Ohio WRC Project Final Report

“Effectiveness of Data Buoys as Early Warning Systems for cHABs (cyanobacterial Harmful Algal Blooms) in Lake Erie”

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Problem and Research Objectives

Harmful cyanobacterial blooms (cHABs) were an annual occurrence in Lake Erie during the mid-1900s due to excessive nutrient loading (Davis 1964). The governments of the United States and Canada agreed to regulate phosphorus (P) loading in the 1970s, which resulted in a lake that was relatively free of cHABs throughout the 1980s and 1990s (DePinto et al. 1986; Makarewicz 1993). However, since the mid-1990s, cHABs have returned, and bloom resurgence has been attributed to increases of dissolved reactive P (Kane et al. 2014; Stumpf et al. 2016), climate change (Michalak et al. 2013). The current cHABs in Lake Erie are dominated by the cyanobacterium Microcystis (Chaffin et al. 2011), which is globally-distributed in eutrophic water and can produce high concentrations of the hepatotoxic microcystins (MCYs) (as reviewed by (Harke et al. 2016b)). In August 2014, MCYs were found in the municipal water of Toledo, Ohio, United States, at concentrations that exceeded the World Health Organization’s guideline of 1 µg/L, causing a loss of safe drinking water for nearly 500,000 residents (Qian et al. 2015; Bullerjahn et al. 2016). Additionally, cHABs can disrupt aquatic food web structure (Tillmanns et al. 2008; Davis et al. 2012), have killed pets and livestock (Huisman et al. 2010; Backer et al. 2013), and can adversely impact local economies (Dodds et al. 2009; Bingham et al. 2015).

Ultimately, preventing cHABs from forming in lakes and rivers by decreasing influx of nutrients from the surrounding watershed will aid in the protection of safe drinking water. However, trends in climate and agricultural practices suggest that cHABs will become more common in the future (Paerl and Huisman 2008; Michalak et al. 2013). Thus, the risk of cyanotoxins in water will continue to be present, and societies will need to rely on water treatment plants to provide safe drinking water. Real-time estimates of cHAB biomass at or near the intake pipes of municipal water treatment plants can aid the operators in adjusting treatment accordingly. Moreover, the real-time information on cHAB biomass could be used to notify lake managers, tourists, and the general public of when cHABs cause water quality problems (Read et al. 2010). Early detection of cHABs creates the opportunity to mitigate health risks and adverse economic impacts by warning people before cHABs are a severe problem (Jochens et al. 2010), and allows people know when a cHAB is not causing a problem at a given area of the lake or time.
In response to the Toledo 2014 “do not drink advisory,” several lake-shore communities in northern Ohio, Ohio universities, and the federal government (NOAA) deployed water quality sondes for cyanobacteria on buoys or in water treatment plant intakes to provide real-time cyanobacterial biomass data. Since summer 2015, 9 water quality sondes attached to buoys have been deployed annually, and 11 water quality sondes have been deployed at the intakes of water treatment plants in the western and central basin of Lake Erie (Fig. 1). The data from this network of water quality sondes is freely available online, and serves as an early warning system used by water treatment plant operators who adjust treatment according to cyanobacterial biomass at the intake, beach managers concerned with cHAB-driven beach closures, scientists researching cHABs, and interested members of the general public (Read et al. 2010).

Figure 1. Locations of data buoys (yellow triangles) and water quality sondes mounted to fixed structures (black circles) in Lake Erie used to track cyanobacteria biomass in real time. The location of the two buoys used in this study are highlighted.

Water-quality sondes have sensors that detect and quantify total algae and cyanobacteria with fluorescence from chlorophyll \(a\) (chl\(a\)) and phycocyanin (a pigment specific to cyanobacteria), respectively (Humbert and Törökné 2016). The users of the water quality sondes network interpret higher fluorescence values as increased chl\(a\) and phycocyanin concentrations and therefore assume higher biomasses of total algae and cyanobacteria. However, there are several potential issues with long-term deployment of water quality sondes that measure cyanobacteria and total algae biomass by fluorescence. The first problem is the use of fluorescence to measure chl\(a\) or phycocyanin concentration. Fluorescence is dependent on the alga’s physiological state because fluorescence per cell can increase under stressful conditions, such as low nutrient concentration and photo-inhibitory high light intensities (Campbell et al. 1998), which could lead to an underestimation or overestimation of cyanobacterial biomass. The second problem arises from the assumption that chl\(a\) or phycocyanin concentration is proportional to algal or cyanobacterial biomass. Algae and cyanobacteria can alter their chl\(a\) and
phycocyanin content (pigment mass per cell) in response to light conditions (MacIntyre et al. 2002). A long-term Lake Erie plankton study found a weak correlation between chla concentration and phytoplankton biomass (Conroy et al. 2005). Another study discovered that Lake Erie *Microcystis* doubled its chla content, and phycocyanin content increased 6 times, in response to low light conditions in the lake (Chaffin et al. 2012). Hence, data buoys are taking measurements of fluorescence without confirmation the sonde data with biomass data from water samples. A third potential issue is instrument drift. Whether water quality sondes are deployed year-around or seasonally (April-November) they are only calibrated a few times a year (< 5 times) due to the difficulties associated with removing and returning the sondes attached to deployed buoys. It is possible that the water quality sondes lose calibration throughout deployment and give inaccurate data. Finally, biofouling from algae and *Dreissena* mussels (Fig. 2) can reduce water exchange around water quality sondes. The densely packed mussels fouling sondes deployed over long-term intervals can result in inaccurate readings.

![Figure 2. Images of biofouling by filamentous green algae and *Dreissena* mussels on the Sandusky buoy (a), on the YSI sonde (b), and on the YSI sensors (c).](image)

The sensors attached to data buoys are located just below the surface of the water (~0.6 to 1 meter); however, water treatment plant intake pipes are near the bottom of the lake in water that is greater than 6 meters in depth. Thus, there is a potential disconnect between water quality data measured at the surface and water quality being drawn into the plant. Moreover, the different buoyancy regulation strategies of the various cyanobacteria in Lake Erie can further exacerbate that disconnect. For example, *Microcystis* is positively buoyant allowing it to accumulate near the surface in calm waters, whereas, *Planktothrix* is neutrally-to-negatively buoyant and will position itself in the center of the water column or sink to the bottom (Konopka et al., 1987; Reynolds et al., 1987). Thus, a data buoy may overestimate cyanobacteria abundance during a *Microcystis* bloom and underestimate cyanobacteria abundance during a *Planktothrix* bloom. This could result in a plant operator to over-treat (which wastes treatment chemicals and money) or under-treat (which could result in microcystins in tap water) the lake water. *Microcystis* and *Planktothrix* are known microcystins producers and bloom in waters that
serve as source water for several large Ohio shoreline cities such as Toledo and Sandusky, respectively.

Wind speed can also impact how water treatment plant operators interpret buoy data. The lake is calm during low wind weather allowing cyanobacteria to position themselves at desired light levels (i.e., *Microcystis* near the surface and *Planktothrix* lower in the water column). High wind speeds create turbulent mixing of the water column and overpower the buoyancy regulation of cyanobacteria resulting in cyanobacteria to be spread evenly from surface to lake bottom. A potential issue in water treatment can arise when a calm day is followed by a windy day. For example, a buoy measures high cyanobacteria biomass due to *Microcystis* at the surface one day, but then high winds the following day mix the bloom throughout the water column. The buoy data will show fewer cyanobacteria biomass, but the intake is actually drawing in more cyanobacteria biomass because the wind mixed the bloom throughout the water column and down to the intake pipe.

The objective of this study was to determine how well water quality sondes attached to buoys measure total algae and cyanobacterial biomass, and water turbidity. Additionally, because the sonde network in Lake Erie cannot measure MCYs, total MCYs concentrations were measured manually to determine potential correlations with sonde data. While several recent studies have found varying relationships between sonde algae data and biomass estimates (McQuaid et al. 2010; Zamyadi et al. 2012; Loisa et al. 2015; Bowling et al. 2016; Zamyadi et al. 2016; Hodges et al. 2017), none have investigated the utility of water quality sondes for tracking MCYs. Additionally, concentrations of dissolved reactive P, total P, nitrate, ammonium, and total N were measured to aid in interpretation of the relationships between sonde and water sample cyanobacterial biomass and MCY data. The final objective was to determine the vertical position of cyanobacteria throughout the water column in relation to buoy cyanobacteria data and wind speed data.

