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A comparison of water sampling and analytical methods in western Lake Erie

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ABSTRACT

Monitoring the water quality of large water bodies requires the efforts of many organizations. However, monitoring organizations often use a variety of sampling and/or analytical methods to measure important parameters such as chlorophyll *a* (chl *a*) and total phosphorus (TP) concentrations that may result in incompatible data sets. In order to assess the compatibility of collection and analytical methods used in Lake Erie, we compared four water sampling methods in side-by-side fashion and conducted a round-robin comparison of field replicates that were analyzed for TP and chl *a* by several laboratories using several methods. For samples collected under mixing conditions, linear regressions among the four sampling methods resulted in nearly 1-to-1, highly correlated ($R^2 > 0.90$) relationships. The methods were less compatible, however, when surface cyanobacterial scums were present. All TP data reported by the different laboratories were in agreement. Chl *a* data from 4 of the 6 laboratories were in agreement (< 20% relative difference) despite the use of different combinations of organic solvents, cell lysis, and quantification methods. In conclusion, we found that sampling methods and TP and chl *a* data from multiple organizations examined were generally compatible. These results should build confidence in the compatibility of data sets generated by Lake Erie monitoring organizations and should be beneficial to any combined analysis of historical, long-term data.

Introduction

Tracking changes in water quality in lakes requires thorough monitoring of water quality over time and space. Two frequently measured parameters are chlorophyll *a* (chl *a*) and total phosphorus (TP) concentrations, as surrogates for total phytoplankton biomass and a constraining factor for maximum lake primary production, respectively. Because of Lake Erie's large size (25,700 km^2), monitoring involves many agencies and organizations that have been independently building long-term (multiple years) data sets since the early 1900s (Charlton and Milne, 2004; Conroy et al., 2005; Davis, 1964; Fraleigh et al., 1975; Herdendorf, 1983; Makarewicz et al., 2000). These long-term data sets cover different areas of the basin, with differing sampling methods, and at differing frequencies and dates. Although a combined data set would provide great benefit for the analysis of large-scale spatial and temporal trends, integration of the individual data sets has never been attempted.

One challenge that may arise when comparing data from several organizations is their use of different water sampling protocols. Factors such as sampling depth and time of day could influence the determination of both chl *a* and TP concentrations between agencies (Martin et al., 1992). Some algae and cyanobacteria can regulate their buoyancy to position themselves at a desired light level within the water column. Bloom-forming cyanobacteria such as *Microcystis* and *Dolichospermum*, which are at times both abundant in Lake Erie, can accumulate near the surface of the water (Reynolds et al., 1987). Diatoms, on the other hand, are negatively buoyant and will sink to deeper depths in calm water (Huisman et al., 2002). Thus, a surface-only grab sample may overestimate or underestimate the chl *a* concentrations relative to the water column average. Concentrations of TP can also vary with depth due to stratified algae and different sampling methods may or may not reflect those differences. Furthermore, higher concentrations of TP may be found near the lake bottom when sediments release phosphate due to anoxic water (Mortimer, 1941) if sediments are resuspended by benthic invertebrates (Chaffin and Kane, 2010), or by other mechanical and chemical pathways (Havens, 1991; Søndergaard et al., 2003).

Another challenge to comparing data from multiple organizations is the use of different analytical procedures. Besides the "standard" method to determine chl *a* concentration, which involves grinding a filter in acetone (i.e. American Public Health Association, 2012; Lorenzen, 1967; Wetzel and Likens, 1991), there are numerous methods to determine chl *a* concentration extracted from a filtered water sample. However,

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the differences in instrumentation and solvents used can lead to significantly different reported concentrations (Hagerthey et al., 2006; Jacobsen and Rai, 1990; Schagerl and Künzl, 2007). For TP concentration, the only accepted method is oxidizing all P to phosphate, usually with persulfate (Menzel and Corwin, 1965) and subsequent quantification with reagents of molybdate, antimony, and ascorbic acid that produce a blue color proportional to the P concentration (Murphy and Riley, 1962).

The objectives of this study were to determine (1) if the different water sampling protocols used by the various organizations resulted in different TP and chl *a* measured concentrations at a given location in the lake and (2) whether there were inherent differences in the TP and chl *a* data produced by different labs due to different analytical procedures. Total P and chl *a* were chosen because these parameters are the most commonly measured parameters by all researchers monitoring water quality in lakes. If the TP and chl *a* data across sampling methods and laboratory analysis protocols are compatible, then pooling data for basin-wide or long-term analyses becomes an easier task. Conversely, revealing conflicting results due to differences in water sampling or laboratory analysis will highlight the need for modified procedures. If results from different organizations vary in a consistent manner, it would be possible to generate conversion factors for the benefit of those who are pooling historical data. Large-scale integrated data sets would provide increased understanding of trophic changes over a greater area and over longer time spans than is currently possible except indirectly by satellite data.

