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ORIGINAL RESEARCH

AWWA WATER SCIENCE

Carlson's Trophic State Index is a poor predictor of cyanobacterial dominance in drinking water reservoirs

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Abstract

A 20-month survey of 71 surface drinking water utilities across 44 waterbodies was conducted to determine whether the commonly used Trophic State Index (TSI) is a reliable indicator of eutrophication in drinking water sources. Raw water quality results showed that cyanobacteria, cyanotoxins (i.e., microcystin), and taste and odor (T&O) compounds (i.e., 2-methylisoborneol and geosmin) were generally low in the utilities sampled. TSI values based on chlorophyll concentrations (TSI Chl-*a*) were closely related to phytoplankton, cyanotoxin, and T&O concentrations and indicated that most drinking water sources were mesotrophic or eutrophic. However, TSI values based on total phosphorus (TSI TP) indicated that the drinking water sources were eutrophic or hypereutrophic. These results suggest that TSI Chl-*a* is a better predictor of cyanobacteria and their compounds than TSI TP. Phytoplankton abundance decreased with depth; therefore, managers should consider switching to deeper intakes when TSI Chl-*a* values increase to reduce removal costs.

KEYWORDS

cyanobacteria, depth, harmful algal blooms, taste and odor compounds

1 | INTRODUCTION

Determining the trophic state of aquatic ecosystems is useful for properly managing surface drinking water sources as it quantifies the biological response of a system to increased nutrient availability. Water quality indexes, such as Carlson's Trophic State Index (TSI), are commonly used for determining the trophic state of aquatic ecosystems and can be used to estimate the abundance or potential abundance of phytoplankton, including potentially toxic cyanobacteria, which are becoming an increasing risk to surface drinking water utilities worldwide. The occurrence of cyanobacterial blooms is increasing worldwide as a result of elevated surface water temperatures and increased nutrient inputs from agricultural, industrial, and urban sources (Glibert, 2020; Heisler et al., 2008; O'Neil, Davis, Burford, & Gobler, 2012). Several bloom-forming cyanobacteria produce toxic secondary metabolites (i.e., cyanotoxins), such as the hepatotoxins microcystin and cylindrospermopsin, that are linked to adverse health effects, such as upset stomach, diarrhea, vomiting, and liver and kidney damage in humans, livestock, and domestic animals (Briand, Jacquet, Bernard, & Humbert, 2003; DeVries, Galey, Namikoshi, & Woo, 1993; Elleman, Falconer, Jackson, & Runnegar, 1978). To manage the potential health risks of cyanotoxin exposure, the US Environmental Protection Agency (USEPA) created a 10-Day Drinking Water Health Advisory for cyanotoxins encouraging some surface drinking water utilities to issue health advisories for children of preschool age and younger (i.e., <6 years old) when microcystin and cylindrospermopsin

concentrations exceed 0.3 and 0.7 μg/L, respectively (USEPA, 2015), whereas the 10-Day Drinking Water Health Advisory for school-aged children and adults is 1.6 μg/L for microcystin and 3 μg/L for cylindrospermopsin. Yet, there are no federal standards for monitoring, response, and management of cyanotoxins that enter surface drinking water utilities, although such standards have been created in some states (Roberts, 2020; Yeager & Carpenter, 2019). A 2019 national survey found that monitoring and management practices vary widely across states and that these approaches are more rigorous in states that record frequent harmful algal bloom (HAB) issues, such as Ohio (Yeager & Carpenter, 2019).

Cyanobacteria further impair drinking water sources through the production of taste and odor (T&O) compounds, such as geosmin and 2-methylisoborneol (MIB) (Dietrich & Burlingame, 2015; Dunlap, Sklenar, & Blake, 2015; Olsen, Chislock, & Wilson, 2016). Geosmin and MIB are volatile terpenes that give water a musty or muddy scent and flavor that can be detected by consumers at concentrations as low as 10 and 30 ng/L, respectively (Izaguirre, Hwang, Krasner, & McGuire, 1982). Detection at such low concentrations causes consumer complaints as many gauge the quality and safety of their drinking water on aesthetic criteria (McGuire, 1995). However, geosmin and MIB have no known health effects at environmentally relevant concentrations (Burgos et al., 2014). Therefore, there are no regulatory guidelines for managing geosmin and MIB in recreational waters or drinking water sources (Watson, Monis, Baker, & Giglio, 2016). Rather, drinking water utilities manage T&O compounds at their own discretion to meet customer demands and instill confidence in finished and raw water quality.

The abundance of cyanobacteria, cyanotoxins, and T&O compounds can vary greatly seasonally and across years (Stumpf, Wynne, Baker, & Fahnenstiel, 2012). For example, cyanotoxins and T&O compounds tend to increase during the warmer summer months when elevated temperatures and prolonged solar radiation stimulate cyanobacterial growth (Jöhnk et al., 2008; Watson, Ridal, & Boyer, 2008). Trophic state can also play a major role as nitrogen and phosphorus inputs facilitate cyanobacterial growth, and it is considered one of the main predictors of cyanobacterial and T&O compound abundance (Downing, Watson, & McCauley, 2001). However, the presence of cyanobacteria is not always indicative of elevated levels of cyanotoxin and T&O compounds as not all cyanobacterial species are able to synthesize cyanotoxins or T&O compounds (Watson et al., 2008).

