0.05$. The best fit lines (linear or $\log_{10}$) are included on each graph.

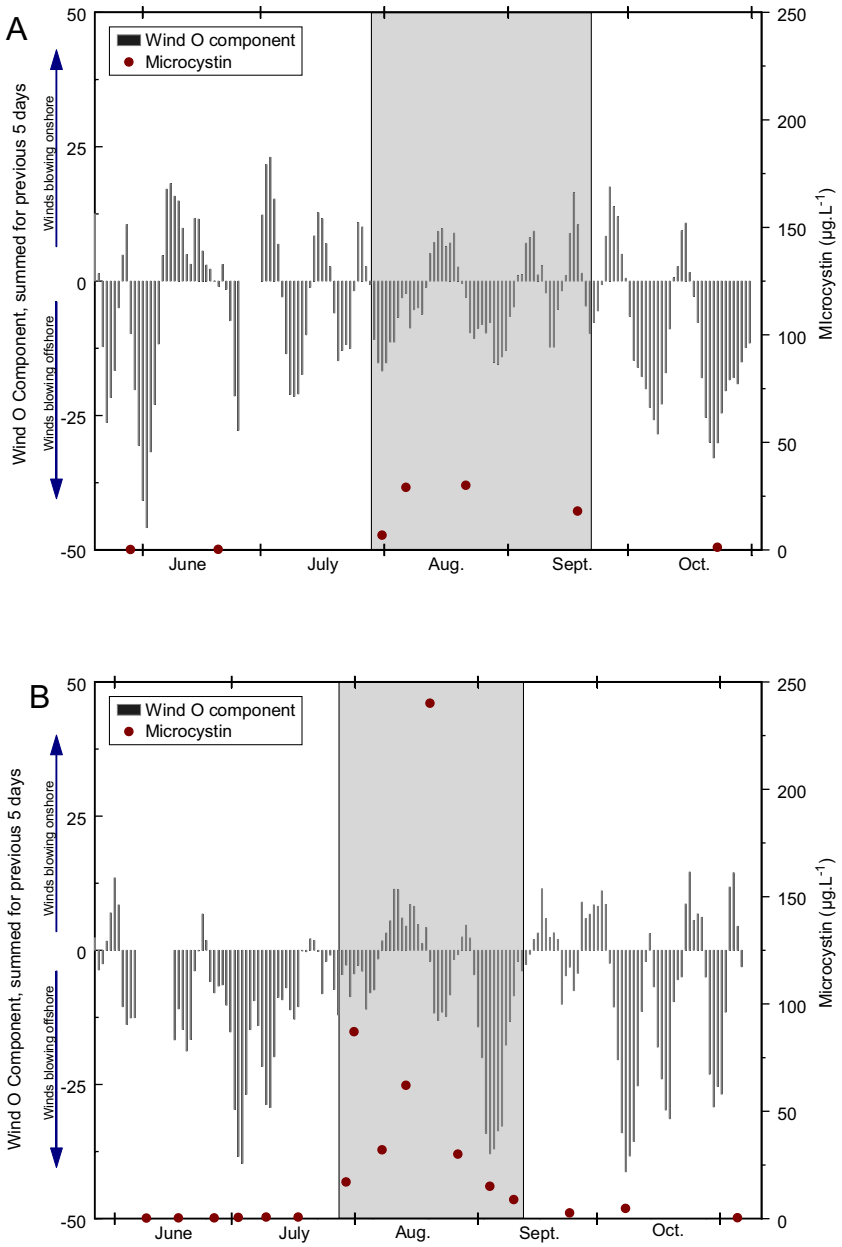

Fig. 3. Time–series plots of wind O component and microcystin concentrations at Maumee Bay State Park Lake Erie Beach in (A) 2013 and (B) 2014. The shaded area shows the cyanoHAB period indicated when microcystin concentrations were >6 µg L^{-1}.