Methods

Sampling method comparison

Several organizations routinely collect water samples in western Lake Erie but do so using differing methods. We compared four of these water collection methods in side-by-side fashion (Fig. 1). The full water column method utilizes a tube sampler constructed of clear, flexible polycarbonate tubing (2.5 cm internal diameter) with a 5-lb weight attached at one end in order to collect a vertically integrated water column sample from the surface to near-bottom or to the thermocline (or up to 8 m maximum depth). The sampler is lowered slowly into the water to the desired depth, and then the surface end is plugged with a rubber cork. The sampler is then quickly lifted, and water drains from the weighted-end into a rinsed bucket as the cork is removed. This method is used routinely by University of Toledo's Lake Erie Center, Ohio State University's Stone Laboratory, and the USGS's Great Lakes Science Center. Because the water column method has the greatest chance to capture a stratified algal bloom (i.e. surface scum or deep chlorophyll maximum), all other sample methods were collectively termed "comparison methods" for our study. The 0- to 2-m integrated method utilizes a 2-m long tube sampler constructed of rigid polyvinylchloride and is deployed to collect an integrated surface to 2 m water sample. This method is used by charter boat captains on Lake Erie who volunteer to collect water samples for researchers. The Twice-the-Secchi disk-depth (2× SD) method is used to collect water representing the photic zone and utilizes an integrated tube sampler lowered to a depth twice that of the Secchi disk (depth). Depending on the Secchi disk depth, either a long and flexible or a short and rigid sampler can be used to collect the 2× SD sample. This method is used by the Ohio Department of Natural Resources and has been used by other Lake Erie researchers (Conroy et al., 2007). The sampling method of the Ohio EPA utilizes a Van Dorn sampler to collect and pool water from three discrete depths (1 m below the surface, 1 m above the lake bottom or thermocline, and mid-depth between the first two samples).

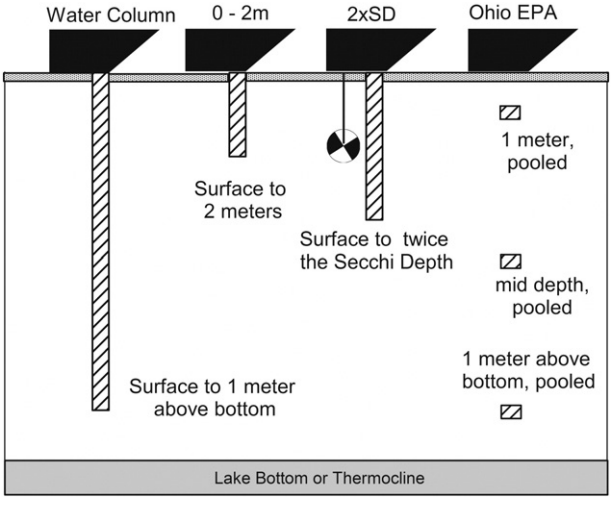

Fig. 1. Four water sampling methods used in this study. Bars indicate the depths of water collected by each method.

During summers of 2013 and 2014 (15 July to 29 October 2013 and 27 June to 1 October 2014), we sampled 23 study locations in Lake Erie with conditions ranging from eutrophic-nearshore (Maumee Bay and Sandusky Bay) to oligotrophic-offshore (center of the central basin) (Fig. 2). Most sites were sampled on multiple dates. A total of 82 water column samples with comparison method samples were collected. At every sample location, GPS coordinates, site depth, Secchi disk depth, and a profile of water temperature were recorded. Water temperature profile was recorded at 0.5-m intervals with a water quality sonde (YSI 6600v2) to determine if a thermocline was present. All sample bottles were rinsed with surface water, and all water sampling equipment was deployed to the appropriate depth. The water column sample was collected first (to a depth of 1 m above the lake bottom, or thermocline if present, or down to 8 m if water column was deeper than 9 m), and water was deposited into a 18.9-L rinsed bucket. Water intended for chl *a* analysis was poured into a 2-L dark polyethylene bottle, and water for TP was poured into a 250-mL polyethylene bottle. Next, the comparison method samples were collected to the appropriate depths and were handled in the same manner as the water column sample. In a few cases, two sampling methods were duplicated. For example, if the Secchi disk depth was 1 m, then the 2× SD and the 0- to 2-m sample methods were identical. In these cases, only one sample was collected and analyzed, and the data were used to represent both methods. All bottles were stored on ice during transportation back to the laboratory for processing. Between 1 h and 6 h passed between sample collection and processing.

Upon returning to the lab, the 250-mL bottles for TP analysis were placed in a −20 °C freezer until analysis. Equal volume aliquots of water from the 2-L bottle were filtered onto GFF filters (47 mm diameter) for chl *a* analysis. Between 50 and 1000 mL of lake water were filtered and then stored at −80 °C until analysis. All TP and chl *a* samples collected during the side-by-side were analyzed at Stone Laboratory using methods described below.

Total P concentration was quantified following an acid-persulfate digestion and quantification via the molybdate-ascorbic acid method (EPA method 365.1) on a SEAL Analytical QuAAtro nutrient analyzer. Seven known concentration phosphate solutions (including zero P) were used for the standard curve ($R^2 > 0.999$), and every-tenth sample was spiked with a known amount of P to ensure high accuracy and precision throughout the analysis (99%–101% recovery).

