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BLUE-GREEN ALGAE (CYANOBACTERIA) PATTERNS AND PREDICTION IN 12 LAKES IN KOSCIUSKO COUNTY, INDIANA

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ABSTRACT. Blue-green algae, or cyanobacteria, can produce microcystin toxin, a threat to human and animal health. Twelve popular lakes in Kosciusko County, Indiana, were studied during 2015–17 to determine spatial and temporal patterns of algae abundances and microcystin toxin concentrations as well as potential predictive methods. All 12 lakes were dominated by blue-green algae compared to other types of algae and often at abundances in the moderate to high risk ranges for human health (up to 625,650 cells/mL). Microcystin levels were often quite different between lakes (ranging from < 0.15 to 11 μg/L) and reached the moderate risk range on 17 occasions. Some lakes typically had low microcystin levels despite large blue-green algae abundance. Algae abundance and microcystin concentrations were typically highest in July when the recreational exposure risk is likely at its highest. Blue-green algae abundances and microcystin toxin levels were found to be similar in Kosciusko County lakes compared to lakes in other parts of Indiana and throughout the U.S. Predictive efforts using rapid screening and complex models provided better understanding of microcystin occurrence and blue-green algae abundances in northern Indiana but were not strong enough to use for public health and safety determinations. These results have led the Lilly Center to pursue rapid lab analysis of microcystin levels instead of relying on predictive methods. Exploration of individual algae species abundances and potential trends in other lake-specific influences are warranted given the results of this study.

Keywords: Cyanobacteria, nutrients, microcystin, chlorophyll, phycocyanin

INTRODUCTION

Cyanobacteria, or blue-green algae, are prokaryotic organisms that contain chlorophyll and phycocyanin pigments. Cyanobacteria can produce harmful toxins, such as microcystin, that have been known to affect humans, their pets, and aquatic life in freshwater and marine environments. Cyanobacteria have been identified as a global threat and can often be unpredictable and perplexing. For example, in a study of 800 lakes, European researchers sought to minimize health risks from cyanobacteria by controlling phosphorus levels (Carvalho et al. 2013). As expected, high phosphorus levels in a lake can intensify algal blooms, including potentially toxic cyanobacteria. However, the same study reported that in about 50% of the lakes, scientists observed low concentrations of cyanobacteria even at times when total phosphorus concentrations in those lakes were high. A study focused on four major South Korean rivers (the Geum, Han, Makdong, and Yeongsan) found that temperature and residence time of a body of water may be better predictors of cyanobacteria abundance in rivers than the availability of nutrients (Cha et al. 2017). A review of Canadian cyanobacteria research found that microcystin toxin levels were highly variable across time and space though microcystin levels tended to increase with lake productivity (Kotak & Zurawell 2007).

Research to better predict toxic blue-green algal blooms and prevent cases of human and animal intoxication is occurring in the United States as well (Anderson et al. 2000; Boyer 2007; Jacoby & Kann 2007; Touchette et al. 2007; Williams et al. 2007; Stephens et al. 2009; Butler et al. 2009; Bishop & Willis 2017; UDAF 2017; Walls et al. 2018). Research has sought to identify why cyanobacteria produce toxins and which factors are important for the prediction and prevention of these toxins (Graham et al. 2004). In 2013, a group of Montana scientists performed what is thought to be the first successful treatment of "canine cyanobacterial

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(microcystin) toxicosis" through oral cholestyramine therapy (Rankin et al. 2013). This breakthrough is important to pet owners who live around lakes, since there have been many documented cases of canine mortality caused by microcystin toxin (DeVries et al. 1993; Harding et al. 1995; Simola et al. 2011).

In Indiana, a study exploring cyanobacterial ecology and microcystin concentrations in three public drinking water supply reservoirs in the Indianapolis area (Eagle Creek, Geist, and Morse) revealed that each contained cyanobacteria and experienced blooms throughout the summer months (Tedesco & Clercin 2008). According to the study, microcystin was detected in 61% of samples taken from these reservoirs. Additionally, by the second week of July researchers determined that cyanobacteria abundances in all three reservoirs exceeded 100,000 cells/mL, levels which could pose health risks for humans and animals coming into contact with the water.

Human health guidelines have been established in Indiana based on microcystin toxin concentrations and cyanobacteria abundances in recreational waters (WHO 2003; IDEM 2019). The low risk conditions prevail when microcystin concentrations are less than 4 μg/L and cyanobacteria abundances less than 20,000 cells/mL. The moderate risk guideline recommends reducing recreational contact with water containing microcystin concentrations of 4–20 μg/L and cyanobacteria abundances of 20,000–100,000 cells/mL. The high risk guideline recommends avoiding recreational contact with water containing microcystin concentrations greater than 20 μg/L and cyanobacteria abundances greater than 100,000 cells/mL. More recently the U.S. Environmental Protection Agency has published draft guidelines for recreational waters (EPA 2017). They recommend prohibiting swimming when microcystin toxin levels exceed 4 μg/L and consider lakes to be impaired when this level is exceeded more than 10% of days during the recreational season.