be used in real-time and comprehensive models to estimate microcystin levels. Microcystin was detected in 94% of the 51 samples collected during this study at three different recreational freshwater sites. The sites included an offshore boater swim area on a shallow inland lake (Buckeye Onion Island), a beach on an inland reservoir (Harsha Main), and a beach in the western basin of Lake Erie (MBSP Lake Erie). Factors for real-time models are those that are easily and quickly measured, including those from hand-held and continuous monitor measurements and observations and environmental data from other sources. Factors for comprehensive models require that a sample be collected and analyzed in a laboratory. Although more effort and time is required to obtain laboratory results, as more data are collected in future studies, comprehensive factors may provide valuable information about the dynamics of cyanobacterial community structure and toxin production. With more frequent data collection, they may also be used to provide advanced warnings of a cyanoHAB.

Among measurements and observations for real-time models made at the site, phycocyanin and Secchi depth were significantly correlated with microcystin concentrations at all three sites (average rho = 0.86 and −0.71, respectively); turbidity, pH, and algae category were significant at two sites. It has been shown (Hansen, 2002) that cyanobacterial blooms significantly increase the pH of the water column due to consumption of dissolved CO_2. Although algal category is an easy observation to record, its usefulness may be limited by subjectivity among different field staff. In a shallow inland lake in the northwest U.S, Lee et al. (2015) also found that turbidity was positively associated and Secchi depth was negatively associated with microcystin concentrations. Cyanobacteria have an affinity for low light conditions and can also reduce water clarity. Significant correlations were found between microcystin concentrations and laboratory-measured chlorophyll-a in a Korean Reservoir, s (r = 0.80) (Joung et al., 2011) and in Lake Taihu, China (r = 0.91) (Otten et al., 2012). In the present study,

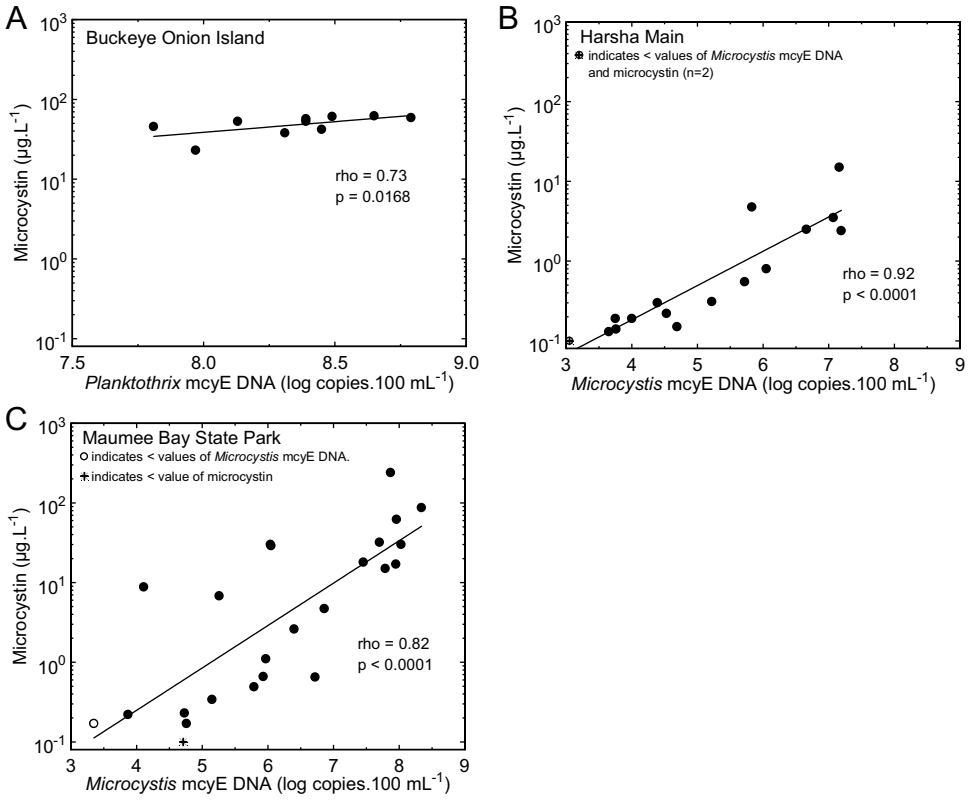

Fig. 4. Graphs showing the highest Spearman's rank correlation between cyanobacterial genes and microcystin concentrations at each of the three lake sites. Spearman's rank correlations, the significance of the correlation (p), and best fit lines are shown on each graph.