Chlorophyll *a* concentration was determined by placing the filtered sample into 10 mL of dimethyl sulfoxide (DMSO), heating to 70 °C for 45 min, centrifuging at 21,000 *g*, and absorbance measured at 665.1 nm and 649.1 nm on a Shimadzu-1240 spectrophotometer and chl *a* concentration calculated as

$$\text{Chl}\,a = ([12.47 \times \text{ABS}_{665.1}] - [3.62 \times \text{ABS}_{649.1}])/\text{Vol}_{\text{DMSO}} \times \text{Vol}_{\text{Lake Water}}$$

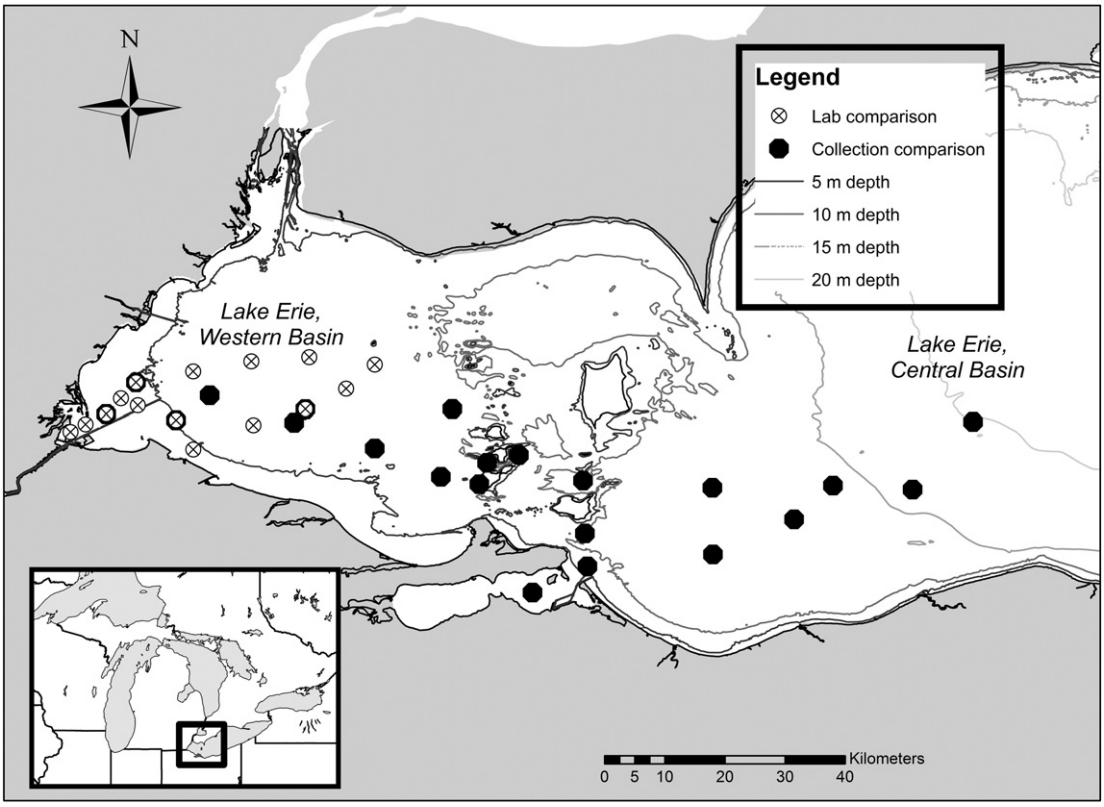

Fig. 2. Sample site locations for the side-by-side methods comparison and the intra-laboratory comparison study. Side-by-side method comparison sites are shown as filled circles and analytical method comparison is shown as a circle with an X. Four sites were sampled for both studies.

where chl *a* is the chlorophyll *a* concentration in μg/L, ABS is absorbance measured at 665.1 nm and 649.1 nm, Vol_{DMSO} is the volume of DMSO used in mL, and $Vol_{Lake\ Water}$ is the volume of lake water filtered in L (Chaffin et al., 2012; Wellburn, 1994). During 2013, one bottle replicate was used, whereas in 2014 two bottle replicates (i.e. 2 separate filters for chl *a* and 2 separate digestions for TP) were analyzed and averaged.

Analytical method comparison

To determine if different analytical methods from different laboratories could result in differences in the data, in 2014 we performed a round-robin inter-laboratory study in which identical samples were sent to each of the participating laboratories for analysis. In order to include a wide range of chl *a* and TP concentrations, we sampled 15 locations between the Maumee River mouth (typically high in chl *a* and TP concentration) and the center of the western basin (Fig. 2). Sampling was conducted twice, once at the beginning of the annual *Microcystis* spp. bloom (25 July 2014) and once during the middle of the bloom (7 August 2014). At each site, GPS coordinates, Secchi depth, and enough raw lake water (~20 L) were collected using a 2-m integrated tube sampler to provide institutions with an adequate sample. Lake water from the sampler was dispensed into a 20-L bucket and then poured into 21 250-mL bottles for TP analysis and 15 1-L bottles for chl *a* analysis. At each of three of the sites, triplicate samples were collected for TP analysis. All samples were held on ice during transportation back to the laboratory. In the laboratory, sample water was filtered (50 to 100 mL per filter; 25 mm GF/F) in duplicate for chl *a* analysis and stored at −80 °C on silica gel. Total P samples in the 250-mL bottles were stored at −20 °C.