Conventional drinking water treatment methods remove cyanobacterial cells and low levels of cyanotoxins and T&O compounds from raw water (He et al., 2016). However, elevated and persistent levels of cyanotoxins and T&O compounds in source water can impose serious logistic and economic challenges to drinking water treatment (Dunlap et al., 2015; Khiari & Watson, 2007). A survey of 800 surface drinking water utilities across the United States and Canada found that 4.5%, on average, of their annual budget was spent on T&O compound removal and management (Khiari & Watson, 2007). Therefore, monitoring and preventing HABs at the reservoir level are imperative as they determine the effectiveness and cost of water treatment and, ultimately, the final quality and safety of drinking water. Despite the potential health and economic impacts of HABs, there are limited data on the presence of cyanotoxins in raw and finished drinking water as cyanotoxins only recently became classified for health advisories (USEPA, 2015), and there are no national monitoring programs for these compounds in the United States.

This study aims to determine the value of Carlson's TSI for estimating eutrophication, as well as characterizing the prevalence of phytoplankton including cyanobacteria, cyanotoxins, and T&O compounds, in all surface drinking water sources in the state of Alabama. Located in the southeastern United States, Elevated summer temperatures and high nutrient inputs from agricultural runoff in Alabama could potentially promote high cyanobacterial abundance. To characterize the trophic state of Alabama reservoirs, the Alabama Department of Environmental Management (ADEM) calculated the average TSI (Carlson, 1977) based on growing season (i.e., summer) chlorophyll-*a* concentrations for 41 reservoirs from 1997 to 2007 and found that 58% of such reservoirs were eutrophic, with higher chlorophyll-*a* values observed throughout the warmer summer months (Alabama Department of Environmental Management, 2018). While elevated nutrient availability signifies an increased threat of HABs, there are limited data on the prevalence of HABs and the presence of cyanotoxins and T&O compounds in Alabama (Graham, Dubrovsky, & Eberts, 2017). To estimate reservoir eutrophication status and to assess the threat of cyanotoxins and T&O compounds to drinking water across space and time, raw water samples were collected from the intake of every surface drinking water utility in Alabama from April 2017 to November 2018 to determine the presence of cyanobacterial toxins (i.e., microcystin, cylindrospermopsin, and saxitoxin), phytoplankton and cyanobacterial abundances, T&O compounds, and nutrients. The goal of this study was to generate monitoring and management criteria at the reservoir and processing plant levels to support state-wide surface water resource management.

2 | MATERIALS AND METHODS

2.1 | Study site and sampling

Raw water samples were collected from the intakes of 71 surface drinking water utilities throughout the state of Alabama, United States, from April 2017 to November 2018 (Figure 1). One utility is located in Tennessee but was included in the study because it provides drinking water to Alabama residents located close to the border of the two states. Sixty-two utilities provided samples from a single intake, seven utilities provided samples from 2 different water intakes, one utility provided samples from 3 intakes, and one utility provided samples from 4 intakes for a total of 83 raw water intakes. All participating utilities were asked to provide raw water samples in July of 2017 and 2018, and several utilities voluntarily provided additional samples throughout the year, resulting in a total of 367 samples.

Drinking water utility managers were provided sampling kits that included standard operating procedures and all of the necessary sampling equipment. Managers were instructed to allow raw intake water to run for at least 20 min before filling the provided acid-washed plastic bottles with the specified sample volume. Managers were also asked to provide intake depth relative to current surface level for every sample submitted for analysis. The managers did not specify whether the system was experiencing a visible algal bloom. Raw water samples were then placed in coolers with ice and shipped overnight to be processed immediately in the laboratory.

2.2 | Sample preparation and analysis

Chlorophyll-*a*, phycocyanin, microcystin, saxitoxin, and cylindrospermopsin samples were prepared by filtering a known volume of well-mixed raw water through Pall