Table 4
Best models based on real-time and comprehensive variables for estimates of microcystin levels at Maumee Bay State Park Lake Erie beach.

Types of variables used	Adjusted R^2	Threshold probability	Model parameters	Parameter statistics		
				Coefficient	Standardized coefficient	*P*-value
Real-time	0.82	35%	*Y*-intercept	−0.2211	NA[c]	NA
			Phycocyanin, cells/mL	−0.00002	0.6799	<0.0001
			Algae category[a]	0.2851	0.2646	0.0238
			Wind speed, miles per h[b]	−0.0028	−0.2712	0.0140
Comprehensive with all variables	0.90	25%	*Y*-intercept	−5.9	NA	NA
			Phycocyanin, cells/mL	−0.00001	0.3306	0.0038
			pH	0.5747	0.3281	0.0033
			Microcystis biovolume, log $\mu m^3\,mL^{-1}$	0.2067	0.4781	<0.0001
Comprehensive with cyanobacterial gene assays	0.81	24%	*Y*-intercept	−10.8	NA	NA
			pH	1.0282	0.5869	<0.0001
			Microcystis mcyE DNA, log copies. $100\,mL^{-1}$	0.3705	0.5081	<0.0001

[a] An observed qualitative algae category was recorded as 0, none; 1, mild; 2, moderate; 3, serious; and 4, extreme.
[b] Wind speed is the wind speed at 8 a.m. on the date of sampling at Toledo Executive Airport (TDZ).
[c] Not applicable.

however, chlorophyll fluorescence was significantly correlated with microcystin concentrations at only one site, Harsha Main, and this correlation was weaker ($r = 0.63$) than those for several other factors. Otten et al. (2012) recommended that chlorophyll-a measurements be routinely used as a first screening step to estimate microcystin concentrations; phycocyanin was not measured during their study. Chlorophyll-a laboratory measurements take time and are not practical for real-time estimates. Alternatively, the results of the present study suggest that measurements of phycocyanin, turbidity, Secchi depth, and pH are effective real-time measurements for estimating microcystin levels at some freshwater lakes.

Continuous monitor measurements at Harsha Main and MBSP Lake Erie were promising for real-time models. The highest Spearman's correlations among all factors were between microcystin concentrations and the 7-day (rho = 0.98) and 14-day average (rho = 1.00) phycocyanin continuous-monitor measurements for Harsha Main and MBSP Lake Erie, respectively. In a reservoir in Poland, Izydorczyk et al. (2005) found a significant correlation between continuously-monitored phycocyanin fluorescence and microcystin concentrations ($r = 0.51$); however, this weak correlation included only samples with microcystin concentrations $<3\,\mu g\,L^{-1}$. In the small dataset from MBSP Lake Erie in the present study ($n = 8$), oxidative reductive potential was one of

two continuously-measured variables that were significantly correlated with microcystin concentrations. Paerl and Otten (2013) hypothesized that oxidative stressors, such as superoxide and hydrogen peroxide, select for toxigenic strains over nontoxic strains of cyanobacteria during periods of high light intensity. They assert there is evidence that microcystin has a protective role by binding to proteins in the cell in order to resist degradation during oxidative stress. One would expect, therefore, to find higher microcystin concentrations as ORP increased, the opposite of what was found in the present study where there was a negative correlation between microcystin concentrations and ORP. A closer examination of the data, however, indicated that this association was probably due to temporal patterns in the small dataset collected in August–September, 2014. Patterns in ORP should be investigated further in a larger dataset collected throughout the season. Overall, continuous monitoring of phycocyanin, pH, temperature, dissolved oxygen, and ORP and compiling antecedent values for 24-h, 3-day, 7-day, and 14-day before a microcystin measurement, may be used to develop real-time models for estimating microcystin concentrations.