Kosciusko County, Indiana contains over 100 lakes. Many of these lakes are surrounded by secondary single-family homes and are popular for fishing, boating, swimming, and watersports. A 2016 study by the Lilly Center for Lakes & Streams (Bingham & Bosch 2016) estimated that these lakes add over $313 million into the county's economy annually. This amount is subject to increase or decrease based on lake health. The study projected that if lake quality were to decline up to 40% (as observed around Grand Lake Saint Marys, Ohio), the economic loss to Kosciusko County could be over $84 million (Bingham & Bosch 2016).

In a 2009 pilot study, cyanobacteria were identified as a potential human health threat in Kosciusko County when microcystin concentration in a Lake Wawasee sample was discovered to be 63 μg/L (L. Tedesco, Pers. Comm., July 26, 2010). A subsequent Lilly Center study from 2010–2013 found an elevated risk for encountering harmful microcystin levels in the recreational waters during the summer months (Bosch et al. 2014). The study indicated that cyanobacteria did not pose a consistent health threat in Kosciusko County lakes at that time, but that strong potential for harmful toxin levels did exist since elevated algal abundances were relatively common. Based on these findings, the Lilly Center began to pursue options for rapid screening techniques and predictive relationships that could warn community members of toxic blooms, as well as provide a fuller understanding of the cyanobacteria in order to better prevent the future occurrence of microcystin. The present study explores current spatial and temporal patterns in cyanobacteria abundances and microcystin concentrations in Kosciusko County.

METHODS

Study sites.—There are 104 lakes with surface area greater than five acres in Kosciusko County (Fig. 1). Many of these lakes are kettle lakes, formed by glaciers, and have various bottom substrates consisting mostly of sand, muck, and marl. For the present study, the 12 all-sports lakes (lakes that allow skiing, tubing, wakeboarding, etc.) in Kosciusko County were sampled weekly during the summers of 2015–2017. These lakes were Beaver Dam, Big Barbee, Big Chapman, Dewart, James, Oswego, Syracuse, Tippecanoe, Wawasee, Webster, Winona, and Yellow Creek (Table 1).

Field sampling.—In 2015 and 2016, lake sampling started in the last week of May and continued through the first week of September; 26 sampling events were included over those two years. In 2017, sampling started the first week of June and continued through the first week of August for a total of nine sampling events. Each lake sampling event was completed at the deepest point on each lake, which was deter-