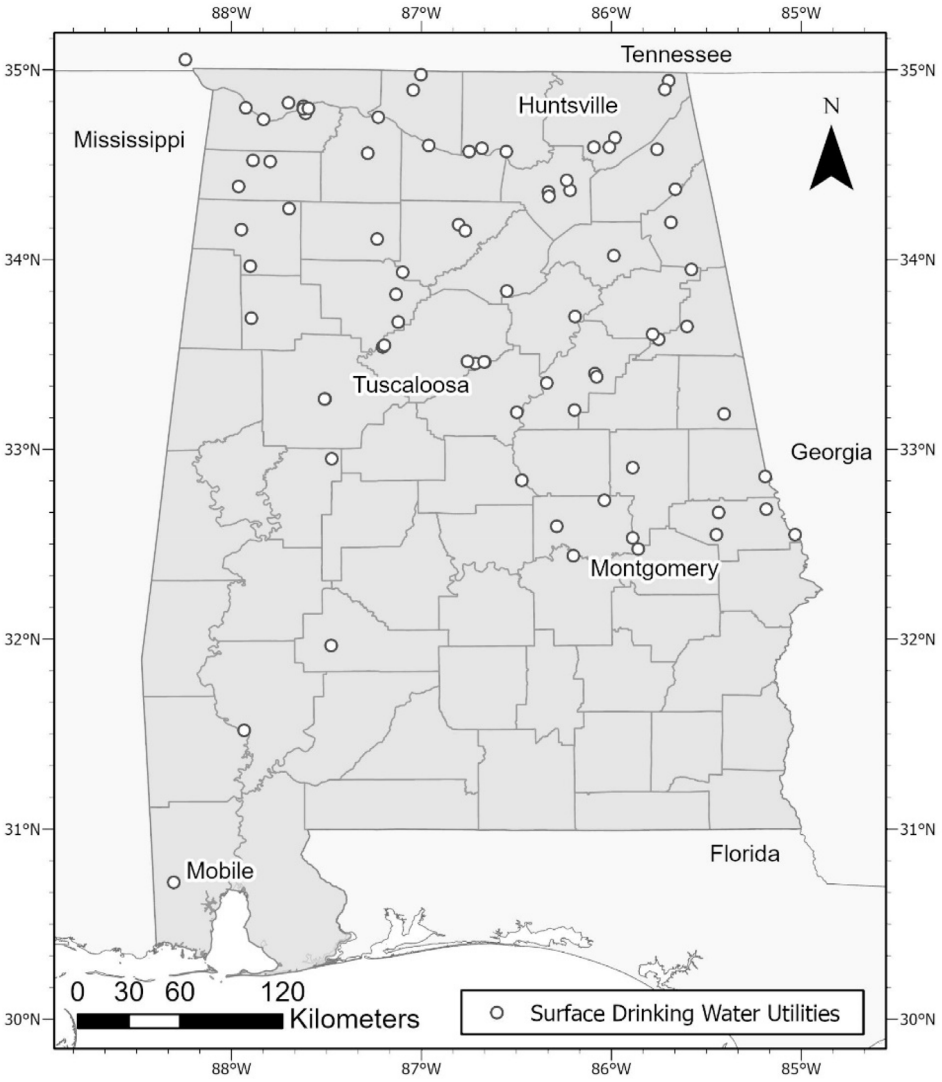

FIGURE 1 Map of the 83 raw water intake locations from the 71 surface drinking water utilities sampled in this study

A/E glass fiber filters and were stored frozen in the dark until analysis. Phytoplankton abundance was estimated using chlorophyll-*a* concentrations (μg/L) that were determined via fluorometric analysis (Turner Designs Trilogy fluorometer, non-acidification chlorophyll module) after extraction from filters with 90% aqueous ethanol in the dark at 4 °C (Sartory & Grobbelaar, 1984). Cyanobacterial abundance was estimated using phycocyanin concentrations (μg/L) that were determined via fluorometric analysis (Turner Designs Trilogy fluorometer, orange module) after extraction from filters that were ground and extracted in a 50-mM phosphate buffer in the dark for 4 hr, centrifuged, and filtered through a <0.2-μm filter prior to fluorometric analysis (Kasinak, Holt, Chislock, & Wilson, 2015; Sarada, Pillai, & Ravishankar, 1999). Microcystin, saxitoxin, and cylindrospermopsin concentrations (μg/L) were determined via enzyme-linked immunosorbent assay (ELISA) after extraction from filters with acidified 75% aqueous methanol (An & Carmichael, 1994) and dried until analysis. Toxin extracts were redissolved in 5 ml of phosphate buffer prior to ELISA.

Total nitrogen (TN) and total phosphorus (TP) raw water samples were stored frozen until analysis. Nutrient concentrations were determined via spectrophotometry using ultraviolet (total nitrogen; μg/L) or colorimetric (total phosphorus; μg/L) standard methods (Gross & Boyd, 1998). Raw, whole-water samples were stored in glass vials sealed with Parafilm at 4 °C and analyzed within 7 days of collection for MIB and geosmin concentrations (ng/L) using solid-phase microextraction combined with gas chromatography/mass spectrometry (Zimmerman, Ziegler, & Thurman, 2002).

Trophic status was determined using Carlson's TSI calculations on the basis of TP and chlorophyll-*a* concentrations as follows:

$$\mathrm{TSI\,(TP)} = 14.42 \times \ln(\mathrm{TP}) + 4.15 \quad (1)$$

$$\mathrm{TSI\,(Chl-}a\mathrm{)} = 9.81 \times \ln(\mathrm{CHL}) + 30.6 \quad (2)$$

where TP = total phosphorus concentration (μg/L) and CHL = chlorophyll-*a* pigment concentration (μg/L). TSI values less than 30 typically indicate oligotrophic conditions, 50–70 typically indicate eutrophic conditions, and over 70 indicate a hypereutrophic lake or reservoir (Carlson, 1977). Mean TSI for each reservoir was calculated by averaging the July 2017 and 2018 TSI values of each intake.

2.3 | Statistical analysis

TSI values were determined by averaging the July 2017 and 2018 samples as this was the only month in which every utility provided raw water samples. Pearson correlation coefficients were used to determine the relationship between chlorophyll-*a*, phycocyanin, microcystin, MIB, geosmin, TN, TP, TN:TP (by weight), and depth throughout the entire study period. Saxitoxin and cylindrospermopsin concentrations were low or undetectable through the study period and were therefore not included in statistical analyses. Differences in measured pigments, cyanotoxins, and T&O compounds between the warmer summer months (July through October) and the rest of the sampling season were determined through analysis of variance. The *stats* package for the statistical software R Studio version 4.0.2 (RStudio Team, 2015) was used for all statistical analyses.