**Methodology**

**Buoy location and sonde calibration**

Two data buoys were used in this study. The Gibraltar buoy was deployed about 200 meters northwest of Gibraltar Island, and the Sandusky buoy was deployed 100 meters off the City of Sandusky lakeside municipal water intake (Fig. 1). The Gibraltar buoy was equipped with a YSI 6600v2 multiprobe sonde during 2015 and a YSI EXO2 sonde during 2016 and 2017. The Sandusky buoy had an EXO2 sonde all three years. The sondes were calibrated together with approximately 20 to 25 other sondes of the Lake Erie sonde network at the University of Toledo’s Lake Erie Center to facilitate comparison across sondes. The EXO2 sondes were calibrated together for relative fluorescence units (RFU) for chla and cyanobacteria-PC (surrogates for total algal and cyanobacterial biomass, respectively) with rhodamine dye and for nephelometric turbidity units (NTU) for water clarity (according to YSI instructions) with a YSI NTU standard, and deionized water was used for the 0.0 point. In 2015, the 6600v2 sonde was calibrated to chla µg/L and bluegreen algae cells/mL with rhodamine dye, which also calibrates RFU in the process. The Gibraltar sonde was calibrated and cleaned three times throughout deployment whereas the Sandusky sonde was not cleaned or calibrated until buoy retrieval due to difficulties accessing the sonde from a large vessel while deployed.
Because the Gibraltar buoy had two different sonde models, a laboratory comparison was conducted to determine a PC RFU conversion factor. A rhodamine dye standard was created following the EXOs instruction manual, and RFUs of that standard were recorded using EXO2 and 6600v2 sondes. The standard was diluted by 50% and RFU were recorded for both sondes. This process was repeated 10 times by diluting the standard by 50% each step (0.19% concentration of original standard). This experiment was repeated twice with two different EXO2 and 6600v2 sondes. The 6600v2 RFU values were converted to EXO2 RFU data because all sondes in the Lake Erie sonde network use EXO2 sondes.

Water sample collection

The Gibraltar buoy was visited several times a week by small boats (< 4 meters) to collect samples adjacent to the buoy manually. A total of 56, 81, and 54 samples were collected next to the Gibraltar buoy (May through October) in 2015, 2016, and 2017, respectively. The Sandusky buoy was sampled six and nine times during summers 2015 and 2016, respectively, aboard the RV Erie Monitor; the vessel anchored within 20 meters of the buoy. To determine the relationship between buoy sonde data and water sample data, a 0-2 meter intergraded tube sampler was used to collect surface water. The 0-2 meter sample represents the ‘average’ conditions experienced by the buoys’ sonde as the buoy bobs up and down with waves (the sonde is between 0.7 and 1.0 meters depth). Clean samplers and sample bottles were triple rinsed with surface water before collection. Water from the sampler was dispensed into a clean 5-gallon bucket and then poured into 1) 2-L dark bottle for chla, PC, total suspended solids (TSS) concentrations, and algal group-specific chla, 2) 500-mL glass jar and preserved with Lugol’s solution for analysis of phytoplankton identification and quantification, 3) 40-mL amber glass vial for total microcystins concentration, and 4) two separate 250-mL acid washed polycarbonate bottles for analysis of TP and TN. About 50 mL of lake water was filtered upon collection using a 0.45µm polycarbonate membrane syringe filter and stored in a 60-mL bottle for analysis of dissolved reactive P, nitrate, nitrite, and ammonium. An ice chest was used to store samples during transportation back to the laboratory, which was 5 minutes for the Gibraltar buoy and 1.5 hours for the Sandusky buoy. Secchi disk depth was also measured.

On a subset of dates (n = 36), water samples were collected at every meter throughout the water column to determine vertical phytoplankton position. Water was collected with a Van Dorn bottle and poured into 250-mL polycarbonate bottles. Water was analyzed for algal group-specific chla concentration.

Water sample analysis methods

Total chla analysis began by filtering 0.25-1.0 L (depending on the density of phytoplankton and suspended solids) onto GF/F filters (47 mm diameter, 0.7 µm pore size), which were then stored on silica gel at -80ºC until analysis. Chlorophyll a was extracted with dimethyl sulfoxide and quantified with spectrophotometry (Golnick et al. 2016). Algal group-specific chla was determined within 10 minutes of returning to the laboratory using a FluoroProbe benchtop reader (Chaffin et al. 2013). The FluoroProbe is a fluorometric device that uses chla and accessory pigment fluorescence to partition total chla among four functional phytoplankton groups (green algae, cyanobacteria, diatoms, and cryptophytes; (Beutler et al. 2002)). Because the FluoroProbe can underestimate algal biomass (Gregor and Marsálek 2004),

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algal group-specific chla concentration was corrected for on each sample date by dividing the algal group-specific chla concentration by the FluoroProbe total chla concentration and then multiplying by the chla concentration determined by DMSO extraction (Bridgeman et al. 2012).

Plankton and solids were filtered (0.25-1.0 L depending on density) onto pre-combusted and weighed GF/F filters (47 mm diameter, 0.7 µm pore size) for analysis of total suspended solids (TSS). Filters with plankton and solids were dried at 103°C overnight and reweighed to determine TSS.

Phytoplankton was quantified with automated imaging flow cytometry (FlowCAM) under 40x, 100x, and 200x magnification on auto-image mode. FlowCAM is a fluid imaging device that was created for the study of phytoplankton that captures images of particles (i.e., plankton) as they flow through the objective lens carried in a medium (i.e., lake water) (Poulton 2016). FlowCAM has been shown to provide results similar to traditional phytoplankton counts (light microscopy with the Utermohl method) (Álvarez et al., 2014). FlowCAM software has a semi-automated image recognition system to aid the user in the sorting of phytoplankton based on 31 recorded parameters for each particle, including length, diameter, and area which were used for calculating biomass (Fluid Imaging Technologies INC. 2011). Sample runs were terminated after 8000 images were captured; the volume analyzed and length of image collection was dependent on the density of particles. Images were first classified using the Auto-Classification function of the Visual Spreadsheet (version 4.0.27) and then manually checked to sort unclassified or incorrectly classified phytoplankton. Cyanobacteria were identified according to Rosen and St. Amand (2015). Areal measurements were converted to biovolume by assuming a sphere for single cells, a cylinder for filamentous cyanobacteria, and by multiplying by the average cell diameter for colonial cyanobacteria.

Total MCYs were determined after 3 freeze/thaw cycles, filtered with a glass microfiber filter (GMF, 0.45 µm) to remove cellular debris, and quantified using a microcystin/nodularin specific enzyme-linked immunosorbent assay (ELISA) (Abraxis #520011, Fischer et al. 2001)). When needed, samples were diluted to bring the MCY concentration within the ELISA working range of less than 5 µg/L.

Concentrations of nitrate, nitrite, ammonium, and dissolved reactive P were quantified with a SEAL Analytical QuAAttro continuous segmented flow analyzer (SEAL Analytical Inc., Mequon, WI) using standard U.S. EPA methods (EPA 353.1, 354.1, 350.1, and 365.1, respectively). Total P and total Kjeldhal N (TKN) were determined on unfiltered water following digestion and quantified on the SEAL analyzer following standard methods (EPA 365.3, 351.2, respectively). Total N was calculated as the sum of TKN, nitrate, and nitrite, and the TN to TP ratio was calculated by dividing TN by TP.