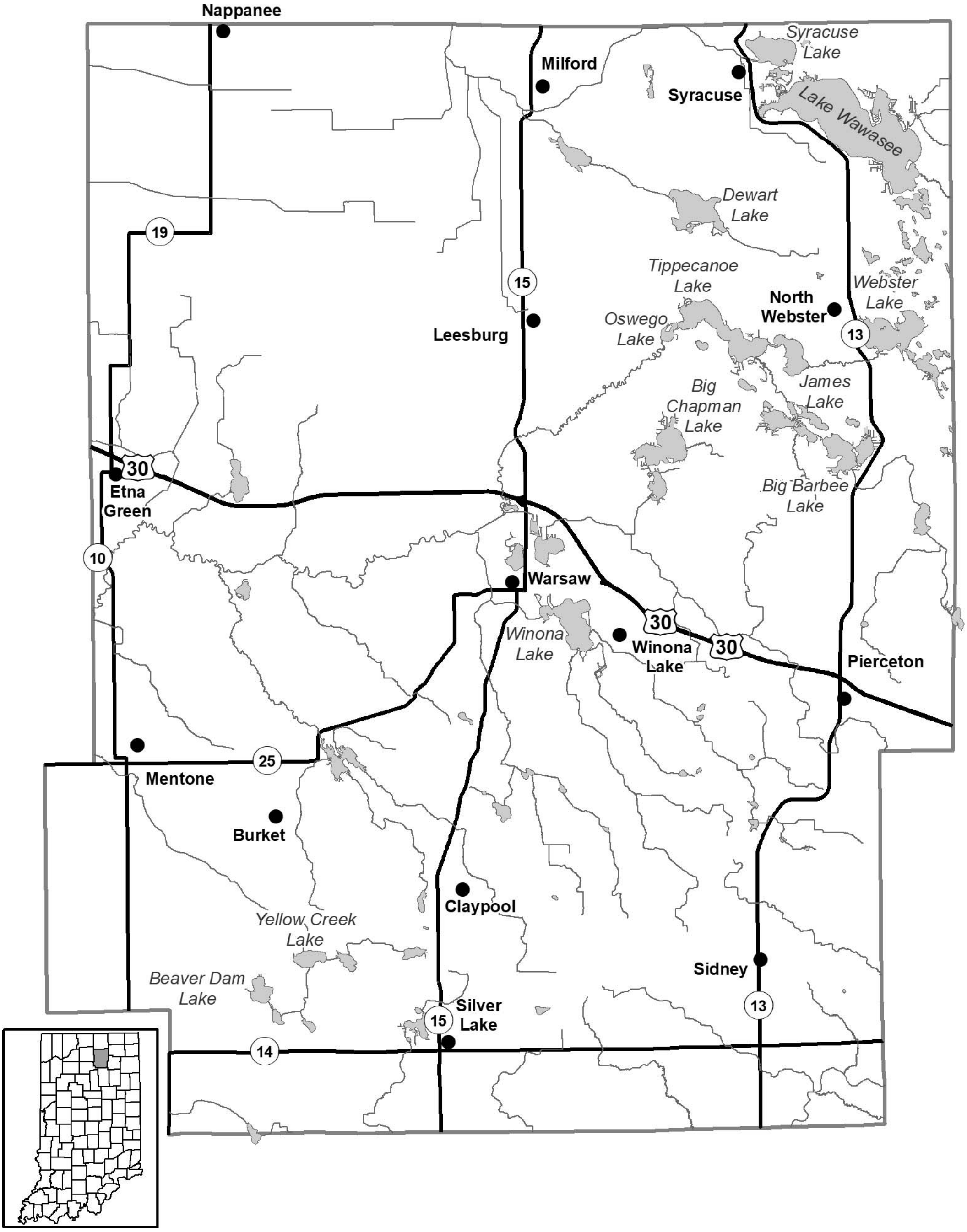

Figure 1.—Map of Kosciusko County, Indiana, showing major lakes and streams, including the 12 lakes studied in this report.

mined by consulting bathymetric maps and confirmed using a handheld portable sounder to check depth on-site. When the deepest point in each lake was identified, latitude and longitude coordinates were recorded in a handheld GPS unit for future sampling events.

A Hydrolab Quanta multi-probe sonde was lowered to 1 m above the lake bottom and depth,

Table 1.—Physical characteristics for each lake.

Lake name	Surface area (ha)	Watershed size (ha)	Max depth (m)	Average depth (m)	Volume (1,000 m^3)
Beaver Dam	63	512	18.6	4.8	2,983
Big Barbee	126	11,630	13.7	4.8	5,984
Big Chapman	204	1,821	11.9	3.8	7,771
Dewart	224	2,047	25.0	5.0	11,139
James	113	14,478	18.9	8.2	9,259
Oswego	32	29,480	11.3	4.2	1,318
Syracuse	166	9,914	10.4	4.0	6,590
Tippecanoe	318	29,480	37.2	11.3	35,872
Wawasee	1,217	9,894	24.7	6.7	81,572
Webster	264	12,731	15.8	3.8	10,068
Winona	231	7,580	24.1	9.1	21,130
Yellow Creek	63	874	20.4	9.6	6,042

water temperature, conductivity, dissolved oxygen, and pH were recorded each meter from the bottom of the lake to the surface. A Secchi disk was used to record water clarity for each of the lakes during each sampling event. Weather conditions were recorded using a SpeedWatch Skymaster weather meter in 2015 and 2016, and a Kestrel 3500 weather meter in 2017. Barometric pressure, altitude, air temperature, maximum wind speed, average wind speed, and wind direction were recorded for each sampling event for each lake.

A Wildco Beta 1930 Vertical Van Dorn sampler was used to capture a nutrient sample for each lake during each sampling event from 1 m below the surface to represent the epilimnion layer. In addition to the water collected at 1 m, water from the surface and 1.7 m were collected and mixed to produce an integrated sample of the water that swimmers and skiers typically come into contact with. This integrated sample was mixed well before a microcystin sample was taken. Three algae samples were then collected from a surface water sample to primarily allow comparison to fluorometer measurements. Lugol's iodine was added to two of the three algae sample bottles to preserve the samples. All nutrient, algae, and microcystin samples were placed on ice in a dark cooler immediately following collection.

A Turner Designs AquaFluor Handheld Fluorometer ("box" style with cuvettes), which measures chlorophyll a and phycocyanin, was used for sampling during all three summers, 2015–2017. Additionally, starting in 2016, the research team utilized a Turner Designs FluoroSense Handheld Fluorometer set ("pen" style), which also measures chlorophyll a and phycocyanin, as a potential rapid screening technique for microcystin and cyanobacteria abundances. All fluorometer measurements were taken from water captured at the surface, and duplicate measurements were taken on both devices until readings were within five percent of each other.

Lab analysis.—Nutrient samples taken from the epilimnion layer were analyzed weekly by the National Center for Water Quality Research at Heidelberg University in Ohio. The lab analyzed the samples for chloride (Cl), silica (SiO_2), sulfate (SO_4), suspended sediments (SS), nitrite (NO_2), nitrate (NO_3), ammonia (NH_3), total Kjeldahl nitrogen (TKN), soluble reactive phosphorus (SRP), and total phosphorus (TP). Total nitrogen (TN) was calculated as the sum of TKN, NO_2, and NO_3. The nitrogen (N) to phosphorus (P) ratio was calculated as TN concentration divided by the TP concentration and denoted as N/P.

Algae samples from each lake were transferred to individual slides and analyzed under a fluorescence microscope to identify species of algae and abundance concentration. One mL of sample was passed through a membrane filter to make each permanent slide. A total of 73 algae types were identified and quantified under 20–1,000× magnification. After undergoing three freeze/thaw cycles, microcystin samples were analyzed via Abraxis ELISA test kits by the National Center for Water Quality Research.

Data analysis.—Collected data were used to produce several lake-specific predictive models for microcystin and cyanobacteria abundances to screen for possible health risks as well as to better understand local cyanobacteria. Only variables measured quickly in the field were

Table 2.—Average summer background measurements by lake at 1 m depth during 2015–2017. Measurements include Secchi disk depth (SD), water temperature (WT), dissolved oxygen (DO), pH, and specific conductivity (SpC).