3 | RESULTS

The temporal dynamics of chlorophyll-*a*, phycocyanin, microcystin, and T&O compound concentrations

	Minimum	Mean	Maximum	*SD*	*n*
Chlorophyll-*a* (μg/L)	0.03	6.68	66.05	8.55	367
Phycocyanin (μg/L)	0.00	1.43	26.51	2.76	358
Microcystin (μg/L)	0.00	0.01	0.21	0.02	367
MIB (ng/L)	0.00	2.81	115.91	10.17	358
Geosmin (ng/L)	0.00	0.97	21.11	2.23	357
TN (μg/L)	12.00	463.84	1,697.92	243.82	348
TP (μg/L)	9.24	63.98	212.10	33.7	366
TN:TP (by weight)	0.10	9.19	44.14	7.04	363
TSI TP	36.22	62.06	81.40	8.06	365
TSI Chl-*a*	0.00	42.64	71.71	12.12	367
Raw water intake depth (meters)	0.00	4.81	25.91	4.91	355

TABLE 1 Photosynthetic pigment (chlorophyll-*a* and phycocyanin), cyanotoxin (microcystin), taste and odor compound (2-methylisoborneol [MIB] and geosmin), total nitrogen (TN), total phosphorus (TP) and nitrogen to phosphorus ratio (TN:TP; by weight), trophic status index based on total phosphorus (TSI TP) and chlorophyll-*a* concentrations (TSI Chl-*a*), and raw water intake depth data for the 71 surface drinking water utilities sampled from April 2017 to November 2018 for this study. Undetected analytes were considered to be 0

throughout the study are summarized in Table 1 and Figure 2. In general, phycocyanin, cyanotoxins, and T&O compounds remained low throughout the study despite a large range in reservoir nutrient concentrations. Chlorophyll-*a* and phycocyanin concentrations ranged from 0.03 to 66.05 μg/L (average 6.68 μg/L) and 0–26.51 μg/L (average 1.43 μg/L), respectively, throughout the entire study period (Table 1 and Figure 2). Chlorophyll-*a* concentrations were statistically lower

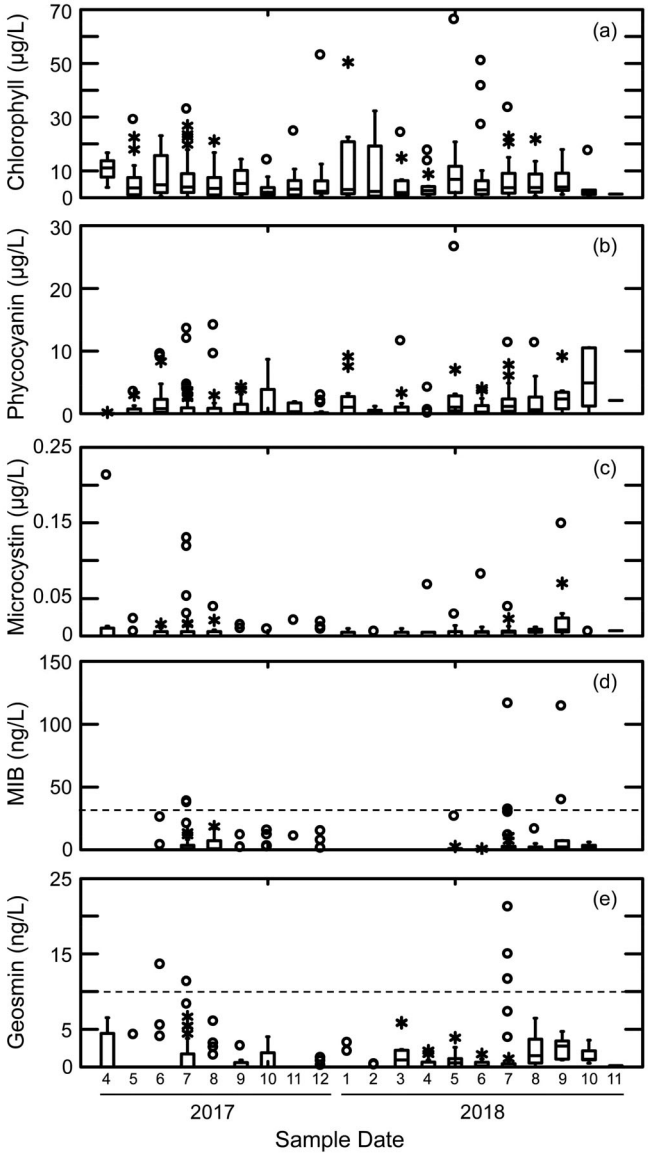

FIGURE 2 Chlorophyll-*a* (a), phycocyanin (b), microcystin (c), 2-methylisoborneol (MIB) (d), and geosmin (e) concentrations in raw water collected from the intakes of 71 surface drinking water utilities from April 2017 through November 2018. Dashed lines represent the human odor concentration threshold of MIB (30 ng/L) and geosmin (10 ng/L). Data presented as box plots. Horizontal lines within box (represent interquartile range) are median values. Upper and lower quartiles are represented by whiskers. Dots beyond whiskers are outliers

(p = .03) through the warmer summer months (July–October; 5.87 μg/L ± 0.83 95% confidence interval [CI]; Table 2) than the rest of the year (7.79 μg/L ± 1.74 95% CI; Table 2). Phycocyanin was marginally higher during the summer (1.60 μg/L ± 0.37 95% CI) compared with the rest of the year (1.21 μg/L ± 0.46 95% CI), although this trend was not statistically significant (p = .18). There was a significant positive correlation between the presence of the two pigments (r^2 = .12, p < .0001), with a 0.11 μg/L (±0.03 95% CI) increase in phycocyanin for every 1 unit increase in chlorophyll-*a* (μg/L; Table 3).