Environmental data for real-time models were compiled from other sources for rainfall, wind speed and direction, barometric pressure, water level, streamflow, and solar radiation. Only three environmental factors were significantly correlated with microcystin concentrations—lake level change at Buckeye Onion Island, and streamflow and the wind O component at MBSP Lake Erie. The negative relations with microcystin for the two measures of water quantity (lake level and streamflow) affirm the previous association between cyanobacterial proliferation and low or stagnant waters. In a shallow Mediterranean lake (mean depth 1.2 m), Romo et al. (2013) found that longer water residence times in dry years resulted in higher microcystin concentrations. As for winds, Wood et al. (2011) measured an increase in *Microcystis* cell counts that coincided with gentle onshore winds in a New Zealand lake, hypothesizing that buoyant *Microcystis* cells formed wind-blown scums. In the case of MBSP Lake Erie, winds can concentrate the bloom and associated toxins along the Ohio coastline, or they can disperse the bloom throughout the western basin, depending on speed and direction. Temporal changes in wind direction and speed may influence microcystin concentrations near MBSP Lake Erie, but further research would need to be done to better understand this factor's influence on microcystin concentrations.

Among nutrient constituents for comprehensive estimates, ammonia was significantly correlated with microcystin concentrations at all three sites and nitrate + nitrite was significant at two sites; both had negative correlations to microcystin concentrations. Dissolved nutrients are readily taken up by cells and are lower during periods of high productivity and rapid recycling of nutrients. In a review of multi-year data from Lake Erie, nitrate and soluble reactive phosphorus were generally depleted during the August cyanoHAB period (Gobler et al., 2016). Specific to each lake in the present study, total nitrogen (rho = 0.72) was significant at Buckeye Onion Island, total phosphorus (rho = 0.78) and nitrite (rho = −0.68) at MBSP Lake Erie, and orthophosphate (−0.48) at Harsha Main. In contrast to Buckeye Lake, total nitrogen was significantly negatively correlated with microcystin concentrations (rho = −0.59) in one of two South African reservoirs studied (Conradie and Barnard, 2012). In agreement with results from MBSP Lake Erie, total phosphorus was significantly correlated with microcystin concentrations ($r = 0.58$) in a Korean reservoir with a 73 km^2 similar surface area (Joung et al., 2011). Although nutrient and toxin production dynamics may be similar among some lakes, all of these correlation results suggest that relations between nutrients and toxin concentrations need to be examined on a site-specific basis. Indeed, in a review of laboratory and field studies, Gobler et al. (2016) concluded that nutrient control of cyanoHAB proliferation and toxin production is likely a function of watershed nitrogen and phosphorus loads, lake geomorphology, and resident cyanobacterial community structure.

Among measures of the cyanobacteria themselves, cyanobacteria by qPCR were significantly correlated with microcystin concentrations at all three sites. All measures of cyanobacteria and *Microcystis* abundance and biovolume were significantly correlated with microcystin concentrations at Harsha Main and MBSP Lake Erie, but none were significant at Buckeye Onion Island. The highest correlations for a molecular assay were *Microcystis* mcyE DNA at Harsha Main (rho = 0.92) and MBSP Lake Erie (rho = 0.82) and *Planktothrix* mcyE DNA (rho = 0.73) at Buckeye Onion Island. Molecular assays are advantageous for comprehensive estimates because results are available within 3 h and they remove any subjectivity associated with microscope counts. The utility of using molecular assays for identifying cyanobacteria associated with elevated microcystin concentrations has been demonstrated in other studies. At three lakes and a pond in the northeast USA, investigators found that the *Microcystis mcyD* gene was significantly correlated with microcystin concentrations ($p < 0.001$) and was a better predictor than total cell counts or chlorophyll-a, measurements recommended by WHO to protect against human exposure (Davis et al., 2009). Similarly, in a study in Missisquoi Bay in Canada (Fortin et al., 2010), significant correlations between *mcyD* copy numbers and microcystin concentrations were found for both pelagic ($r = 0.87$) and littoral ($r = 0.93$) sampling stations during one of two growing seasons. This suggests that these relations may differ from year to year. As was found in the present study, significant correlations were found between microcystin concentrations and general *Microcystis* or *Microcystis mcyE* genes at two South African Reservoirs (rho = 0.33–0.53; Conradie and Barnard, 2012) and at Lake Taihu, China ($r = 0.93$; Otten et al., 2012).