Buoy data analysis

The buoys recorded data every 15 minutes, which was automatically transmitted to WQDataLive, a website platform (wqdatalive.com). Water sample data were compared to the buoy data on 4 time scales: 1) the single data point closest to the time that the water sample was collected, 2) the average of five buoy data points one hour prior to water sample collection (for example, if water sample was collected at 11:00 the buoy measurements at 10:00, 10:15, 10:30, 10:45, and 11:00 were averaged), 3) averaged for 4 hours prior to water sample, and 4) the daily average (00:00 – 23:45) of the day of sample. Analysis of covariance test (ANCOVA) for homogeneous slopes was conducted to determine if slopes between buoy data (the covariate) and
water sample data (the dependent data) differed among the 3 years (the factors). Then, when ANCOVA indicated non-parallel slopes among years, linear regression was used to determine the relationships between buoy and water sample data separately for each year. IBM SPSS Statistics v23 were used for all data analysis.

*Every-meter phytoplankton and wind data analysis*

Because the every-meter cyanobacteria biomass data was determined with a FluoroProbe and the data buoy measured cyanobacteria biomass as RFU, buoy RFU data was converted to cyanobacteria-chla with the relationship found below (Table 1). This data analysis was only conducted on data collected next to the Gibraltar buoy because no relationship was found between buoy RFU and cyanobacteria biomass at the Sandusky buoy. Next, the percent relative difference (\%RD) between the buoy-converted data and every-meter cyanobacteria biomass data was calculated as:

\[
\%RD = \frac{(Chl_{a@z} - Chl_{buoy})}{Chl_{buoy}} \times 100\%
\]

Where Chl$_{a@z}$ is the cyanobacteria chla concentration measured at depth z (0, 1, 2, 3, 4, or 5 meters) and Chl$_{buoy}$ is the cyanobacteria chla concentration that was converted from the buoy cyanobacteria RFU data. The \%RD will always for comparisons between low and high biomass data.

Wind speed data were obtained from NOAA’s National Buoy Data Center (http://www.ndbc.noaa.gov/station_page.php?station=sbio1) using South Bass Island site for Gibraltar buoy. We initially proposed to use the buoy’s weather station wind data for this project, but we had to find alternate wind data source due to malfunctions to both buoys’ weather stations during summer 2016. The average wind speed 1 h, 4 h, 8 h, 12 h, and 24 h before sample collection were calculated. The %RD between buoy and cyanobacteria chla at each depth was plotted against wind speed for each time frame investigated.

**Principal Findings and Results**

**Sonde RFU comparison**

In the sonde comparison study, there was a significant linear relationship between PC RFUs values of diluted calibration standards measured by the YSI 6600v2 and EXOv2 sondes (P < 0.001, $R^2 = 0.986$); however, the 6600v2 sondes RFU values were 8.3 times greater than that measured by the EXOv2 sondes. There was no difference in slopes for the 2 separate experiment trials with different sondes. Therefore, the conversion factor for converting 6600v2 PC RFU (used in 2015) values to EXOv2 PC RFU (used in 2016 and 2017) values was 0.1204.

**Temporal patterns**

Cyanobacterial biomass measured by the Gibraltar buoy peaked at 10 cyanobacteria-PC RFUs in late July 2015 and RFUs were between 0.5 and 3.0 during August and September 2015 (Fig. 3a). Cyanobacteria-PC RFUs throughout the entire 2016 season and during May through early September 2017 were between 0.0 to 0.3 RFUs, suggesting lower cyanobacterial biomass
than 2015. Cyanobacteria-PC RFUs increased in September and October 2017 peaking at 1.0 RFUs. Water sample cyanobacteria-chla followed a similar temporal pattern as buoy cyanobacteria-PC RFU measurements with greatest concentrations in late July 2015 (> 30 µg/L), low concentrations (< 5 µg/L) throughout 2016, and a late summer peak in 2017 (10.5 µg/L). Cyanobacterial biomass measured at the Sandusky buoy was more variable and reached greater PC RFUs than the Gibraltar buoy (Fig. 3b). Cyanobacteria-chla in the water samples did not follow the temporal pattern of the Sandusky buoy PC RFUs.

Total MCY concentrations followed similar patterns as Gibraltar buoy cyanobacteria-PC RFUs and water sample cyanobacterial biomass (Fig. 3a). Highest MCY concentrations (5.9 µg/L) occurred during the largest peak of cyanobacteria during late-July 2015. Lower total MCY concentrations occurred in 2016 and 2017.

Figure 4. Total phytoplankton abundance at the Gibraltar (A) and Sandusky (B) data buoys during summer 2015, 2016, and 2017 estimated by total chlorophyll a sensors (relative fluoroscence units; gray lines), and total chlorophyll a (green dots) measured in water samples collected next to the data buoys.
In 2015 chlorophyll RFUs followed a similar pattern as cyanobacteria RFUs, except for a minor peak associated with a diatom bloom in late June (Fig. 4a). Chlorophyll RFUs peaked at 9.5 during 2015. Two peaks occurred in each of 2016 and 2017 of similar RFUs values. Diatoms were dominant during the June peaks, whereas cyanobacteria were dominant during the August peaks. Water sample chla concentration followed the pattern of the buoy RFUs measurements with greatest concentrations in late July 2015 (> 50 µg/L) and the bimodal peaks in 2016 and 2017 had concentrations around 20 µg/L. Chlorophyll RFUs measured at the Sandusky buoy was more variable throughout the summers with several peaks per year that reached greater RFUs than the Gibraltar buoy (Fig. 4b). Chlorophyll concentrations in the water samples did not track with buoy RFUs.

Figure 4. Total phytoplankton abundance at the Gibraltar (A) and Sandusky (B) data buoys during summer 2015, 2016, and 2017 estimated by total chlorophyll a sensors (relative fluorescence units; gray lines), and total chlorophyll a (green dots) measured in water samples collected next to the data buoys.

Water clarity (NTUs) measured at the Gibraltar buoy during 2015 followed a similar pattern as cyanobacterial biomass peaking 336.8 on July 25, but there were a few minor peaks in May and June (Fig. 5a). NTUs measured in 2016 and 2017 were much lower than 2015, with a
few smaller peaks detected early and late summers. Water sample TSS temporal pattern aligned with buoy NTU each summer. At the Sandusky buoy, extremely high NTUs (>1000 NTUs) were recorded each summer after mid-August (Fig. 5b); however, these high values could be the result of fouling on the water quality sondes (Fig. 2). Before mid-August, higher NTUs were measured during 2015 than 2016 and 2017.

![Figure 5. Turbidity (nephelometric turbidity units; gray line) at the Gibraltar (A) and Sandusky (B) data buoys during summer 2015, 2016, and 2017, and total suspended solids (brown dots) measured in water samples collected next to the data buoys.](image)

**Correlations between buoy and water samples**

For Gibraltar buoy data, an ANCOVA test for homogeneous slopes found significant interactions (P < 0.05), among year, buoy data (the covariate) and water sample data (the dependent variable) indicating that regression trends were different each year, and this was true for all 4 time frames investigated and all buoy-water sample data pairs (Table 1). In general, the 1-hour time frame had the most robust relationship (highest R² value) for all years and parameters, and Figure 6 displays the relationships between 1-hour averaged buoy data and water sample data.
Table 1. Linear regression statistics between water sample data collected next to the Gibraltar buoy data and the buoy data over 4 time frames. All coefficients were significantly different among years as indicated by failed ANCOVA test for homogeneity each year. Asterisks indicate P values of the regression: * P < 0.1; ** P < 0.01; *** P < 0.001. Bold and italics indicate the best relationship between buoy and water sample data.