Cyanotoxin concentrations were generally low throughout the study. Saxitoxin concentrations were below the 0.67 ng/L limit of detection (LOD) for our assay throughout the study period, and cylindrospermopsin ranged from undetectable (below LOD of 1.7 ng/L) to 3.1 ng/L, suggesting that these cyanotoxins were either low or not present in our samples. Microcystin concentrations ranged from undetectable (below LOD of 0.005 μg/L) to 0.21 μg/L (average 0.01 μg/L; Table 1 and Figure 2) and were generally higher during the warmer summer months, although the highest recorded concentration occurred in April 2017 at one site. There was no statistically significant relationship between microcystin and chlorophyll-*a* (r^2 < .01, p = .21) or phycocyanin (r^2 < .01, p = .78; Table 3).

MIB and geosmin concentrations ranged from below the LOD (1 ng/L) to 115.91 ng/L (average 2.81 ng/L) and 21.11 ng/L (average 0.97 ng/L), respectively, throughout the sampling period (Table 1 and Figure 2). There was a significant positive correlation between the two T&O compounds (r^2 = .25, p < .0001), with a 2.25 ng/L (±0.42 95% CI) increase in MIB for each 1 unit increase in geosmin (ng/L) (Table 3). MIB and geosmin concentrations were 3.64 ng/L (±2.16 95% CI) and 0.55 ng/L (±0.48 95% CI) higher from July to October than the rest of the year, respectively ($p \leq .02$; Table 2). There was a significant positive relationship between MIB and chlorophyll-*a* (r^2 = .01, p = .03) and phycocyanin (r^2 = .02, p = .01), as well as geosmin and chlorophyll-*a* (r^2 = .03, p < .0005; Table 3). Geosmin and phycocyanin were not significantly correlated.

TN, TP, and TN:TP (by weight) ranged from 12.00 to 1,697.92 μg/L (average 463.84 μg/L), 9.24 to 212.1 μg/L (average 66.04 μg/L), and 0.1 to 44.14 (average 9.19), respectively, throughout the sampling period (Table 1 and Figure 2). Chlorophyll-*a*, phycocyanin, and microcystin had a nonlinear relationship with nutrients and TN:TP ratios. However, chlorophyll-*a* concentrations were 1.83 μg/L (±1.63 95% CI) higher when TN:TP ratios were under 10 (p = .03; Table 2). There was also a trend of higher phycocyanin and MIB when TN:TP was less than 10, although these trends were not statistically significant (Figure 3 and Table 3).

TABLE 2 Mean (±95% confidence interval) values for chlorophyll-*a*, phycocyanin, microcystin, 2-methylisoborneol (MIB), and geosmin between the warmer summer months (July through October) and the rest of the year (November through June), above and below the apparent 10 nitrogen to phosphorus ratio (total nitrogen [TN]:total phosphorus [TP]; by weight) and cutoff above and below the apparent productivity cutoff for depth (5 m)

	Chlorophyll (µg/L)	Phycocyanin (µg/L)	Microcystin (µg/L)	MIB (ng/L)	Geosmin (ng/L)	*n*
July to October	5.87 (0.83)	1.60 (0.37)	0.005 (0.003)	4.29 (1.72)	1.19 (0.34)	212
November to June	7.79 (1.74)	1.21 (0.46)	0.006 (0.002)	0.65 (0.55)	0.64 (0.28)	155
p-value	.03	.18	.45	<.01	.02	
Below 10 TN:TP	7.26 (1.16)	1.44 (0.36)	0.004 (0.002)	2.87 (1.19)	0.90 (0.30)	252
Above 10 TN:TP	5.42 (0.93)	1.41 (0.47)	0.009 (0.005)	2.45 (2.18)	0.95 (0.30)	111
p-value	.03	.12	.04	.15	.87	
Depth < 5 m	7.58 (1.16)	1.50 (0.35)	0.005 (0.002)	3.42 (1.48)	1.03 (0.30)	258
Depth > 5 m	4.64 (1.05)	1.24 (0.52)	0.004 (0.002)	0.83 (0.34)	0.71 (0.39)	97
p-value	.004	.45	.55	.04	.23	

TABLE 3 Pearson correlation coefficients between chlorophyll-*a*, phycocyanin, microcystin, 2-methylisoborneol (MIB), geosmin, trophic state index (TSI) values based on chlorophyll-a (TSI Chl-*a*) and total phosphorus (TSI TP) collected from the raw water intakes of 71 surface drinking water utilities from April 2017 through November 2018

Variable 1	Variable 2	Effect estimate	±95% CI	*p*-value	r^2
Chlorophyll-*a*	Phycocyanin	0.11	0.03	<.0001	.12
Microcystin	Chlorophyll-*a*	0.00	0.00	.21	<.01
Microcystin	Phycocyanin	0.00	0.00	.78	<.01
MIB	Chlorophyll-*a*	0.14	0.13	.03	.01
MIB	Phycocyanin	0.47	0.38	.01	.02
Geosmin	Chlorophyll-*a*	0.05	0.03	<.0005	.03
Geosmin	Phycocyanin	0.07	0.08	.08	.01
Geosmin	MIB	2.25	0.42	<.0001	.25
Microcystin	MIB	0.00	0.00	.69	<.01
Microcystin	Geosmin	0.00	0.00	.31	<.01
Chlorophyll-*a*	TSI Chl-*a*	0.56	0.05	<.0001	.63
Phycocyanin	TSI Chl-*a*	0.05	0.02	<.0001	.05
Microcystin	TSI Chl-*a*	0.00	0.00	.03	.01
MIB	TSI Chl-*a*	0.11	0.09	.01	.02
Geosmin	TSI Chl-*a*	0.04	0.02	<.0001	.04
Chlorophyll-*a*	TSI TP	0.23	0.11	<.0001	.04
Phycocyanin	TSI TP	0.02	0.04	.32	.00
Microcystin	TSI TP	0.00	0.00	.69	.00
MIB	TSI TP	0.06	0.13	.39	.00
Geosmin	TSI TP	−0.01	0.03	.57	.00

Abbreviation: CI, confidence interval.