Frozen samples were delivered to each laboratory in person. Laboratories that participated in TP comparison were the National Center for Water Quality Research at Heidelberg University (NCWQR), NOAA Great Lakes Environmental Research Laboratory (NOAA), and Ohio State University Stone Laboratory (OSU-SL). TP samples were delivered to a fourth laboratory; however, because the sample preservation method (freezing) was not compatible with the lab's protocol (sample preservation with acid), results were incompatible and are not included here. All laboratories utilized a variation of the same TP method (EPA method 365.1), which involved a digestion step with persulfate followed by quantification by colorimetry. For chl *a*, the laboratories that participated were University of Toledo Lake Erie Center (UT-LEC), USGS Great Lakes Science Center (USGS), NOAA, Ohio EPA, OSU-SL, and Ohio State University Aquatic Ecology Laboratory (OSU-AEL). The laboratories used several different organic solvents (acetone, dimethylsulfoxide (DMSO), or *N*,*N*-dimethylformamide (DMF)), extraction methods (mechanical grinding, or chemical lysis), and quantification methods (fluorescence or absorbance) (Table 1).

During the 2014 side-by-side sampling, two chl *a* methods were compared. A subset of water column samples ($n = 30$) were analyzed using the DMSO method and the more traditional acetone method (EPA method 446.0). Equal aliquots of water from a 2-L bottle were filtered on four filters. The filters were frozen, and then two filters were analyzed by DMSO and the other two by acetone. The two filters

Table 1
Laboratories that participated in our round-robin inter-laboratory comparison and their respective method for quantification of chlorophyll *a* where superscript 1 = EPA method 446.0, 2 = Wellburn, 1994, 3 = Lorenzen, 1967; 4 = Speziale et al. 1984.

Institution	Chl extraction solvent	Chl filter ground?	Chl quantification
Ohio EPA	Acetone	Yes	Fluorescence—acidified[1]
NOAA-GLERL	DMF	No	Fluorescence—non-acidified
OSU-Stone Lab	DMSO	No	Absorbance—non-acidified[2]
USGS-GLSC	Acetone	No	Fluorescence—non-acidified
OSU-AEL	Acetone	Yes	Absorbance—acidified[3]
UT-LEC	DMF	No	Fluorescence—acidified[4]

that were analyzed by DMSO followed the procedure outlined above. The remaining two filters were placed in 10 mL of 90% acetone and sonicated for 20 s, incubated for 3 h at 4 °C, centrifuged at 4000 rpm, and absorbance measured at 750 nm, 664 nm, 647 nm, and 630 nm. Chlorophyll *a* concentration calculated as

$$\text{Chl}\,a = ([11.85 * \text{ABS}_{750\text{-}664}] - [1.54 \times \text{ABS}_{750\text{-}647}] - [0.08 \times \text{ABS}_{750\text{-}630}])/\text{Vol}_{\text{Acetone}} \times \text{Vol}_{\text{Lake Water}}$$

where chl *a* is the chlorophyll *a* concentration in μg/L; ABS is absorbance measured at 750 nm, 664 nm, 647 nm, and 630 nm; $\text{Vol}_{\text{Acetone}}$ is the volume of acetone used in mL; and $\text{Vol}_{\text{Lake Water}}$ is the volume of lake water filtered in L. All of these samples were analyzed at Stone Laboratory.

Data analysis

For the side-by-side sample method collection, the water column sample was used as the reference sample because it had the greatest chance of capturing a stratified algal bloom at any depth. Linear regressions were used to compare the 3 comparison methods against the water column sample. The large range of sample concentrations necessitated the use of paired sample *t*-tests rather than ANOVA to determine if there were significant differences between the water column data and each comparison method.

Water sent to the different laboratories was separated from one large pooled sample; thus, the replicates among the laboratories are termed "field duplicates." Because we did not know the "true" concentration value of a sample and we had no reason to believe one laboratory was more accurate than another, concentrations reported for each sample were averaged. Total P data reported by the NCWQR, NOAA, and OSU-SL and chl *a* data reported by NOAA, Ohio EPA, OSU-SL, and OSU-AEL for each sample were used to calculate the average concentration. In order to avoid skewing the averages, outlying chl *a* data from UT-LEC and USGS were excluded from the average (reasons discussed below). Paired sample *t*-tests were conducted to determine if there were significant differences between the data reported by each laboratory and the average concentration. Because variance among reported concentrations increased with increasing concentration, data were log-transformed to achieve homoscedasticity then subjected to linear regression.

We used percent relative difference (% RD) to test for difference among sample duplicates, calculated as

$$\%\text{RD} = \left| \left(X_1 - X_{\text{avg}}\right) \Big/ \left[\left(X_1 + X_{\text{avg}}\right)/2\right] \right| \times 100\%$$

where X_1 is the concentration reported by a laboratory and X_{avg} is the average concentration reported by the above-mentioned laboratories. All statistics were conducted with IBM SPSS Statistics v. 22.