Cyanobacteria: Buoy PC RFU vs Water Sample Cyanobacteria-Chl a (µg/L)

<table>
<thead>
<tr>
<th>Time Frame</th>
<th>Slope</th>
<th>R²</th>
<th>Slope</th>
<th>R²</th>
<th>Slope</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time of Sample</td>
<td>24.549***</td>
<td>0.796</td>
<td>NS</td>
<td>0.000</td>
<td>13.911***</td>
<td>0.729</td>
</tr>
<tr>
<td>1 hour prior</td>
<td>27.796***</td>
<td>0.850</td>
<td>NS</td>
<td>0.018</td>
<td>14.142***</td>
<td>0.752</td>
</tr>
<tr>
<td>4 hour prior</td>
<td>25.984***</td>
<td>0.828</td>
<td>NS</td>
<td>0.042</td>
<td>15.407***</td>
<td>0.698</td>
</tr>
<tr>
<td>Daily Average</td>
<td>28.973***</td>
<td>0.771</td>
<td>NS</td>
<td>0.091</td>
<td>14.912***</td>
<td>0.736</td>
</tr>
</tbody>
</table>

Cyanobacteria: Buoy PC RFU vs Water Sample Cyanobacterial Biovolume (µm³/mL)

<table>
<thead>
<tr>
<th>Time Frame</th>
<th>Slope</th>
<th>R²</th>
<th>Slope</th>
<th>R²</th>
<th>Slope</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time of Sample</td>
<td>3,358,438***</td>
<td>0.843</td>
<td>NS</td>
<td>0.001</td>
<td>2,794,873*</td>
<td>0.440</td>
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<tr>
<td>1 hour prior</td>
<td>3,293,269***</td>
<td>0.850</td>
<td>NS</td>
<td>0.028</td>
<td>2,414,589*</td>
<td>0.377</td>
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<td>4 hour prior</td>
<td>2,840,735***</td>
<td>0.816</td>
<td>NS</td>
<td>0.066</td>
<td>NS</td>
<td>0.342</td>
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<tr>
<td>Daily Average</td>
<td>3,657,880***</td>
<td>0.807</td>
<td>NS</td>
<td>0.069</td>
<td>NS</td>
<td>0.318</td>
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</tbody>
</table>

Total Algae: Buoy Chl RFU vs Water Sample Chl a (µg/L)

<table>
<thead>
<tr>
<th>Time Frame</th>
<th>Slope</th>
<th>R²</th>
<th>Slope</th>
<th>R²</th>
<th>Slope</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time of Sample</td>
<td>24.072***</td>
<td>0.523</td>
<td>3.999***</td>
<td>0.365</td>
<td>11.640***</td>
<td>0.630</td>
</tr>
<tr>
<td>1 hour prior</td>
<td>24.555***</td>
<td>0.516</td>
<td>4.210***</td>
<td>0.396</td>
<td>10.182***</td>
<td>0.674</td>
</tr>
<tr>
<td>4 hour prior</td>
<td>27.125***</td>
<td>0.537</td>
<td>3.801***</td>
<td>0.363</td>
<td>11.451***</td>
<td>0.660</td>
</tr>
<tr>
<td>Daily Average</td>
<td>27.675***</td>
<td>0.518</td>
<td><strong>5.096</strong>*</td>
<td>0.496</td>
<td>10.805***</td>
<td>0.666</td>
</tr>
</tbody>
</table>

Water Clarity: Buoy NTU vs Water Sample TSS (mg/L)

<table>
<thead>
<tr>
<th>Time Frame</th>
<th>Slope</th>
<th>R²</th>
<th>Slope</th>
<th>R²</th>
<th>Slope</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time of Sample</td>
<td>0.659***</td>
<td>0.738</td>
<td>1.078***</td>
<td>0.870</td>
<td>0.994***</td>
<td>0.822</td>
</tr>
<tr>
<td>1 hour prior</td>
<td>0.703***</td>
<td>0.802</td>
<td><strong>0.938</strong>*</td>
<td>0.896</td>
<td>0.949***</td>
<td>0.830</td>
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<tr>
<td>4 hour prior</td>
<td>0.693***</td>
<td>0.788</td>
<td>0.969***</td>
<td>0.829</td>
<td><strong>0.888</strong>*</td>
<td>0.845</td>
</tr>
<tr>
<td>Daily Average</td>
<td>0.720***</td>
<td>0.752</td>
<td>0.964***</td>
<td>0.799</td>
<td>0.962***</td>
<td>0.792</td>
</tr>
</tbody>
</table>

Significant (P < 0.001) and highly correlated (R² > 0.80) linear relationships occurred between buoy cyanobacteria-PC RFUs and water samples cyanobacterial-chla (Fig. 6a) and cyanobacterial biovolume (Fig. 6b) for all 4 time frames during 2015 and 2017 as separate datasets (Table 1), but 2016 was not significant (likely due to very little cyanobacteria in 2016). The coefficient for the PC RFU cyanobacteria-chla relationship in 2015 (27.796 µg chla/L per PC RFU) was nearly twice that of 2017 (14.142 µg chla/L per PC RFU). Whereas the coefficients for PC RFU-cyanobacterial biovolume were less different among years (2015: 3,293,269 µm³/mL per PC RFU; 2017: 2,794,873 µm³/mL per PC RFU).
Significant (P < 0.001) linear relationships occurred between buoy cyanobacteria-PC RFU and water sample total MCY concentrations during years 2015 and 2017, but not 2016 (Fig. 6c). The relationships between buoy PC RFU and MCY concentration were weaker (2015 $R^2 = 0.593$; 2017 $R^2 = 0.354$) than the buoy PC RFU and cyanobacterial biomass estimate metrics. Cyanobacterial-chla and biovolume correlated significantly (Table 2; Fig. 6d) and ANCOVA indicated that there was no interaction between data and years. This result suggests that FlouroProbe measured cyanobacterial-chla was an appropriate surrogate for cyanobacterial biovolume.
Table 2. Linear regression statistics between FluoroProbe cyanobacteria-chlorophyll a concentration (µg/L) and total cyanobacterial biovolume (µm³/mL). ANCOVA indicated that there was no significant difference among slopes each year.

<table>
<thead>
<tr>
<th>Year</th>
<th>Slope</th>
<th>P value; R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>All years</td>
<td>112,478.420</td>
<td>&lt;0.001; 0.863</td>
</tr>
<tr>
<td>2015</td>
<td>115,666.527</td>
<td>&lt;0.001; 0.840</td>
</tr>
<tr>
<td>2016</td>
<td>212,087.958</td>
<td>0.024; 0.357</td>
</tr>
<tr>
<td>2017</td>
<td>215,913.477</td>
<td>0.020; 0.622</td>
</tr>
</tbody>
</table>

Significant (P < 0.001), but less correlated (R² between 0.27 and 0.67) relationships occurred between buoy chl a RFU and water sample total chl a concentration (Table 1; Fig. 6e). However, as indicated by ANCOVA test for homogeneity, the coefficients were highly different among years ranging from 5.096 µg chl a/L per RFU to 27.125 µg chl a/L per RFU. Additionally, the strongest relationship for each year occurred in different time frames.

Significant (P < 0.001) and highly correlated (R² > 0.80) linear relationships occurred between buoy NTUs and water samples TSS for all 4 time frames during all years of study (Table 1; Fig. 6f). The coefficient in 2015 was 0.703 mg TSS/L per NTU and the coefficients for 2016 and 2017 were nearly identical at 0.938 and 0.949 mg TSS/L per NTU, respectively.

For the Sandusky buoy, ANCOVA found no interactions among year, buoy data, and water sample data (Table 3). The cyanobacterial biomass relationships were only significant for the pooled dataset across all years; however, but the highest the R² was only 0.36, indicating a weak relationship. The total chl a relationship was significant across all years and for 2015 separately (but the all-year relationship was driven by 2015). There was no significant relationship between buoy NTU and water sample TSS at the Sandusky buoy.
Table 3. Linear regression statistics between water sample data collected next to the Sandusky buoy data and the buoy data over 4 time frames. All years included all data pooled together, and ANCOVA indicated there was no significant interaction between year and data suggesting trends were similar each year. Asterisks indicate P values of the regression: * P < 0.1; ** P < 0.01; *** P < 0.001. Bold and italics indicate the best relationship between buoy and water sample data.