The Carlson TSI TP values ranged from 36.22 to 81.40 (average 62.06), while TSI chlorophyll concentrations (TSI Chl-*a*) ranged from 0 to 71.71 (average 42.64) throughout the study period (Table 1). Interestingly, the average summer (July 2017 and 2018) TSI TP and TSI Chl-*a* values differed greatly, with TS TP values 19.26 (±1.51 95% CI) higher than TSI Chl-*a* on average ($p < .0001$). Based on summer TP data, 80% and 16% of our samples originated from a eutrophic or hypereutrophic source, respectively, while chlorophyll-*a*-derived TSI values classified 31% as eutrophic and none as hypereutrophic (Figure 4). TSI Chl-*a* values were significantly correlated ($p < .03$) with chlorophyll-*a*, phycocyanin, microcystin, MIB, and geosmin (Table 3). However, these relationships were not observed when compared with TSI TP. Chlorophyll-*a* was the only

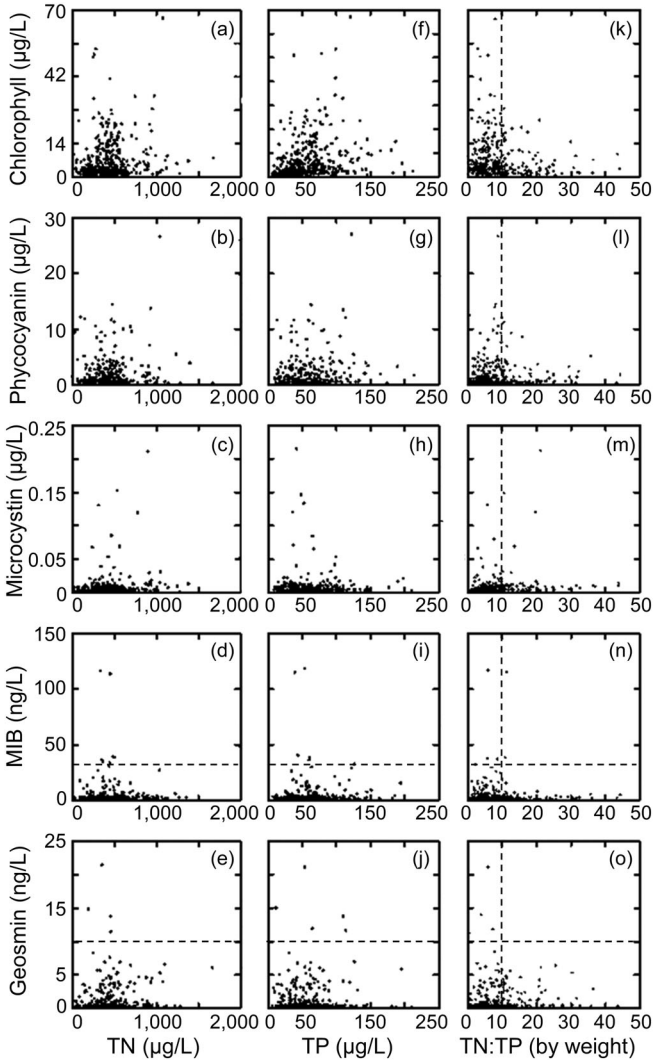

FIGURE 3 Relationship between chlorophyll-*a*, phycocyanin, microcystin, geosmin and 2-methylisoborneol (MIB), and total phosphorus (TP; a–e), total nitrogen (TN; f–j), and total nitrogen to phosphorus ratio by weight (TN:TP; k–o). Black horizontal dashed lines represent the human odor concentration threshold of MIB (30 ng/L) and geosmin (10 ng/L). Vertical dashed lines represent the apparent TN:TP threshold for pigment and secondary metabolite abundance

parameter closely related to TSI TP, with a 0.23 (±0.11 95% CI) unit increase in chlorophyll-*a* for every unit increase in TSI TP ($r^2 = .04$, $p < .0001$).

Surface drinking water intake depths ranged from the surface to 25.91 m, with an average sampling depth of 4.81 m (Table 1). Nineteen samples were collected from surface intakes (i.e., 0 m). As depth increased, the abundance of phycocyanin, microcystin, and geosmin decreased, although these relationships were not statistically significant (Figure 5). However, chlorophyll-*a* and MIB concentrations were significantly higher in samples collected from intakes shallower than 5 m ($p \leq .04$; Table 2).

4 | DISCUSSION

Bloom-forming cyanobacteria often thrive under elevated surface water temperatures, increased nitrogen and/or phosphorus availability, reduced nitrogen to phosphorus ratios (TN:TP), and poor mixing conditions (Downing et al., 2001; Paerl & Huisman, 2008; Smith, 1983). While the surface drinking water sources sampled in the present study had relatively high nutrients and summer surface water temperature conditions, cyanobacterial abundance was generally low, and cyanotoxins remained below health advisory thresholds determined by the USEPA (USEPA, 2015; Figure 1).