Results

Side-by-side method comparison

In the side-by-side method comparison, concentrations of TP ranged from 3.5 to 183.0 μg P/L, whereas chl *a* concentration ranged from 1.5 to 127.8 μg/L, indicating that a wide range of trophic conditions were sampled. The comparison method samples were highly correlated with the water column sample (and among each other) with R^2 values exceeding 0.91 and linear regression slopes of nearly 1.00 (Fig. 3; Table 2). Paired sample *t*-tests indicated no significant difference between the water column methods and the 3 comparison methods (Table 2).

While the full side-by-side comparison data set did not indicate significant differences among the data sets, there were few instances during a cyanobacterial surface bloom when chl *a* concentrations did differ among methods (Fig. 4). The central basin was sampled on 15 July 2013 during a *Dolichospermum* spp. (formerly *Anabaena*) bloom.

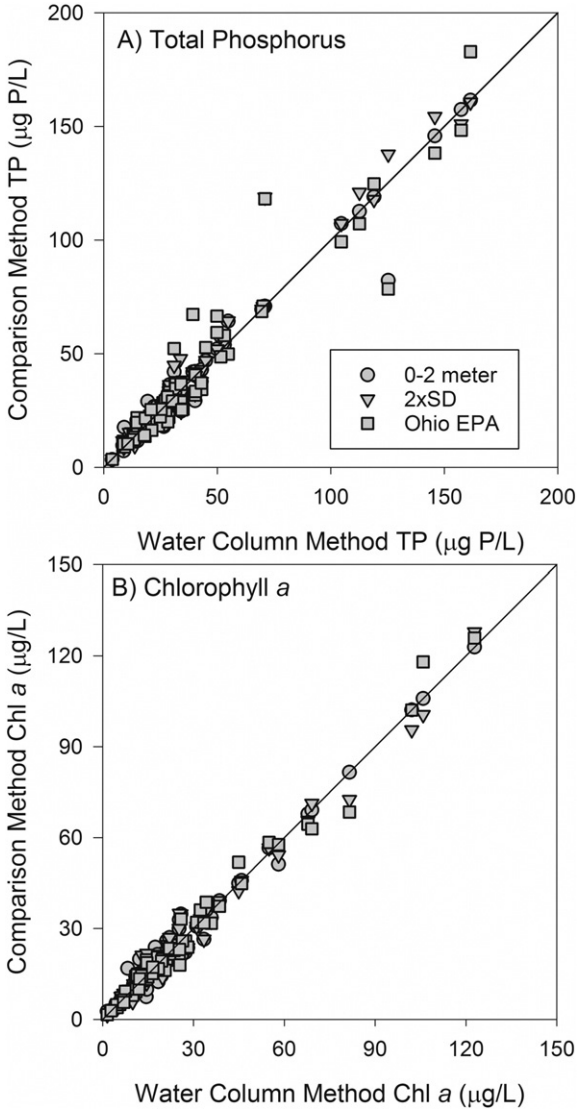

Fig. 3. Total phosphorus (A) and chlorophyll *a* (B) concentrations measured in the side-by-side method comparison. The *x*-axis data are from the whole water column, and the *y*-axis data are the three comparison methods. The black line is the 1-to-1 line.

Cyanobacteria phycocyanin fluorescence vertical profiles on this occasion indicated relatively high cyanobacteria densities near the surface and with densities 10× lower at depths >4 m. The 0- to 2-m sample had a chl *a* concentration nearly twice that of the other 3 methods that included deeper water containing lower concentrations of cyanobacteria (Fig. 4A). By contrast, when high winds mixed the bloom throughout the water column (as indicated by uniform vertical profiles cyanobacteria phycocyanin fluorescence), the 4 sampling methods resulted in very similar chl *a* concentrations (Fig. 4B).

Analytical method comparison

Total P and chl *a* concentrations reported by the different laboratories generally agreed across the broad trophic gradient sampled. However, there were differences among laboratories. For chl *a*, data from the USGS and UT-LEC on 7 August did not agree with the other 4 labs returning data. For both USGS and UT-LEC, we were able to identify likely factors that may have caused the disagreements (discussed below).

We used percent relative difference (% RD) to evaluate our data across a broad range of concentrations. Ohio EPA considers a 20% RD between field duplicates acceptable (Ohio Environmental Protection Agency, 2013), but at low concentrations (<5 times the method

Table 2
Side-by-side water sample collection comparison linear regression and paired sample *t*-test statistics for the 3 comparison methods (0–2 m, twice the Secchi disk depth (2× SD), and method used by Ohio EPA of pooling water from three depths) against the water column method (WC). Degrees of freedom for all regressions and paired *t*-tests were 81. All regressions were significant at $P < 0.001$.