<table>
<thead>
<tr>
<th>Cyanobacteria: Buoy PC RFU vs Water Sample Cyanobacteria-Chl (µg/L)</th>
<th>All years</th>
<th>2015 YSI 6600v2</th>
<th>Slope</th>
<th>P value; R²</th>
<th>2016 YSI EXO2</th>
<th>Slope</th>
<th>P value; R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time of Sample</td>
<td>7.358</td>
<td>0.025; 0.331</td>
<td>NS</td>
<td>0.532; 0.104</td>
<td>NS</td>
<td>0.362; 0.119</td>
<td></td>
</tr>
<tr>
<td>1 hour prior</td>
<td>7.839</td>
<td>0.032; 0.307</td>
<td>NS</td>
<td>0.445; 0.152</td>
<td>NS</td>
<td>0.677; 0.026</td>
<td></td>
</tr>
<tr>
<td>4 hour prior</td>
<td>6.985</td>
<td>0.061; 0.245</td>
<td>NS</td>
<td>0.456; 0.145</td>
<td>NS</td>
<td>0.870; 0.004</td>
<td></td>
</tr>
<tr>
<td>Daily Average</td>
<td><strong>9.408</strong></td>
<td><strong>0.021; 0.348</strong></td>
<td>NS</td>
<td>0.158; 0.429</td>
<td>NS</td>
<td>0.883; 0.003</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Total Algae: Buoy Chl RFU vs Water Sample Chl a (µg/L)</th>
<th>All years</th>
<th>2015 YSI 6600v2</th>
<th>Slope</th>
<th>P value; R²</th>
<th>2016 YSI EXO2</th>
<th>Slope</th>
<th>P value; R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time of Sample</td>
<td><strong>10.316</strong></td>
<td>&lt;0.001; 0.812</td>
<td><strong>10.488</strong></td>
<td><strong>0.012; 0.812</strong></td>
<td>NS</td>
<td>0.894; 0.004</td>
<td></td>
</tr>
<tr>
<td>1 hour prior</td>
<td>10.441</td>
<td>&lt;0.001; 0.762</td>
<td>10.638</td>
<td>0.023; 0.765</td>
<td>NS</td>
<td>0.531; 0.083</td>
<td></td>
</tr>
<tr>
<td>4 hour prior</td>
<td>9.945</td>
<td>&lt;0.001; 0.674</td>
<td>10.490</td>
<td>0.051; 0.650</td>
<td>NS</td>
<td>0.389; 0.151</td>
<td></td>
</tr>
<tr>
<td>Daily Average</td>
<td>8.648</td>
<td>0.002; 0.608</td>
<td>7.386</td>
<td>0.187; 0.388</td>
<td>NS</td>
<td>0.761; 0.020</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Water Clarity: Buoy NTU vs Water Sample TSS (mg/L)</th>
<th>All years</th>
<th>2015 YSI 6600v2</th>
<th>Slope</th>
<th>P value; R²</th>
<th>2016 YSI EXO2</th>
<th>Slope</th>
<th>P value; R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time of Sample</td>
<td>TSS data only in 2016</td>
<td>TSS data only in 2016</td>
<td>NS</td>
<td>0.209; 0.358</td>
<td>NS</td>
<td>0.218; 0.347</td>
<td></td>
</tr>
<tr>
<td>1 hour prior</td>
<td>TSS data only in 2016</td>
<td>TSS data only in 2016</td>
<td>NS</td>
<td>0.237; 0.325</td>
<td>NS</td>
<td>0.702; 0.040</td>
<td></td>
</tr>
</tbody>
</table>

**Nitrogen and Microcystins: chla ratio**

Nitrate concentrations at Gibraltar buoy decreased throughout summer each year (Fig. 7), although the maximum concentration measured in 2015 was approximately 100 µmol/L greater than maximum levels measured in 2016 and 2017. Nitrate concentration declined to levels less than 10 µmol/L by mid-August every year, but in 2017 nitrate concentrations increased to 20 µmol/L by early September and then decreased to low levels throughout September (Fig. 7c).
Figure 7. The relationship between nitrate concentration and the ratio of microcystins to cyanobacteria-chla displayed as seasonal patterns for summers 2015 (a), 2016 (b), and 2017 (c), and as a scatter plot (d). Note that highest microcystins to cyanobacteria-chla occurred at intermediate nitrate concentrations and decreased with decreased nitrate concentrations.

The MCY per cyanobacteria-chla ratio (MCY:chl\(_a\)) was calculated to indicate the toxin production per cyanobacteria biomass. During 2015, MCY:chl\(_a\) was less than 0.14 throughout the entire summer and ratio values decreased to less than 0.07 after September 1 (Fig. 7a). During 2016, MCY:chl\(_a\) ranged from 0.08 to 0.30 during July and August, and then decreased to values less than 0.06 in September (Fig. 7b). During July and mid-August of 2017 the MCY:chl\(_a\) was highly variable but, in general, decreased to values less than 0.08 by the end of August (Fig. 7c), and then MCY:chl\(_a\) increased from 0.08 to highest values of 0.39 by mid-September and then again decreased throughout the second half of September. The decreases in MCY:chl\(_a\) were associated with decreases in nitrate concentration in all 3 years, and the lowest MCY:chl\(_a\) values occurred when nitrate concentration was less than 10 \(\mu\)mol/L (Fig. 7d). A similar pattern was observed between MCY:chl\(_a\) and total nitrogen concentration and the ratio of total nitrogen to total phosphorus, but not total phosphorus, dissolved reactive phosphorus, and ammonium-nitrogen concentrations (not shown).

Every-meter phytoplankton data
Buoy measured cyanobacteria RFU and the converted RFU-to-cyanobacteria chla concentrations are displayed in Fig. 8. Highest biomasses of cyanobacteria during late July through September 2015.

![Cyanobacteria abundance](image)

*Figure 8. Cyanobacteria abundance at the Gibraltar buoy during summers 2015 as phycocyanin relative fluorescence units (RFU) and RFU converted to cyanobacteria chlorophyll a concentration.*

The every meter sampling indicated that cyanobacteria were not evenly distributed throughout the water column on some dates but were evenly distributed on other dates (Fig. 9). For example, on July 28, 2015, cyanobacteria chla concentration peaked at 71.5 µg/L at 0 m and declined throughout the water column to 10.5 µg/L at 5 m. An example of cyanobacteria evenly distributed throughout the water column occurred on August 7, 2015, when chla concentration ranged from 11.2 to 13.8 µg/L. Much lower cyanobacteria chla concentrations were recorded in 2016 and 2017.

![Cyanobacteria isopleths](image)

*Figure 9. Isopleths of cyanobacteria chlorophyll a concentration measured at every meter from the surface to 5 meters throughout summers 2015, 2016, and 2017. Note the difference in color scale among the three years.*

A comparison of buoy RFU converted-cyanobacteria chla to cyanobacteria chla measured throughout the water column showed that were occurrences when the buoy both underestimated and overestimated the cyanobacteria chla at specific depths (Fig. 10). Data points that lay above the 1 to 1 line (dotted line) indicate the buoy underestimated cyanobacteria chla concentration at the particular depth, whereas those beneath the 1 to 1 line indicate the buoy overestimated
cyanobacteria chla concentration. For example, on July 28, 2015, the buoy estimated the cyanobacteria chla concentration to be 32.9 µg/L, but the 0 m and 1 m cyanobacteria chla concentrations exceeded the buoy estimate (71.5 and 51.2 µg/L, respectively), whereas the 5 m cyanobacteria chla concentration was much less than the buoy (10.5 µg/L). Overall, the buoy tended to underestimate cyanobacteria chla concentrations at 0 m while overestimating the deeper cyanobacteria chla concentrations.

Wind speed data was used to determine if the differences observed between cyanobacteria chla concentrations measured by the buoy and measured at depth could be explained (Fig. 11). The percent relative difference (%RD) between cyanobacteria chla concentration measured by the data buoy and throughout the water column showed how much cyanobacteria biomass at depth differed from the buoy measurement. The greatest range of wind speed occurred 1 hour before sampling (0.85 to 10.25 m/s) and the wind speed range decreased with longer time frames. There was high %RD across the range of the 1-hour window before sample collection (Fig. 11a), which indicates that cyanobacteria position in the water column was not affected by wind over a short time span. As longer time frames were considered, the %RD decreased with increased wind speed.
Because the %RD decreased with increased wind speed, the average %RD for each depth at wind speeds less than and greater than 4.5 m/s were calculated (Table 4). There was a greater difference in %RD between low and high wind speeds near the surface and less difference in %RD between the wind speeds deeper in the water column. For example, 12 hours before sample, buoy and chl\(a\) at 0 meter were 52.9% different at low wind speeds and only 18.1% at high wind speeds, whereas, at 5 meters, the differences were 38.9% and 31.2% for low and high winds, respectively. Additionally, surface chl\(a\) deviated more from the buoy than bottom chl\(a\) in low vs. high winds, but high winds resulted in the surface chl\(a\) to be more similar to buoy than bottom chl\(a\). The smallest difference (16.8%) between buoy and water sample occurred at the 1 meter depth during high wind speeds when the buoy data 12 hours before sample collection was average.
Table 4. The average of the absolute value of percent relative differences between cyanobacteria chla concentration measured by the data buoy and throughout the water column as a function of wind speed less than 4.5 m/s and greater than 4.51 m/s and as time before sample collection.