Lake name	SD (m)	WT (°C)	DO (mg/L)	DO (% sat)	pH (units)	SpC (μS/cm)
Beaver Dam	1.17	25.9	9.0	111	8.88	368
Big Barbee	1.14	25.3	9.4	114	8.60	484
Big Chapman	2.44	25.3	8.5	102	8.60	434
Dewart	2.75	25.0	8.3	100	8.74	338
James	2.13	25.1	8.4	101	8.52	475
Oswego	2.38	25.2	8.8	104	8.57	459
Syracuse	3.20	25.1	8.4	101	8.77	370
Tippecanoe	2.48	25.1	8.4	101	8.55	465
Wawasee	2.62	24.5	8.4	101	8.72	381
Webster	1.90	25.7	8.5	104	8.60	459
Winona	1.29	25.0	9.5	115	8.72	532
Yellow Creek	1.26	25.6	9.8	121	8.84	403

used: Secchi disk, air temperature, water temperature, dissolved oxygen (mg/L and % sat), maximum wind speed, average wind speed, AquaFluor phycocyanin and chlorophyll a channels, pH, and conductivity. Predictive models for microcystin concentrations and cyanobacteria abundances were evaluated by the coefficient of determination (R^2) values as well as a comparison based on set thresholds for each measurement, including percentage of predictions correctly above the threshold, correctly below the threshold, incorrectly above the threshold (false positive), and incorrectly below the threshold (false negative). Thresholds were set to have about half of the dataset above and below the threshold as well as to reflect lab analysis detection level (microcystin, 0.15 μg/L), and human health guidelines (cyanobacteria, 20,000 cells/mL).

Rapid screening options were explored with one or two rapid field measurements to predict microcystin and cyanobacteria abundances. First, simple linear regression analysis was used to test for the best single variable prediction. Second, using two variables, every combination of two factors (x and y) were tested with the following regression form: $a_0 + a_1x + a_2y + a_3xy + a_4x^2 + a_5y^2$.

Complex prediction models were developed using stepwise binary logistic regression. To create the more complex prediction models, multiple variables that would be measured quickly in the field were used (see list above) as well as all of the squared and interaction terms. Model development included sample month and lake name as categorical data. The stepwise aspect of the model added and removed variables algorithmically based on a p-value of 0.15 for each variable. The binary logistic aspect of the model calculated a probability that the variables would be above or below threshold categories. When numerous variables are used, it is possible that quasi-complete separation exists which causes the maximum likelihood estimator to not exist. To avoid this, some variables were removed by hand as suggested by the model software.

It was also determined that complex prediction models should be developed for predicting microcystin concentrations and cyanobacteria abundances. These models required a cutoff probability to determine whether they were predicting correctly or not; we used cutoff values of 50% and 35% probability that the microcystin and cyanobacteria levels would be predicted as above or below the thresholds. The 50% cutoff probability would help achieve the least number of false positive and negative predictions, and the 35% cutoff would sacrifice some correct predictions to minimize the risk to human health and safety. The 35% cutoff probability would result in a prediction of above the threshold more often but with fewer false negatives, thus creating a safer, more conservative model.

RESULTS

Spatial patterns.—Average water clarity, as measured by Secchi disk depth, varied greatly between lakes during summers of 2015–2017 (Table 2). Highest average water clarity was observed in Syracuse Lake at 3.20 m. Lowest average water clarity was found in Big Barbee Lake at 1.14 m. The highest single Secchi

Table 3.—Average summer nutrient measurements by lake at 1 m depth during 2015–2017. Measurements include chloride (Cl), silica (SiO_2), sulfate (SO_4), suspended sediments (SS), nitrite (NO_2), nitrate (NO_3), ammonia (NH_3), total Kjeldahl nitrogen (TKN), total nitrogen (TN), soluble reactive phosphorus (SRP), total phosphorus (TP), and the ratio of total nitrogen to total phosphorus (N/P). All units in mg/L.

Lake name	Cl	SiO_2	SO_4	SS	NO_2	NO_3	NH_3	TKN	TN	SRP	TP	N/P
Beaver Dam	17.6	0.53	21.0	4.6	0.005	0.063	0.090	1.32	1.39	0.006	0.040	38.4
Big Barbee	19.5	3.71	32.6	6.7	0.027	0.534	0.193	1.17	1.73	0.014	0.045	39.3
Big Chapman	26.0	6.90	33.1	3.2	0.001	0.373	0.074	0.59	0.96	0.022	0.018	62.6
Dewart	16.0	2.59	14.5	1.6	0.001	0.017	0.109	0.77	0.79	0.004	0.019	43.8
James	21.6	5.32	28.8	1.7	0.006	0.069	0.043	0.82	0.89	0.005	0.024	38.1
Oswego	23.5	4.02	33.5	0.7	0.082	0.419	0.040	0.79	1.29	0.004	0.020	88.4
Syracuse	18.0	2.83	24.8	1.6	0.000	0.019	0.050	0.59	0.61	0.003	0.018	40.4
Tippecanoe	20.7	3.85	30.8	1.6	0.009	0.077	0.046	0.75	0.83	0.004	0.021	42.2
Wawasee	17.6	2.51	23.7	2.4	0.001	0.039	0.062	0.61	0.65	0.004	0.014	55.5
Webster	17.3	9.30	27.8	2.6	0.002	0.009	0.080	0.88	0.89	0.004	0.028	32.5
Winona	28.4	2.50	46.0	4.4	0.023	0.479	0.061	1.05	1.55	0.005	0.030	52.2
Yellow Creek	15.9	1.09	27.5	5.2	0.019	0.331	0.105	1.23	1.58	0.015	0.042	43.9