		Regression			Paired *t*-test	
	Method	R^2	Equation	Slope 95% CI	Average difference	*P*
Water column total *P v.*	0–2 m	0.965	$TP_{method} = (0.958\ TP_{WC}) + 0.001$	0.92–1.00	0.390	0.568
	2× SD	0.964	$TP_{method} = (1.038\ TP_{WC}) - 0.001$	0.99–1.08	−1.350	0.074
	OEPA	0.914	$TP_{method} = (0.978\ TP_{WC}) + 0.001$	0.91–1.05	−0.160	0.558
Water column chlorophyll *a v.*	0–2 m	0.98	$Chl_{method} = (0.989\ Chl_{WC}) + 0.385$	0.96–1.02	−0.120	0.736
	2× SD	0.98	$Chl_{method} = (0.963\ Chl_{WC}) + 1.101$	0.93–1.00	−0.223	0.536
	OEPA	0.981	$Chl_{method} = (1.002\ Chl_{WC}) - 0.049$	0.97–1.03	−0.010	0.977

detection limit), 50% RD is acceptable (Ohio Environmental Protection Agency, 2013). Total P data reported by each laboratory plotted against the across-laboratory average showed that nearly all samples are within the acceptable 20% RD range (Fig. 5). For TP, NCWQR, NOAA, and OSU-SL each had 28 of the total 30 samples (93.3% of samples) that were within the acceptable 20% RD range (Table 3). However, 5 of the 6 samples outside the 20% RD range occurred when average TP concentration was <13 μg P/L. For chl *a*, UT-LEC and USGS had 50% and 3.4% samples, respectively, within the 20% RD range. The other four laboratories had between 86.7% and 93.3% of samples within the 20% RD range, but half of those samples outside the acceptable range occurred when average chl *a* concentration was <6 μg/L.

The regression equations for the side-by-side method comparisons (Table 2) and inter-lab comparison (Table 3) were very similar. Y-intercepts were near zero, and the 95% confidence interval for slopes included 1.0 in every case except 1 (TP, OSU-SL). The average differences between individual labs and the overall average were <1.5 μg/L (with the exception of USGS chl *a* data).

All 30 field duplicates analyzed with the DMSO and acetone methods at OSU-SL were within 20% RD of each other (Fig. 6). In a previous study, UT-LEC analyzed 60 field duplicates collected from the same sample locations as the inter-lab comparison using the DMSO and DMF-acid method. Those samples were also within 20% RD of each other and highly correlated ($R^2 = 0.93$) (Chaffin, 2009).

Discussion

Organizations that collect water quality samples in western Lake Erie have been aware for many years that their collection methods differ. If the water column, including phytoplankton and suspended sediments, is well mixed then collecting samples from differing depths would not present an issue. However, especially since the return of harmful algal blooms and their accompanying surface scums in the past 15–20 years, concerns have increased regarding varying sampling methods that may mischaracterize water column averages by either over or under-sampling highly concentrated layers of buoyant cyanobacteria near the surface.

Our results indicate that under well-mixed conditions, different sampling methods did not produce differing chl *a* and TP measurements, but when surface scums are present, differences do appear. Sampling during the summers of 2013 and 2014 occurred during relatively well-mixed conditions and cyanobacteria were not, in general, densely concentrated at the surface in a scum despite "severe" *Microcystis* blooms (updated data sets from Bridgeman et al., 2013 and Stumpf et al., 2012). Wind speeds must be very low (< 3 m/s, Webster and Hutchinson, 1994) for several consecutive hours during a dense cyanobacterial bloom in order for a thick surface scum to occur. These conditions rarely align with pre-determined sampling dates; however, we were able to collect a few samples during scums. Differences in chl

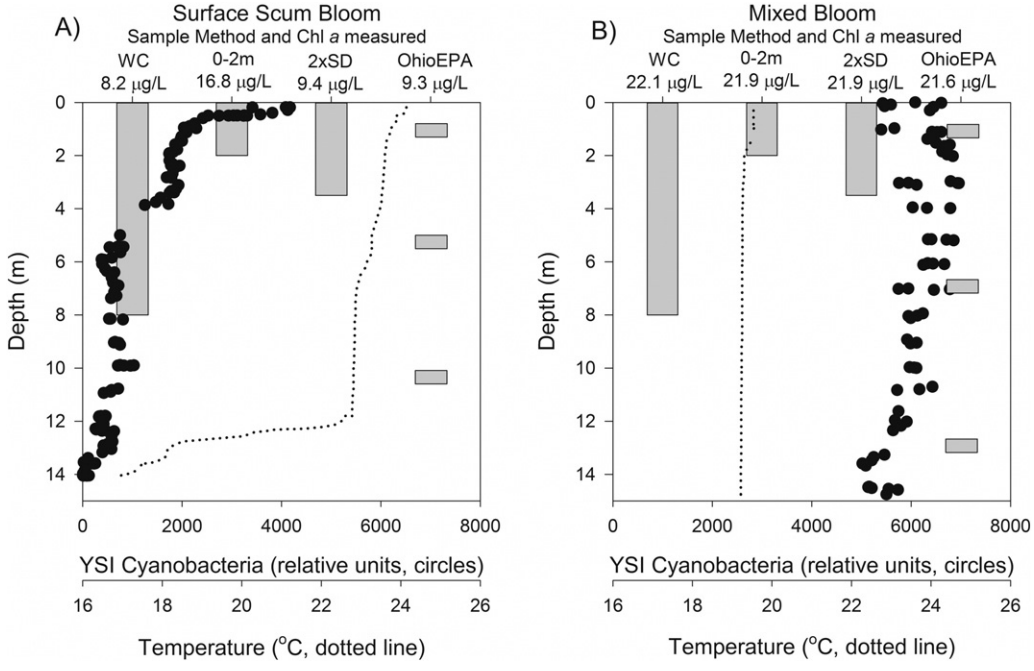

Fig. 4. Vertical profiles of cyanobacteria and temperature and the chlorophyll *a* concentration measured in the 4 water sampling methods used for (A) surface scum bloom on July 13, 2013, and (B) a bloom mixed throughout the water column on October 2, 2013. Measured chlorophyll a concentrations are given at top of bars indicating sampling depths.