HABs dominated by cyanobacteria are common during the warmer summer months when elevated temperatures and prolonged solar radiation facilitate their growth (Jöhnk et al., 2008). These trends were observed throughout the 20-month sampling period with higher phycocyanin concentrations from July to October than the rest of the year (Table 2). Interestingly, chlorophyll-*a* concentrations were significantly higher from November to June, although the difference in average chlorophyll-*a* was <2 µg/L. Due to the temporal and spatial variability of cyanobacterial abundance and cyanotoxin production, it is possible that the annual sampling of the utilities was not sufficient to capture the episodic trends of cyanobacterial growth in our systems.

Cyanotoxins were typically present at low to undetectable levels throughout the 20-month study. Microcystin did not exceed the USEPA drinking water health advisory of 0.3 µg/L for children under 6 years old (USEPA, 2015) over the duration of this project, with the highest observed concentration being 0.21 µg/L (Table 1). Microcystin was poorly correlated with algal abundance throughout the study period (Table 3), likely because not all cyanobacterial species have the ability to produce toxins, and the triggers for cyanotoxin synthesis, storage, and release vary by cyanobacterial species and even strain, leading to poor spatial and temporal relationships between cyanobacteria and their metabolites (Downing et al., 2001; Smith, 1983; Watson et al., 2008). While some cyanobacteria can produce both cyanotoxins and T&O compounds, there was also no clear relationship between microcystin and T&O compound abundance in our study (Table 3). There are conflicting data on the cooccurrence of phytoplankton, cyanotoxins, and T&O compounds, and weak relationships between the three parameters are not uncommon (Graham et al., 2017; Watson et al., 2008).

MIB and geosmin production by cyanobacteria are the main biological sources of T&O compounds in surface drinking water sources, particularly during elevated temperature and nutrient conditions (Jüttner & Watson, 2007; Watson et al., 2008). MIB and geosmin

were both significantly higher during the warmer summer months, which has been observed in previous field studies (Table 2; Sugiura, Iwami, Inamori, Nishimura, & Sudo, 1998; Westerhoff, Rodriguez-Hernandez, Baker, & Sommerfeld, 2005). Only 2% of the samples exceeded the human detection threshold concentration for MIB (30 ng/L), all of which occurred between July and September. MIB was closely related to chlorophyll-*a* and phycocyanin concentrations, which suggests that cyanobacteria were the main producers of MIB in our systems (Table 3). Only 1% of the samples exceeded the human detection threshold concentration for geosmin (10 ng/L), all of which occurred between June and July. Geosmin concentrations were not significantly correlated to phycocyanin concentrations, which suggests the presence of a benthic geosmin producer, such as benthic cyanobacteria or actinobacteria, in the studied systems (Izaguirre et al., 1982). Benthic MIB and geosmin producers may contribute to T&O compounds in the water column, although their contribution to drinking water sources is not as well studied as pelagic cyanobacterial species, and this topic requires further study (Cai et al., 2017). There was a significant relationship between MIB and geosmin, although they did not occur exclusively in tandem. These results are surprising as T&O problems are common in the state of Alabama. A survey of rural Alabama drinking water consumers that rely on groundwater or surface water sources found that 20% of consumers experience aesthetic issues (Wedgworth et al., 2014). The findings from our study suggest that aesthetic issues reported by Alabama drinking water consumers are not related to cyanobacterial off-flavors.

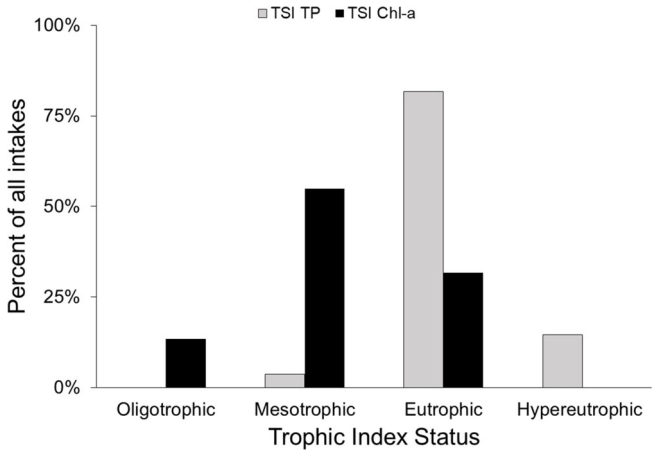

FIGURE 4 Trophic index status (TSI) estimates based on average total phosphorus concentrations (TSI TP) compared to chlorophyll-*a* concentrations (TSI Chl-*a*) collected from 83 raw surface water intakes from 71 drinking water utilities in July 2017 and 2018

Carlson's TSI values varied greatly depending on whether TSI TP or TSI Chl-*a* concentrations were used. TSI TP values suggested that most of the samples were collected from either eutrophic (80%) or hypereutrophic (16%) systems, while TSI Chl-*a* values reflected conditions common of more mesotrophic (53%) or eutrophic (31%) systems (Figure 4). The discrepancy between the two calculated TSI values suggests that our systems are nutrient-rich but phytoplankton-poor. Typically, this difference is observed in systems that have high turbidity, which reduces light availability for phytoplankton (Carlson, 1991). However, these patterns could also be related to summer stratification, in which surface water is warmer; high phytoplankton abundance reduces nutrient concentrations; and deeper water is cooler, with low productivity due to low light availability, and therefore has higher nutrient concentrations. In our study, chlorophyll-*a* and MIB were significantly higher in shallow (<5 m) intakes (Table 2). Chlorophyll-*a*, phycocyanin, microcystin, MIB, and geosmin were all significantly