measurement, at 4.78 m, was from Syracuse Lake; the lowest was in Big Barbee Lake at 0.61 m. Other background measurements, including water temperature, dissolved oxygen, pH, and specific conductivity remained more consistent across all lakes (Table 2).

Epilimnion nutrient samples were analyzed for spatial patterns among the lakes (Table 3). The highest average soluble reactive phosphorus level was found in Big Chapman Lake at 0.022 mg/L; the lowest in Syracuse Lake at 0.003 mg/L. Big Chapman Lake showed the highest average soluble reactive phosphorus level of all the lakes, despite relatively low total phosphorus levels (the same average total phosphorus as Syracuse Lake, which had the lowest average soluble reactive phosphorus).

Suspended sediments varied greatly between lakes, with the highest average suspended sediments level recorded in Big Barbee Lake at 6.7 mg/L and the lowest recorded in Oswego Lake at 0.7 mg/L (Table 3). The four lakes with highest average suspended sediments levels, Beaver Dam, Big Barbee, Winona, and Yellow Creek, all also had the lowest Secchi disk measurements (Table 2). Additionally, these four lakes had the highest average total nitrogen levels with the largest average in Big Barbee at 1.73 mg/L (Table 3). Oswego Lake had the highest average N/P ratio at 88.4, which was higher than James Lake and Tippecanoe Lake (the other lakes in the Tippecanoe lakes chain); the lowest average N/P was 32.5 on Webster Lake.

Microcystin toxin averages varied substantially between lakes (Table 4). Over the three-year

Table 4.—Average summer algae measurements by lake for integrated sample of top 2 m of lake during 2015–2017. Measurements include microcystin (MC), blue-green (BG) algae (or cyanobacteria) count, total algae count, relative phycocyanin (PC), and chlorophyll a (Chl a) using AquaFluor.

Lake name	MC (μg/L)	BG algae (cells/mL)	Total algae (cells/ml)	PC (units)	Chl a (units)
Beaver Dam	1.24	43,989	52,145	6.3	8.9
Big Barbee	0.17	53,830	66,670	6.5	10.5
Big Chapman	1.64	33,864	37,849	2.1	3.4
Dewart	1.31	19,376	23,319	2.9	4.4
James	0.49	33,539	38,588	3.2	6.4
Oswego	0.26	34,301	39,195	1.4	3.9
Syracuse	0.33	38,703	41,895	1.3	2.8
Tippecanoe	0.26	33,575	38,371	2.2	4.7
Wawasee	1.07	22,306	25,612	2.2	3.2
Webster	0.35	44,203	48,122	2.4	5.0
Winona	0.23	83,663	91,073	5.9	8.9
Yellow Creek	1.28	38,400	47,353	8.5	9.1

Table 5.—Average monthly and annual background measurements for all 12 lakes combined at 1 m depth during 2015–2017. Measurements include Secchi disk depth (SD), water temperature (WT), dissolved oxygen (DO), pH, and specific conductivity (SpC).

Month/year	SD (m)	WT (°C)	DO (mg/L)	DO (% sat)	pH (units)	SpC (μS/cm)
May	3.40	20.0	10.4	113	8.49	471
June	2.05	24.3	8.8	105	8.70	444
July	1.76	26.2	8.9	111	8.75	419
August	1.96	26.5	8.3	103	8.67	418
September	2.35	25.7	8.5	103	8.40	425
2015	2.10	24.8	9.0	108	8.73	432
2016	2.06	25.8	8.7	106	8.60	430
2017	2.03	24.6	8.7	104	8.78	430

study, the highest concentrations of microcystin were 11 μg/L observed once on Big Chapman Lake and once on Beaver Dam Lake. Yellow Creek Lake had one measurement over 10 μg/L. Altogether, there were 17 occurrences where microcystin levels exceeded 4 μg/L (moderate risk guideline, IDEM 2019) out of 346 samples, including occurrences on Beaver Dam, Big Chapman, Dewart, Wawasee, and Yellow Creek lakes. There were 169 occurrences where microcystin levels were below the level of detection (0.15 μg/L).

The highest average microcystin level was 1.64 μg/L (Table 4) at Big Chapman Lake, a lake with above average water clarity. The lowest average microcystin level was 0.17 μg/L at Big Barbee Lake, a lake with below average water clarity. However, microcystin levels and water clarity were not always proportional, e.g., Dewart Lake and Yellow Creek Lake had very similar microcystin levels, despite water clarity in Dewart Lake being more than twice the clarity in Yellow Creek Lake.