<table>
<thead>
<tr>
<th>Depth (m)</th>
<th>1 hour &lt;4.5m/s</th>
<th>1 hour &gt;4.5m/s</th>
<th>4 hours &lt;4.5m/s</th>
<th>4 hours &gt;4.5m/s</th>
<th>8 hours &lt;4.5m/s</th>
<th>8 hours &gt;4.5m/s</th>
<th>12 hours &lt;4.5m/s</th>
<th>12 hours &gt;4.5m/s</th>
<th>24 hours &lt;4.5m/s</th>
<th>24 hours &gt;4.5m/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 Meter</td>
<td>49.8%</td>
<td>29.4%</td>
<td>52.1%</td>
<td>22.9%</td>
<td>50.7%</td>
<td>20.0%</td>
<td>52.9%</td>
<td>18.1%</td>
<td>47.7%</td>
<td>30.0%</td>
</tr>
<tr>
<td>1 Meter</td>
<td>35.6%</td>
<td>27.0%</td>
<td>38.1%</td>
<td>21.3%</td>
<td>37.7%</td>
<td>18.6%</td>
<td>39.3%</td>
<td>16.8%</td>
<td>37.6%</td>
<td>20.7%</td>
</tr>
<tr>
<td>2 Meter</td>
<td>29.2%</td>
<td>32.9%</td>
<td>32.1%</td>
<td>27.4%</td>
<td>32.8%</td>
<td>24.5%</td>
<td>33.3%</td>
<td>24.2%</td>
<td>35.1%</td>
<td>20.1%</td>
</tr>
<tr>
<td>3 Meter</td>
<td>36.9%</td>
<td>32.2%</td>
<td>38.5%</td>
<td>28.7%</td>
<td>38.4%</td>
<td>26.7%</td>
<td>38.8%</td>
<td>27.0%</td>
<td>38.6%</td>
<td>27.5%</td>
</tr>
<tr>
<td>4 Meter</td>
<td>33.9%</td>
<td>35.4%</td>
<td>34.8%</td>
<td>33.7%</td>
<td>35.1%</td>
<td>32.5%</td>
<td>35.9%</td>
<td>31.0%</td>
<td>37.7%</td>
<td>27.0%</td>
</tr>
<tr>
<td>5 Meter</td>
<td>37.9%</td>
<td>34.2%</td>
<td>38.6%</td>
<td>32.4%</td>
<td>38.2%</td>
<td>32.2%</td>
<td>38.9%</td>
<td>31.2%</td>
<td>38.0%</td>
<td>33.2%</td>
</tr>
</tbody>
</table>

Overall: 37.2% 31.8% 39.0% 27.7% 38.8% 25.8% 39.8% 24.7% 39.1% 26.4%

Microcystins throughout the water column

Total microcystins were measured at every meter on two dates during the 2015 bloom (Fig. 12). On both dates, microcystin concentrations at depth as 0 m, 1 m, and 2 m were 4 to 6 times greater than microcystins at deeper depths. The higher concentrations of microcystins at the surface corresponded to greater biovolumes of cyanobacteria, especially Microcystis, near the surface (Fig. 13).

Figure 12. Total microcystin concentrations throughout the water column on two dates in July 2015 at the Gibraltar Island data buoy.
Figure 13. Phytoplankton biovolume measured throughout the water column on July 30, 2015, at the Gibraltar Island data buoy.

Total microcystins were measured weekly from the depth of 5 m throughout summer 2016 (Fig. 14). Microcystins at 5 m followed a similar temporal pattern as surface (0-2 intergraded sample) microcystin concentration with both depths peaking in mid-August. However, there were 3 occurrences when microcystin concentrations differed between surface and 5 m depth. On August 11, 2016, surface microcystins were 1.03 µg/L whereas the 5 m concentration was 0.30 µg/L. The opposite pattern was observed on August 12 and 15, 2016, as 5 m microcystin concentration exceed the surface concentrations by nearly a factor of 3.

Figure 14. Total microcystin concentrations at the surface (0-2 m intergraded sample) and at 5 meters depth measured throughout summer 2016 at the Gibraltar data buoy.
Finding Significance

Buoys as surrogates for water samples

Since the August 2014 “do not drink” advisory in Toledo, Ohio many data buoys and sondes attached to permanent structures have been deployed in western Lake Erie to serve as an early warning system for potentially toxic ChABs. Cyanobacterial biomass and MCY concentration followed a very similar temporal pattern as buoy cyanobacteria phycocyanin RFUs, suggesting the buoys can serve as an early warning system for ChABs. However, buoy data should only be interpreted as relative biomass data because the trends between buoy and water sample data differed among the 3 years. For example, if a water treatment plant operator noticed increased cyanobacteria RFUs at the intake, it would be safe to assume that more cyanobacteria were entering the water system and treatment should be adjusted to remove higher levels of potentially toxic cyanobacteria. On the other hand, a researcher who wished to use buoy data to calibrate a ChABs-model would first need the exact relationship between buoy data and water sample data. Although, that researcher could compare the temporal trends of model output to in situ buoy data.

Buoy chla and cyanobacteria data on the 15-minute time scale was very irregular, which could have led to flawed interpretations. For example, during this study cyanobacteria PC at the Gibraltar buoy ranged from 0.07 to 4.97 RFU and had an average of 1.19 RFU. These large spikes in RFU data were likely due to large Microcystis colonies drifting past the sonde as it was recording a measurement (Hodges et al. 2017). Viewing the single most recent value could be misleading, users should consider several data points to get a better interpretation of actual biomass. Indeed, buoy data averaged over a 1-hour before water sample collection had a better correlation with water sample data than the buoy data at the time of sample collection. Furthermore, data over the past 24 hour or several days should be considered to determine trends in potentially toxic cyanobacteria.

This research highlighted two main flaws of in situ buoy data. First, data from different buoys may not be directly comparable. There were positive linear relationships found between buoy data and water sample data for the Gibraltar buoy for every parameter tested, but those same correlations did not occur at the Sandusky buoy. This difference could have been due to the lack of service or cleaning of the Sandusky buoy (Fig. 2), which would have minimized water exchange across the water quality sondes and affected results. Alternatively, the difference may have been because of the different cyanobacteria communities at the two buoys (Bowling et al. 2016). Microcystis was the only dominant cyanobacteria at the Gibraltar buoy, but a more diverse cyanobacteria community was present at the Sandusky buoy. For example, Planktothrix, a filamentous bloom-forming cyanobacterium associated with shallow, light limited waters (Kurmayer et al. 2016), was often found at the Sandusky buoy due to outflow from Sandusky Bay (Conroy et al. 2017). Additionally, in late summer (Aug-Oct) Microcystis was found at Sandusky buoy due to the easterly spread of the western basin Microcystis bloom (Chaffin et al. 2014b). It is plausible that these two cyanobacteria have different fluorescence properties that result in different RFU readings. The second flaw was year-to-year differences. All three parameters investigated showed significantly different coefficients over the studied years. User error during standard preparation or calibration is a potential source of year-to-year variation that cannot be ruled out, but environmental variables may also be a factor. Cyanobacterial colony morphometry can have significant impacts on the relationship between fluorescence values and
biovolume. Similarly, (Hodges et al. 2017) showed there was a stronger linear relationship between Microcystis single cells and fluorescence than colonial Microcystis forms. Indeed, single cells of Microcystis made up more than 50% of the total cyanobacterial biovolume throughout summer 2015, which had tighter correlations between fluorescence and cyanobacteria biovolume (Table 1). Turbidity was high in 2015, and because algae can alter pigment content per cell with light climate (MacIntyre et al. 2002; Chaffin et al. 2012), cyanobacteria in 2015 could have had more PC and chla per cell than cyanobacteria in 2016 and 2017. Nutrient status of algae can also impact fluorescence (Beardall et al. 2001), and nutrient concentrations during 2015 were much higher than 2016 and 2017. Additionally, variations in the content of PC and chla per cell due to growth phase (Chang et al. 2012) and the presence of extracellular PC (Bastien et al. 2011) can also lead to inaccuracies between sonde data and cyanobacterial biomass estimates (Zamyadi et al. 2016). While calibration of the sondes of the Lake Erie sonde network occurs together (Fig. 1), the data from each sonde may not be directly comparable across time and space.