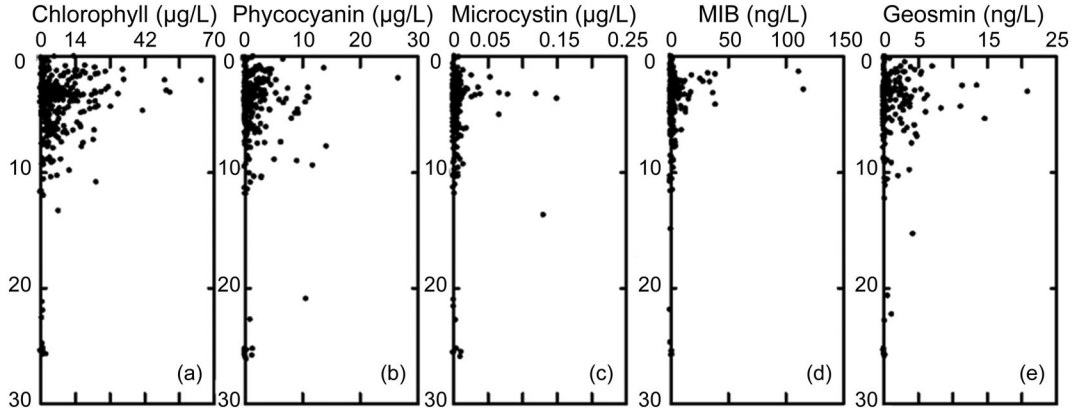

FIGURE 5 Chlorophyll-*a* (a), phycocyanin (b), microcystin (c), 2-methylisoborneol (MIB) (d), and geosmin (e) concentrations by intake depth (m) of raw water collected from 71 drinking water utilities from April 2017 through November 2018

correlated to TSI Chl-*a* values (Table 3). The same relationship was not observed for TSI TP values. Therefore, TSI Chl-*a* was a better predictor of cyanobacteria and their metabolites in the drinking water sources monitored in this study than TSI TP. This is consistent with past studies which have shown that TSI Chl-*a* values are the best predictors of MIB and geosmin outbreaks in lentic systems (Downing et al., 2001).

There was a general trend in our systems of higher phytoplankton and cyanobacterial secondary metabolite abundance when TN:TP ratios (by weight) were below 10 (Figure 3). In general, there is a tendency for cyanobacteria to dominate lentic systems when the TN:TP ratios are below 30 (Smith, 1983). Observational studies have found that MIB, geosmin, and microcystin concentrations were highest when TN:TP values were low (Harris et al., 2016; Perkins et al., 2019; Smith et al., 2002). Currently, the state of Alabama has not implemented state criteria for nitrogen and phosphorus concentrations in drinking water sources, although there are several lake-specific chlorophyll-*a* criteria implemented throughout the state (Alabama Department of Environmental Management, 2017).

Due to the low abundance of cyanobacteria and cyanotoxins in our systems, it is difficult to propose predictive models for HABs, but differences in vertical distribution present an interesting management strategy for drinking water utilities. Cyanobacteria thrive and produce a higher concentration of cyanotoxins, MIB, and geosmin under elevated light conditions (Saadoun, Schrader, & Blevins, 2001; Wang & Li, 2015). Westerhoff et al. (2005) found that the highest concentrations of MIB occurred in the upper 10 m of the water column. In this study, chlorophyll-*a* and MIB were more abundant in shallow intakes (<5 m; Table 2). These findings present a low-cost, preventative solution for reducing the cost of removing cyanobacteria and their secondary metabolites from drinking water. Switching to a deeper intake, when available, could reduce the abundance of phytoplankton entering water treatment plants at the intake level, thereby reducing the economic and logistic complications associated with removing these compounds from drinking water.

5 | CONCLUSIONS

HABs threaten drinking water sources through the production of cyanotoxins and T&O compounds. The results of this 20-month, state-wide survey suggest that cyanobacteria, cyanotoxins, and T&O compounds were scarce in surface drinking water sources. TSI values based on chlorophyll-*a* concentrations suggest that the majority of the drinking water sources were mesotrophic or eutrophic and correlated well with phytoplankton, cyanotoxin, and T&O compound abundance. Yet, TSI values based on TP suggest that the systems were eutrophic or hypereutrophic and did not correlate well with cyanotoxins and T&O compounds. The discrepancy between the two TSI values suggests that there may be an additional factor, such as stratification, suppressing cyanobacterial growth. When monitoring surface water sources, managers should prioritize chlorophyll-*a* over nutrient measurements as TSI Chl-*a* values were closely related to cyanotoxins and T&O compounds. Moreover, calculating the TSI Chl-*a* from several intakes, when available, can be useful for determining which intake has the lowest chance of containing cyanotoxin and T&O compounds without the need to directly measure these parameters. For this study, deeper intakes tended to have lower phytoplankton and T&O compound abundance. Although not tested in this study, directly measuring Secchi depth can also be a reliable and low-cost indicator of trophic state and could be used as an early warning sign to initiate more rigorous sampling.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Edna Gabriela Fernandez-Figueroa: Data curation; formal analysis; validation; investigation; visualization; methodology; writing-original draft. **Riley P Buley:** Investigation. **Mario U G Barros:** Methodology. **Matthew F Gladfelter:** Formal analysis. **William D McClimans:** Conceptualization; funding acquisition; methodology. **Alan E Wilson:** Conceptualization; data curation; formal analysis; supervision; funding acquisition; methodology; project administration.

DATA AVAILABILITY STATEMENT

Data collected from this study are available from the corresponding author upon reasonable request.

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