Cyanobacteria abundances varied substantially among lakes as well (Table 4). The highest cyanobacteria abundance measured among the 360 samples was 625,650 cells/mL in Winona Lake. Altogether there were 32 occurrences where abundances exceeded 100,000 cells/mL (high risk guideline, IDEM 2019), including all of the lakes except Dewart and Wawasee, and there were 179 occurrences where abundances exceeded 20,000 cells/mL (moderate risk guideline, WHO 2003).

While all lakes were dominated by cyanobacteria (87%) compared to other types of algae (Table 4), microcystin levels did not always reflect cyanobacteria abundances. For example, Winona Lake and Big Barbee Lake often had low microcystin levels despite an abundance of cyanobacteria, while Dewart Lake and Lake Wawasee had higher microcystin levels and the lowest cyanobacteria counts. Of the 12 lakes sampled, Winona Lake, with an average of 83,663 cells/mL, had the highest cyanobacteria abundance; Dewart Lake, with an average of 19,376 cells/mL, had the lowest (Table 4).

Temporal patterns.—According to an analysis of lake surface water background measurements by month, most lakes experienced large changes between May and June (Table 5). Average water clarity dropped from 3.40 m to 2.05 m. Dissolved oxygen and conductivity dropped as well, while pH and water temperature increased. In year to year comparisons for each of the 12 lakes, all parameters were fairly consistent, with slightly higher water temperature in 2016 and slightly higher dissolved oxygen in 2015 (Table 5).

Analysis of nutrient parameters for all lakes averaged by month showed similar results in May, June and September, but dramatic changes were observed in July and August (Table 6). For example, there were higher suspended sediments and lower NH_3 averages in the middle of the summer (July) compared to other months (Table 6). There were similar NH_3 averages for May and September, but there was a drop in average from May to June, and a large increase between July (0.038 mg/L) and August (0.139 mg/L). Additionally, there was a substantial decrease in average NO_3 between July (0.153 mg/L) and August (0.054 mg/L), and then a sharp increase between August (0.054 mg/L) and September (0.394 mg/L). July and August were also found to have the lowest average N/P for all lakes, with the months of May, June, and September significantly higher.

Year to year trends across the 12 lakes proved remarkably similar (Table 6). Total Kjeldahl nitrogen was lower in the summers of 2015 and

Table 6.—Average monthly and annual nutrient measurements for all 12 lakes combined at 1 m depth during 2015–2017. Measurements include chloride (Cl), silica (SiO_2), sulfate (SO_4), suspended sediments (SS), nitrite (NO_2), nitrate (NO_3), ammonia (NH_3), total Kjeldahl nitrogen (TKN), total nitrogen (TN), soluble reactive phosphorus (SRP), total phosphorus (TP) and the ratio of total nitrogen to total phosphorus (N/P). All units in mg/L.

Month/year	Cl	SiO_2	SO_4	SS	NO_2	NO_3	NH_3	TKN	TN	SRP	TP	N/P
May	20.9	1.13	32.2	1.9	0.006	0.185	0.101	0.80	0.99	0.005	0.019	69.2
June	20.3	2.35	28.9	3.4	0.014	0.328	0.050	1.01	1.35	0.005	0.029	51.8
July	19.2	3.98	26.7	4.4	0.010	0.153	0.038	0.90	1.06	0.004	0.029	37.9
August	19.7	5.22	29.2	2.1	0.003	0.054	0.139	0.78	0.83	0.015	0.025	37.6
September	23.3	6.01	28.3	1.2	0.086	0.394	0.101	0.72	1.20	0.004	0.023	86.6
2015	18.9	4.25	27.5	3.3	0.010	0.155	0.060	0.84	1.00	0.002	0.028	40.4
2016	21.4	3.58	31.6	2.5	0.017	0.198	0.107	0.84	1.06	0.011	0.025	54.1
2017	19.0	3.51	24.2	3.6	0.015	0.265	0.052	1.01	1.29	0.007	0.029	45.6

2016 than 2017. Suspended solids were lower in the summer of 2016 than 2015 and 2017. Average N/P was higher in 2016 compared to the other two years (Table 6), a trend similar to that of water temperature.

Average microcystin varied substantially by month from 0.10 μg/L in May to 1.32 μg/L in July and back to 0.30 μg/L in September (Table 7). Cyanobacteria abundances were also highest on average in July. Year to year data revealed that 2015 microcystin levels and cyanobacteria abundances were substantially higher than those of 2016 and 2017 (Table 7).

Rapid screening prediction.—The AquaFluor and FluoroSense fluorometers were highly correlated for both phycocyanin ($R = 0.83$) and chlorophyll a ($R = 0.91$) measurements. More measurements were taken with the AquaFluor fluorometer, so only AquaFluor results will be included in the remainder of this manuscript.

When using a single variable with quick field measurements, the best predictors for microcystin were phycocyanin ($R^2 = 0.29$) and chlorophyll a ($R^2 = 0.19$), while the best predictors for cyanobacteria abundance were phycocyanin ($R^2 = 0.18$) and pH ($R^2 = 0.10$).