4.2. The feasibility of developing models for real-time and comprehensive estimates of microcystin levels

During this study, it was demonstrated that site-specific models with high sensitivities and specificities could be developed to estimate microcystin concentrations above or below the Ohio Recreational Public Health Advisory level of 6 $\mu g\,L^{-1}$. The model dataset was limited by having only 24 data points over 2 years; R^2 values and other model performance measures may change with subsequent years of data. The high adjusted R^2 values (0.82–0.91) in the models in the present study, however, indicate that this approach has potential. Measures of the algal community were most often included in models; these included phycocyanin, algae category, *Microcystis* biovolume, and *Microcystis mcyE* DNA concentrations. Also included were the independent variables pH and wind speed.

Other studies provide evidence that linear-regression models to estimate toxin concentrations are feasible and promising. Although not site specific, Marion et al. (2012) developed a logistic model for predicting the probability of exceeding 4 $\mu g\,L^{-1}$ microcystin at seven inland Ohio beaches, including one beach included in the present study (Harsha Main). The best model contained phycocyanin and Secchi depth. In contrast to the Marion et al. (2012) study and the present study, McQuaid et al. (2011) did not find significant correlations between phycocyanin and microcystin concentrations in a Canadian lake. Instead, investigators developed alert levels based on (1) the relation between cyanobacterial biovolume and phycocyanin and (2) the calculated maximum potential microcystin concentrations produced by dominant *Microcystis* sp. Using 5 years of data collected in a Kansas water-supply reservoir (19.3 km^2), the best linear regression model for predicting microcystin concentrations contained a seasonal component (sine and cosine variables) and a concomitant

chlorophyll measurement from a continuous monitor ($R^2 = 0.48$); phycocyanin was not measured in the Kansas study (Stone et al., 2013). Larger datasets collected in future studies may be used to evaluate the feasibility of including a seasonal component variable in a model and may be used to develop more robust site-specific models.

5. Conclusions

To our knowledge, this was the first study that showed that models could be developed for estimates of exceeding a microcystin threshold concentration at a recreational freshwater lake site. Site-specific models may provide relevant public health information to water resource managers and the public on the levels of toxin concentrations in both recreational and drinking waters. In addition, such models could be used to augment satellite predictions, target when to collect samples for toxin analysis, warn the public of health risk, modify water-treatment strategies, and understand progression of the cyanoHAB throughout the season.

The results from this study showed that there were a few similarities and many differences among environmental and water-quality factors significantly correlated with microcystin concentrations at three different recreational lakes sites. Measures of the algal community (phycocyanin, cyanobacterial biovolume, and cyanobacterial gene concentrations) and pH were most strongly correlated with microcystin concentrations. For phycocyanin, the relations were different between sites and were different between hand-held measurements on-site and nearby continuous monitor measurements for the same site. Continuous measurements over multiple days showed the highest correlations to microcystin concentrations; they reduced the influence from errors in instantaneous measurements and from diurnal variations in the data. Future studies should focus on multi-year data collection efforts with sample collection on several consecutive days each week before, during, and after the cyanoHAB season to develop robust site-specific models. A user-friendly program called Virtual Beach could be used by water-resource managers to update models from year to year.

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