Turbidity is a commonly measured parameter as an indicator of water quality. Turbidity is primarily associated with phytoplankton biomass and suspended solids (sediments from tributary loading or resuspension from the lake bottom), but high concentrations of colored dissolved organic matter can also impact turbidity (Wetzel 2001). Buoy measured turbidity, and water sample TSS were highly correlated, and the trends were similar but significantly different, among years (Table 1).

**Microcystins correlations**

Cyanobacterial blooms are troublesome due to their potential to produce high concentrations of the toxin MCYs. Many lake managers and water treatment facilities have opted to use real-time water quality sondes that estimate cyanobacterial biomass; however, the water quality sondes cannot directly measure MCYs. Therefore, users interpreting sonde data are left to assume that MCYs and cyanobacterial abundance are proportional; conversely, cyanobacterial biomass and MCYs concentrations often do not correlate in the environment (Dyble et al. 2008; Millie et al. 2009; Wang et al. 2009; Rinta-Kanto et al. 2009). Additionally, the MCY to cyanobacteria-chla ratio decreased throughout summer and differed year-to-year (2015 had lower MCY:cyanobacteria-chla than 2016 and 2017; Fig. 7), which indicates that amount of MCY produced per unit cyanobacterial biomass was not constant. Nonetheless, within each bloom season, peaks in buoy cyanobacteria-PC RFU were often associated with an increase MCY concentrations, but the magnitude of the MCY peak was not scalable with the RFU peak.

Microcystin production by cyanobacteria has been linked to N availability by numerous studies (Orr and Jones 1998; Long et al. 2001; Horst et al. 2014; Gobler et al. 2016). Nitrate concentrations at the Gibraltar buoy decreased to low levels throughout each year of the study (Fig. 7), which is a temporal pattern that has been documented in other years in the western basin of Lake Erie (Chaffin et al. 2013; Gobler et al. 2016). The MCY to cyanobacteria-chla ratio pattern followed nitrate concentrations and only low MCY:cyanobacteria-chla ratios occurred at low nitrate concentrations (Fig. 7d). Additionally, during late August 2017 nitrate concentrations increased by 20 µmol/L and there was a corresponding increase in the MCY:cyanobacteria-chla ratio (Fig. 7c). Furthermore, nitrate enrichments to late summer bloom water under experimental conditions resulted in increased toxin production (Chaffin et al. in review; Harke and Gobler...
2015; Harke et al. 2016a). Taken together, previous studies and the results presented agree that low N availability constrains cyanobacterial bloom toxicity.

Water quality sonde networks have been deployed in Lake Erie and elsewhere to track potentially toxic cyanobacterial blooms, with the goal of protecting the public from cyanobacterial toxins (Jochens et al. 2010). As discussed above, several factors interfere with relationships between PC RFU data and cyanobacterial abundance, and there is no sound correlation between cyanobacteria biomass and toxin concentration. Therefore, due to the multiple layers of uncertainty, sonde PC RFU data should not be used as a direct estimate of MCY concentration. Although, nitrate concentration data or a general understanding of the temporal patterns of nitrate concentrations at a given site can improve the decision-making process. For example, it is known that the highest nitrate concentrations occur in Lake Erie during early summer and decrease to low levels by the end of August (Chaffin et al. 2013), and therefore, spikes of PC RFU during early summer should be considered to be highly toxic whereas peaks of PC RFU later in the season could be assumed to be less toxic. Following that logic, a water treatment plant operator should use higher treatment doses (such as more activated carbon) per PC RFU during early summer blooms to potentially remove more cyanobacterial toxins per PC RFU than late summer blooms. Nevertheless, these guidelines should be used only as a tool, and not a replacement for sample testing where decisions with enormous ramifications (such as drinking water safety) are concerned and need to be confirmed by sample testing.

Water column cyanobacteria and relation to buoy data

Cyanobacteria can migrate throughout the water column and often concentration near the surface of the water (Reynolds et al. 1987; Ganf et al. 1989; Brookes et al. 2003). The vertical migration of cyanobacteria can be problematic for data buoys with water quality sondes fixed at one depth. Indeed, the chla concentration measured at a depth of 1 meter by the data buoy often did not match the chla concentration measured at the surface (above the sonde) or below the sonde at deeper depths (Fig. 10). Therefore, interpretation of a data buoy’s chla data that is recorded from a fixed depth cannot be assumed to be equal to the concentration above or below the sonde.

Wind speed can affect phytoplankton position in the water column. In light winds and calm water, buoyant cyanobacteria will concentrate near the surface (Hutchinson and Webster 1994; Soranno 1997), whereas, negatively buoyant phytoplankton, such as diatoms, will sink towards the lake bottom (Webster and Hutchinson 1994; Huisman et al. 2002). In high winds and rough waters, the buoyancy of cyanobacteria and the sinking rate of diatoms is over-powered by the water turbulence, and the phytoplankton will be evenly distributed throughout the water column (Huisman et al. 2002; Brookes et al. 2003). There were more considerable differences between cyanobacteria chla measured by the buoy at depth during low wind speeds and the relative difference decreased as wind speed before sampling increased (Fig. 11, Table 4). This indicates that the cyanobacteria became more-mixed throughout the water column and that the buoy estimates of cyanobacteria biomass were more extractible to other depths.

The period over which wind speed was averaged affected how to interpret the relationship between buoy and at-depth measurements of cyanobacteria chla. In the 1-hour before sampling period, large relative differences between the buoy and at depth occurred at high wind speeds (Fig. 11a), which likely indicates high winds started just recently before sampling and there was not enough time to mix the water column. The decreases in relative difference
between buoy and chla at depth became apparent when 12 and 24 hours of wind speed data were averaged (Fig. 11d and e), and indicates that cyanobacteria vertical position was more affected by long-term (12+ hours) than short-term (< 1 hour) wind speeds.

The relationship between buoy and surface (0 m) cyanobacteria chla was different from the relationship between the buoy and deep chla (5 m). Under calm winds, surface and buoy chla differed by 52.9%, which indicated that buoyant cyanobacteria migrated above the buoy’s water quality sonde (0.6-1.0 m in depth). Buoy data would have misled data users by the presence of noticeable scum at the surface. Contrary to low winds, the smallest difference between the buoy data and chla at the surface and 1 m (18.1% and 16.8%, respectively) was recorded under high winds. This indicated that surface buoy measurements are most accurate at high wind speeds because water turbulence inhibited surface scum formation. However, the difference between the buoy data and chla at the 5 m was not affected by wind speed (31.2% at low wind speeds and 38.9% at high wind speeds). Therefore, there is going to be an error associated with interpretation surface buoy data and cyanobacteria biomass at deeper depths. For example, a water treatment plant operator cannot assume surface buoy biomass data is proportional to biomass being drawn into the plant from deeper depths, and the difference cannot be corrected for by wind speed.