Using two variables that can be measured quickly and categorizing by lake, the best correlation for microcystin ($R^2 = 0.34$) included maximum wind speed and phycocyanin as predictor variables, and the best correlation for cyanobacteria abundance ($R^2 = 0.26$) included phycocyanin and water temperature.

Complex model prediction.—Complex models correctly predicted microcystin concentrations and cyanobacteria abundances relative to their thresholds (50% and 35%) more often than rapid screening models containing one or two variables (Table 8). For microcystin, the single, double, and multiple variable models resulted in total correct predictions 61, 58, and 79% of the time, respectively. For cyanobacteria, the single, double, and multiple variable models resulted in total correct predictions 58, 64, and 80% of the time, respectively.

Table 7.—Average monthly and annual algae measurements with all 12 lakes combined for integrated sample of top 2 m of lake during 2015–2017. Measurements include microcystin (MC), blue-green (BG) algae (or cyanobacteria) count, total algae count, relative phycocyanin (PC), and chlorophyll a (Chl a) using AquaFluor.

Month/year	MC (μg/L)	BG algae (cells/mL)	Total algae (cells/ml)	PC (units)	Chl a (units)
May	0.10	7,045	8,890	1.6	3.5
June	0.60	24,378	28,825	3.7	6.1
July	1.32	65,756	73,758	4.8	7.2
August	0.55	44,849	51,762	3.7	5.6
September	0.30	29,665	33,971	2.4	4.6
2015	1.73	75,466	84,448	4.9	6.9
2016	0.24	24,949	29,864	2.5	4.7
2017	0.41	24,414	28,193	4.6	7.2

Table 8.—Evaluation of lake-specific predictive models of varying complexity for microcystin concentration and cyanobacteria abundances. Values indicate percentage of predictions correctly below the threshold (true negative), correctly above the threshold (true positive), incorrectly above the threshold (false positive), and incorrectly below the threshold (false negative). Thresholds were set to 0.15 μg/L for microcystin and 20,000 cells/mL for cyanobacteria. Multiple variable models were used based on different cutoff probabilities (50% and 35%) that microcystin or cyanobacteria levels would be predicted above or below the thresholds.

	True negative	True positive	False positive	False negative
Single variable				
Microcystin	14	47	36	4
Cyanobacteria	12	46	38	3
Two variable				
Microcystin	11	47	38	4
Cyanobacteria	21	43	29	6
Multiple variable				
Microcystin (50%)	40	39	9	11
Cyanobacteria (50%)	41	39	10	10
Microcystin (35%)	34	43	16	7
Cyanobacteria (35%)	36	43	15	5

Overall, the various model complexities correctly predicted cyanobacteria abundances slightly more often than microcystin concentrations relative to the thresholds (Table 8).

Single and two variable models incorrectly predicted microcystin concentrations and cyanobacteria abundances above the thresholds substantially more often that the multiple variable models (Table 8). These incorrect predictions would be considered false positive results, i.e., the models predict levels above the threshold, but the actual levels are below the threshold.

The multiple variable models based on different cutoff probabilities (50% and 35%) illustrated that when the models were optimized for prediction exactness (50% probability), the models were correct slightly more often than when the models were optimized for health and safety (35% probability) (Table 8). The model optimization for health and safety incorrectly predicted microcystin concentrations and cyanobacteria abundances below the thresholds substantially less than when the models were optimized for exact predictions. These incorrect below threshold predictions would be considered false negative results, where the models predict levels below the threshold, but the actuals levels are above the threshold.

DISCUSSION

Spatial patterns.—Some lake background measurements were similar across all 12 lakes, indicating similar influences. The 12 lakes experienced similar air temperatures and wind conditions over the study period such that similar water temperatures were expected (Table 2). Since groundwater and surface water inflows to these lakes are coming from watersheds with a common limestone geology, it is also understandable that observed pH levels were similar as well.

Other lake measurements varied substantially across the 12 lakes. Water clarity is generally affected by both sediment and algae abundance. In these lakes, lakes with lower water clarity had higher dissolved oxygen (Tables 2 & 3), indicating that algae abundances are primarily affecting water clarity in these lakes. This is further demonstrated by the wide variation in average cyanobacteria abundances observed among the 12 lakes (Table 4).

Specific lake characteristics or their landscape position likely influenced variations among the 12 lakes. Syracuse Lake has the advantage of most of its water being processed and filtered by Lake Wawasee (Fig. 1) before entering Syracuse Lake; this likely contributes to Syracuse having the highest average water clarity (Table 2) of the 12 study lakes. Similarly, Oswego Lake has lowest average suspended sediments (Table 3) as Oswego has the advantage of processing through Tippecanoe and James lakes. Big Barbee Lake has a relatively large watershed size compared to its lake volume (Table 1) which may contribute to it having the lowest average clarity (Table 2) and highest average suspended sediments (Table 3).

More nutrients and sediments entering the lake from the watershed will reduce water clarity from algae growth (fed by nutrients) and directly from the suspended sediments.