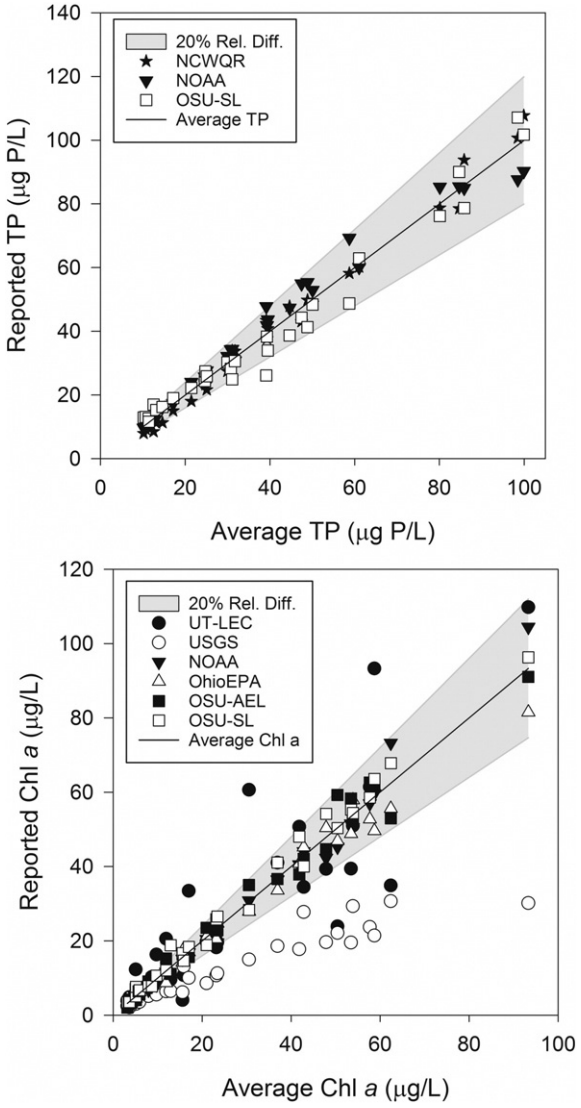

Fig. 5. Sample difference from the average concentration reported by the different laboratories for total phosphorus (A) and chlorophyll *a* (B). Most samples are within the 20% relative difference considered acceptable for field duplicates (Ohio Environmental Protection Agency, 2013), as indicated by the shaded area. UT-LEC and USGS chl *a* data were not included in the average calculation.

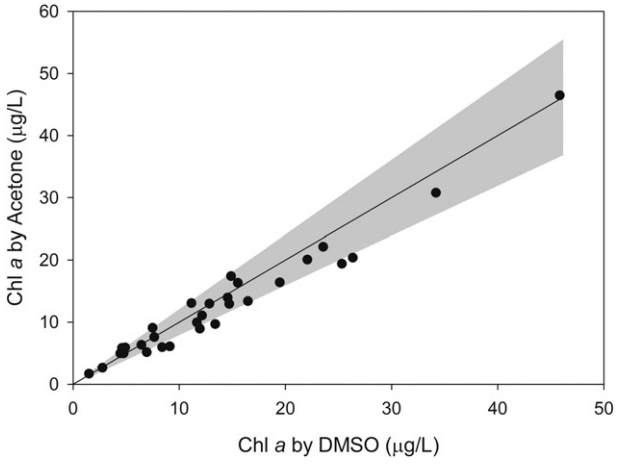

Fig. 6. Comparison of water column sample bottle duplicates analyzed at Stone Laboratory using the DMSO method and the acetone method of EPA 446.0 indicated that methods are within the 20% relative difference considered acceptable for bottle duplicates (Ohio Environmental Protection Agency, 2013).

a concentrations were observed among the four methods when surface scums were present. In some cases, chl *a* concentrations in the 0- to 2-m segment of the water column were double the water column average (Fig. 4). Surface scums were present for only 11 of the 82 sites sampled during 2013 and 2014; thus, these very calm sampling days were relatively infrequent during our sampling.

Although differences in the vertical distribution of chlorophyll may seem to be of concern mainly for monitoring organizations, it should be noted that the method of water sampling may also affect the results of cyanobacterial toxin testing. During cyanobacterial blooms, most of the toxins produced are located within cyanobacterial cells; therefore, the distribution of chlorophyll may be indicative of the distribution of toxin. Sampling methods that preferentially collect water from the scum and near surface may result in very high toxin concentrations that do not reflect the average concentration of toxin in the water and may cause undo concern if reported publicly. During surface scum conditions, we recommend an integrated water column sampler that captures the surface scum and as much of the water column as possible as the most reliable method of obtaining a sample representative of the water column average. However, we recognize that such samplers are not always practical and that monitoring programs may have other priorities that dictate alternate methods of sampling. At minimum, researchers who find themselves collecting surface samples on days when surface scums are present should be aware that their data may not agree with data collected from integrated water column samplers.