Conclusions

Significant correlations occurred between buoy data and water sample data for cyanobacterial biomass, total algae, and turbidity; however, the trends differed between buoys and among years. Further, relative trends in the data over time and space can be gleaned, but these methodologies do not replace laboratory methods for estimation of actual phytoplankton biomass. In spite of that, cyanobacterial biomass and MCY concentration data from water samples collected next to the Gibraltar buoy followed a similar temporal pattern as the buoy cyanobacteria-PC RFU data, which indicates their usefulness as a guidance tool for sectors like water treatment and beach management. Additionally, the inclusion of nitrate concentration data can lead to more robust predictions on the relative toxicity of blooms. Low wind speeds over the previous 12 hours (< 4.5 m/s) led to an underestimation of cyanobacteria biomass at the surface, whereas high wind speeds (> 4.5 m/s) resulted in more accurate measurements. However, cyanobacteria biomass estimates 5 meters below the surface were between 31% to 39% different from the buoy estimate and were not improved when wind speed was considered. Overall, deployed buoys and water quality sondes that are routinely cleaned and calibrated can efficiently track relative cyanobacteria abundance and can be used as an early warning system for potentially toxic blooms.

A flow chart was created to help interpretation of data buoys better cyanobacteria biomass and extrapolate the buoy data to other water depths. Additionally, the relative risk of microcystins was also included. The flow chart is specific to systems dominated by Microcystis. The first division was based on nitrate concentration because highest ratios of microcystin concentration to cyanobacteria-chla corresponded to nitrate concentrations greater than 7 µmol/L (0.1 mg NO₃-N/L, Fig. 7), and phytoplankton growth is considered N-limited at nitrate concentrations less than 7 µmol/L (Chaffin et al. 2014a). Nitrate concentrations higher than 7 µmol/L are to be associated with a higher risk of microcystins, but additional factors could limit microcystin concentration, such as low light levels (Chaffin et al. 2018). Summer 2015 had high nitrate concentrations (Fig. 7) but low water clarity and low microcystin concentrations relative
to the high *Microcystis* biomass. The second division on the flow chart is wind speed and the division is 4.5 m/s (10 miles per hour) averaged over the previous 12 hours. The final division is depth of concern (surface or at depth). At calm wind speeds, *Microcystis* will float above the buoy sensors resulting in an underestimation at the surface, but high wind speeds will mix the water column and result in biomass estimates that are within 20% of the actual surface measurements. Wind speeds did not impact the relationship between buoy and cyanobacteria chl*α* data at depth, and therefore, the users must be aware that there will be an error of 30% to 40% when relying on surface buoy as an estimate of cyanobacteria biomass near the lake bottom, for example, a water intake.

![Flow chart](image)

**Figure 15.** A flow chart for better interpretation of cyanobacteria biomass data measured by surface buoys in a *Microcystis*-dominated system based on nitrate concentration and wind speed.

**References**


Davis CC (1964) Evidence for the eutrophication of Lake Erie from phytoplankton records. Limnol Oceanogr 9:275–283


2. Publication/Presentations Citations

Publications in review:


Publications in preparation:

Presentations:

#1  Event name: Ohio Academy of Science Annual Meeting  
Size of audience: 20  
Date: 4/16/2016  
Title of talk: Effectiveness of data buoys for sampling cyanobacterial harmful algal blooms in Lake Erie  
Type of presentation: Oral

#2  Event name: Lake Erie Water Plant Group Spring 2016 Meeting  
Size of audience: 100  
Date: 4/21/2016  
Title of talk: Stone Lab’s HAB research  
Type of presentation: Oral

#3  Event name: Great Lakes HABS Collaboratory Webinar  
Size of audience: 50  
Date: 6/2/2016  
Title of talk: Let’s Hear It for the Buoys?- Accuracy of data buoys for monitoring cyanobacterial blooms in Lake Erie  
Type of presentation: Oral

#4  Event name: International Association on Great Lakes Research Conference  
Size of audience: 50  
Date: 6/6/2016  
Title of talk: Accuracy of Data Buoys for Monitoring Cyanobacterial Blooms in Lake Erie  
Type of presentation: Oral

#5  Event name: Phycological Society of America Annual Meeting  
Size of audience: 100  
Date: 7/26/2016  
Title of talk: Lake Erie’s re-eutrophication: degradation, adaptation, and restoration  
Type of presentation: Oral

#6  Event name: Western Lake Erie Basin  
Size of audience: 25  
Date: 9/12/2016  
Title of talk: Harmful Algal Blooms in Lake Erie  
Type of presentation: Oral

#7  Event name: Ohio GIS Conference  
Size of audience: 200  
Date: 9/28/2016  
Title of talk: Harmful Algal Blooms in Lake Erie  
Type of presentation: Oral
#8  Event name: Ohio Water Resource Center Luncheon  
Size of audience: 30  
Date: 10/12/2016  
Title of talk: Harmful Algal Bloom Research at Stone Lab: Monitoring Blooms and Determining Drivers of Bloom Toxicity  
Type of presentation: Oral

#9  Event name: São Paulo State University (UNESP)- Botucatu, Brazil  
Size of audience: 20  
Date: 2/19/2017  
Title of talk: The re-eutrophication of Lake Erie: degradation, adaptation, and restoration  
Type of presentation: Oral

#10 Event name: Lake Erie Millennium Network  
Size of audience: 125  
Date: 2/21/2017  
Title of talk: Accuracy of data buoys for tracking cyanobacterial blooms in Lake Erie  
Type of presentation: Oral

#11 Event name: Ohio Academy of Science  
Size of audience: 45  
Date: 4/8/2017  
Title of talk: The efficiency of data buoys for tracking cyanobacterial blooms in Lake Erie  
Type of presentation: Oral

#12 Event name: International Association on Great Lakes Research Conference  
Size of audience: 75  
Date: 5/16/2017  
Title of talk: Yeah buoy: Monitoring cHABS in western Lake Erie using in-situ technology  
Type of presentation: Oral

#13 Event name: SEAL Analytical Workshop  
Size of audience: 30  
Date: 8/25/2017  
Title of talk: Harmful Algal Bloom Research at Stone Lab: Monitoring Blooms and Determining Drivers of Bloom Toxicity  
Type of presentation: Oral

#14 Event name: Ohio State University School of Environment and Natural Resources Freshman  
Size of audience: 45  
Date: 9/16/2017  
Title of talk: Hands-on lesson in HAB research at Stone Lab
Type of presentation: Oral

#15  Event name: Shipwrecks and SCUBA  
Size of audience: 125  
Date: 10/21/2017  
Title of talk: MAST Helps to Monitor Central Basin Cyanobacterial Blooms  
Type of presentation: Oral

#16  Event name: University of Toledo- Lake Erie Center Public Lecture Series  
Size of audience: 30  
Date: 11/16/2017  
Title of talk: From Lake to River: Using Plankton to Assess Water Quality in Lake Erie and Its Tributaries.  
Type of presentation: Oral

#17  Event name: Lakewood High School Watters Seminar  
Size of audience: 50  
Date: 12/14/2017  
Title of talk: Re-eutrophication of Lake Erie and restoration of wetlands in the Maumee River watershed of northwest Ohio.  
Type of presentation: Oral

#18  Event name: Upper Maumee Watershed Partnership Meeting  
Size of audience: 10  
Date: 1/10/2018  
Title of talk: Cyanobacterial Harmful Algal Bloom detection in western Lake Erie and the Maumee River using sensor technology  
Type of presentation: Oral

#19  Event name: Kent State University Biology Department seminar  
Size of audience: 50  
Date: 1/19/2018  
Title of talk: Working towards a forecast of Lake Erie cyanobacterial bloom toxicity  
Type of presentation: Oral

#20  Event name: Midwest Aquatic Plant Management Society  
Size of audience: 230  
Date: 2/27/2018  
Title of talk: The role of phosphorus, nitrogen, and light on Lake Erie cyanobacterial bloom growth and toxicity  
Type of presentation: Oral

#21  Event name: Trumbull County Soil & Water Conservation District Lunch and Learn  
Size of audience: 20  
Date: 3/20/2018  
Title of talk: Development of a Lake Erie HAB toxicity forecast
Type of presentation: Oral

3. Students Supported

Morgan Potts. Undergraduate research student at Stone Lab during 2016. Morgan is currently a senior at Bowling Green State University.

Alex Johnson. Undergraduate research student at Stone Lab during 2017. Alex is currently a senior at Cleveland State University.

4. Profession Placement of Graduates

None.

5. Awards or Achievements

None.

6. Additional Funding

None.