Across the 12 lakes, cyanobacteria dominate the algae communities (an average of 87% of algal cells), but vary among lakes from 81% in Big Barbee to 92% in Syracuse (Table 4). Cyanobacteria abundances were often at the moderate to high risk ranges for human health. Microcystin levels were often quite different between lakes and reached the moderate risk range on 17 occasions. For example, Winona and Big Barbee lakes often had low microcystin levels despite large abundances of cyanobacteria, while Dewart and Wawasee lakes had higher microcystin levels and the lowest abundances of cyanobacteria. This may indicate that certain species of cyanobacteria are present and producing microcystin in Dewart and Wawasee lakes that are not present, or not producing microcystin, in Winona and Big Barbee lakes. Collected algae data for this project included species-level quantification in each sample, and a future study could explore algae species as an indicator in microcystin occurrence in these 12 lakes. Previous research has shown size classes of cyanobacteria are important indicators of microcystin levels (Graham & Jones 2007) which further supports the value of this sort of future study.

Temporal patterns.—As seen in spatial patterns, some interesting temporal patterns emerged from this study. Water clarity was relatively consistent between years but widely variable between months (Table 5), thus indicating that typical seasonal cycles are occurring across the lakes each year.

Cyanobacteria dominated algae communities in these 12 lakes throughout the study period, though there was substantial variation (Table 7). Cyanobacteria abundances and microcystin concentrations were substantially higher in July on average, which is also when there is the most human recreation, increasing the potential health threat. In June, there is more microcystin occurrence leading into July relative to the cyanobacteria abundance compared to August (Table 7), potentially indicating different species composition or microcystin production efficiency before and after the July peak.

Some potential patterns also emerged with nutrient ratios and cyanobacteria measures. Lower N/P ratios were seen in the 12 lakes in 2015 and in mid-summer when microcystin was elevated. Conversely, high N/P in the 12 lakes in 2016 as well as during months of May and September corresponded to the lowest average microcystin levels. A similar pattern was observed for four Midwestern reservoirs, including three in the Indianapolis area, where microcystin levels were highest when N/P fell below 30 (Harris et al. 2016). Not surprisingly cyanobacteria abundances had higher abundances in July and August and in 2015 when the average N/P was lowest. A review of Canadian lake research also found that microcystin tended to increase with lower N/P (Kotak & Zurawell 2007).

The results reported here compare favorably to those reported by sampling of public beaches at Indiana state parks over summer months of 2015–2017 (IDEM 2019). Microcystin levels of many samples were below detection, and a few in the moderate risk range. The highest microcystin concentration measured by IDEM was 7.83 μg/L on Hardy Lake in July, 2015. Similar to the 12 Kosciusko County lakes, IDEM reported higher microcystin levels in 2015 compared to 2016 and 2017 as well as during July compared to other summer months. The study by Harris et al. (2016) of four regional reservoirs from 2001–2012, reported moderate risk microcystin levels up to 9.0 μg/L but an average across all years of only 0.45 μg/L. Finally, a broad survey of 1,161 U.S. lakes in 2007 found lakes exceeded high risk guidelines for microcystin 0.72% of the time and cyanobacteria abundances 6.8% of the time (Loftin et al. 2016) compared to the present study where lakes exceeded the high risk guidelines for microcystin 0% of the time and cyanobacteria abundances 8.9% of the time.

Although most IDEM (2019) reports of cyanobacteria abundance were similar to those found in the 12 Kosciusko County lakes, some were much higher. The highest cyanobacteria abundance reported by IDEM was recorded twice at Cecil M. Harden Lake with an abundance of 1,800,000 cells/mL (IDEM 2019) compared to 625,650 cells/mL in Winona Lake as part of the present study. Seven other IDEM samples were over 1,000,000 cells/mL indicating that some of the lakes in Indiana's state parks have highly elevated cyanobacteria abundances compared to lakes in this study.

Predictions.—Predictive efforts using rapid screening and complex models provided better understanding of microcystin occurrence and cyanobacteria abundances in northern Indiana and elsewhere even though they were not strong enough to use for public health and safety determinations. Potentially promising field tools with rapid results, like fluorometers and Secchi disks, did not result in strong enough correlations for microcystin or cyanobacteria levels, such that visual interpretation of local lakes in relation to human health threats from cyanobacteria is questionable. Even complex model predictions would lead to false negatives (model predicts microcystin or cyanobacteria level below threshold when level is actually above threshold) 5–10% of the time for cyanobacteria abundance thresholds and 7–11% of the time for microcystin concentration thresholds, which is unacceptable for human health considerations.

From this analysis of possible predictive models, certain influential variables were identified which may warrant further exploration. In addition to the more typically assumed variables like water clarity, phycocyanin, chlorophyll a, water temperature, and month of the year, some unexpected variables emerged. Maximum wind speed was a relatively strong predictor variable in both two variable and multiple variable models to microcystin prediction, indicating that water turbulence or increased mixing might increase microcystin occurrence. Similarly, increased conductivity might influence microcystin occurrence positively, while the models indicate increased pH might be important for the overall cyanobacteria abundance growth.

Future work.—The present study informs several potential research efforts for the near future. Exploration of individual algae species abundances and other influences, such as zebra mussel populations (Bierman et al. 2005), local agricultural practices, and climate factors, are all warranted given the results of this study. The present study also influences future organizational focus for the Lilly Center related to alternative options for rapid cyanobacteria toxin results, as well as encouraging individualized management of various lakes throughout Kosciusko County.

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