As with sampling procedures, analytical procedures at the participant laboratories were also largely compatible. Exceptions to the general agreement between laboratories for chl *a* were explainable. Results from the USGS laboratory consistently underestimated actual chl *a* concentration by a factor of 2 or 3. We attributed this result to under-extraction of chl *a* from the glass fiber filters due to the practice of

Table 3
Inter-lab chlorophyll *a* comparison log–log linear regression and paired sample *t*-test statistics for each laboratory against the across-laboratory average. UT-LEC and USGS chlorophyll data were not included in the average calculation (see text). All regressions were significant at $P < 0.001$. Degrees of freedom = 29 for all regressions, except those involving OEPA data where degrees of freedom = 28.

		Log–log regression			Paired *t*-test		% RD
	Laboratory	R^2	Equation	Slope 95% CI	Average difference	*P*	Percent of samples in the 20% RD range
Average TP *v.*	NCWQR	0.989	$TP_{lab} = (1.113\ TP_{ave}) - 0.195$	1.07–1.16	0.46	0.470	93.3%
	NOAA	0.987	$TP_{lab} = (1.009\ TP_{ave}) + 0.003$	0.97–1.05	−1.43	0.084	93.3%
	OSU-SL	0.963	$TP_{lab} = (0.893\ TP_{ave}) + 0.160$	0.83–0.96	0.99	0.261	93.3%
Average Chl *v.*	UT-LEC	0.807	$Chl_{lab} = (0.893\ Chl_{ave}) + 0.140$	0.72–1.06	−1.87	0.517	50.0%
	USGS	0.955	$Chl_{lab} = (0.824\ Chl_{ave}) - 0.059$	0.76–0.89	14.73	<0.001	3.4%
	NOAA	0.994	$Chl_{lab} = (1.069\ Chl_{ave}) - 0.121$	1.03–1.10	0.46	0.476	90.0%
	OEPA	0.988	$Chl_{lab} = (0.960\ Chl_{ave}) + 0.039$	0.92–1.00	1.47	0.031	93.1%
	OSU-AEL	0.979	$Chl_{lab} = (1.009\ Chl_{ave}) + 0.028$	0.95–1.07	−0.46	0.450	86.7%
	OSU-SL	0.987	$Chl_{lab} = (0.985\ Chl_{ave}) + 0.044$	0.94–1.03	−1.42	0.040	93.3%

using acetone as a solvent without grinding the filters. While acetone alone may be provide adequate extraction in some situations, the dense chl *a* concentrations, or large colony sizes frequently found in western Lake Erie apparently require grinding the filter to augment acetone extraction. Others have also reported that acetone is less effective at extracting algal pigments than DMSO (Devesa et al., 2007) and DMF (Speziale et al., 1984). Laboratories that followed acetone extraction with filter grinding obtained chl *a* results that closely matched results from labs that used more efficient solvents. The high variability of the chl *a* results from UT-LEC were more difficult to explain. Bottle duplicates analyzed at this lab were within 20% RD indicating acceptable precision of measurement, and the log–log regression slope and intercept were similar to the other labs. However, overall variability was high with nearly half of the measurements >20% RD from the pooled data of all labs. In a previous comparison in 2008, chl *a* measurements using UT-LEC DMF method and the DMSO method showed good agreement between bottle duplicates (Chaffin, 2009). However, we determined that by 2014 the UT-LEC fluorometer was operating with optical filters that had exceeded their operational life-span and likely needed to be replaced. UT-LEC has since updated its chl *a* procedure, using new filter and lamp sets.

Water quality monitoring organizations have to consider many factors when deciding on which method to use to collect water samples. Researchers may choose methods based on familiarity or they may select a particular method in order to generate a data set that is consistent with others using the same method. Organizations may not necessarily be sampling to address a research question, but rather to determine human health risks. For example, organizations monitoring for *Escherichia coli* or to determine maximum microcystin concentrations for swimmers at public beaches may collect only surface samples. Researchers who are interested in entire water column dynamics may select integrated tube samplers to capture the "average" conditions. Ultimately, the method selected must address the question of particular interest. Because western Lake Erie is usually well mixed, our results suggest that monitoring organizations need not be overly concerned about their different sampling protocols resulting in disparate measurements.

Although our results indicate that the two major factors that we examined – water column sampling methods and lab analytical procedures – are not a major source of variation between lab results, some of the variability between labs may be due to factors not examined in our study. Our round-robin study did not account for the potential effects of lab-specific factors such as personnel, sampling bottles, and sample storage time. These factors could be addressed in future studies by having personnel from the different labs present on the vessel using their own sample bottles, storage coolers, and then transporting and storing samples at their own labs according to their normal procedures.

In conclusion, we found that TP and chl *a* data from multiple organizations' methods were generally compatible. Although not all factors that could affect the data produced by an individual lab were addressed in this study, we suggest that two of the mostly likely factors for differences between labs – water sampling method and laboratory procedures – did not produce large differences in results, with the caveat that sampling during a cyanobacterial surface scum should be done with an awareness of potential bias towards increased chl *a* and TP near the surface. Our results are a positive step towards building confidence in the compatibility of data sets generated by monitoring organizations and should be beneficial to any analysis of historical, long-term data collected from multiple organizations.

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