PLEON

POCONO LAKE ECOLOGICAL OBSERVATORY NETWORK

To the Twin and Walker Creeks Watershed Conservancy

Report of 2022 PLEON Sampling

From the Pocono Lakes Ecological Observatory Network

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I. Summary 2022: Twin and Walker Lakes at a Glance

PLEON partnered with TWCWC to monitor Big Twin, Little Twin, and Walker lakes three times in 2022. PLEON also collected samples for potentially toxigenic cyanobacteria screening from Big Twin Lake on May 26, 2022.

Table 1: Summary of 2022 monitoring. Dates are Twin Lakes and Walker samplings, respectively.

	Variables Monitored	**Crew**
26 May	- PTOX screen from Big Twin and swampy inflow.	Beth Norman (PLEON Director) Alexandra Bros (PLEON intern)
18 June 24 June	- Profiles: temperature, dissolved oxygen, conductivity, pH - Secchi Depth - Chlorophyll a (0.5 m, composite) - Total N, Total P, dissolved organic carbon (0.5 m, composite)	Collection: TWCWC Analysis: PLEON
19 July	- Profiles: temperature, dissolved oxygen, conductivity, pH, light - Secchi Depth - Chlorophyll a (0.5 m, composite) - Total N, Total P, dissolved organic carbon (0.5 m, composite) - Zooplankton community (Twin Lakes only) - Phytoplankton community (Twin Lakes only)	Beth Norman (PLEON Director) Jillian Groff (PLEON intern) Alexandra Bros (PLEON intern)
20 Aug 21 Aug	- Profiles: temperature, dissolved oxygen, conductivity, pH - Secchi Depth - Chlorophyll a (0.5 m, composite) - Total N, Total P, dissolved organic carbon (0.5 m, composite)	Collection: TWCWC Analysis: PLEON

Table 2: Summary of Big Twin Lake in 2022

	26 May	**18 June**	**19 July**	**20 Aug**
Thermally stratified?	—	YES	YES	YES
Epilimnion depth (m)	—	5	3	4
Metalimnion depth (m)	—	8	9	8
Secchi depth (m)	—	1.5	1.75	0.8
Vertical extinction coefficient (k)	—	—	0.88	—
$Z_{10\%}$ (m)	—	—	2.62	—
$Z_{1\%}$ (m)	—	—	5.23	—
Mean hypolimnetic DO (mg/L)	—	1.25	0.08	0.10
Epilimnetic chlorophyll concentration (µg/L)	—	2.35	2.84	9.30
Epilimnietic TN (mg/L)	—	0.29	0.35	0.79
Epilimnetic TP (µg/L)	—	7.78	9.44	16.5
TSI_{secchi}	—	53.2	51.9	63.2
$TSI_{chlorophyll}$	—	39.0	40.8	52.5
TSI_{TP}	—	33.3	36.0	44.0
Trophic classification*	—	OLIGOTROPHIC	MESOTROPHIC	EUTROPHIC
PTOX cyanobacteria found?	NO	—	—	—
Toxin testing recommended?	—	—	—	—

*according to $TSI_{chlorophyll}$

Table 3: Summary of Little Twin Lake in 2022

	18 June	19 July	20 Aug
Thermally stratified?	YES	YES	YES
Epilimnion depth (m)	4	3	4
Metalimnion depth (m)	10	9	10
Secchi depth (m)	2.5	3	1.8
Vertical extinction coefficient (k)	—	0.97	—
$Z_{10\%}$ (m)	—	2.38	—
$Z_{1\%}$ (m)	—	4.76	—
Mean hypolimnetic DO (mg/L)	1.03	0.31	0.14
Epilimnetic chlorophyll concentration (µg/L)	1.35	0.07	4.33
Epilimnietic TN (mg/L)	0.22	0.25	0.32
Epilimnetic TP (µg/L)	2.80	1.38	6.24
TSI_{secchi}	46.3	44.2	51.5
$TSI_{chlorophyll}$	33.6	3.91	45.0
TSI_{TP}	18.8	8.76	30.1
Trophic classification*	OLIGOTROPHIC	OLIGOTROPHIC	MESOTROPHIC

according to $TSI_{chlorophyll}$

Table 4: Summary of Walker Lake in 2022

	24 June	19 July	21 Aug
Thermally stratified?	YES	YES	PARTIAL
Epilimnion depth (m)	3.5	2	3
Metalimnion depth (m)	5	5.5	—
Secchi depth (m)	1.4	1.25	1.2
Vertical extinction coefficient (k)	—	1.66	—
$Z_{10\%}$ (m)	—	1.38	—
$Z_{1\%}$ (m)	—	2.77	—
Mean hypolimnetic DO (mg/L)	0.71	0.08	0.1*
Epilimnetic chlorophyll concentration (µg/L)	0.72	2.96	3.53
Epilimnietic TN (mg/L)	0.32	0.35	0.78
Epilimnetic TP (µg/L)	12.7	14.3	18.4
TSI_{secchi}	55.2	56.8	57.4
$TSI_{chlorophyll}$	27.3	41.3	43.0
TSI_{TP}	40.3	41.9	45.5
Trophic classification**	OLIGOTROPHIC	MESOTROPHIC	MESOTROPHIC

**single reading at deepest point of profile.   **according to $TSI_{chlorophyll}$*

II. Chemical Profiles

A. Temperature

Big Twin was thermally stratified during all 2022 samplings (Figure 1). The epilimnion, or the well-mixed surface layer, extended to 5 m, 3 m, and 4 m during the June, July, and August samplings, respectively. The average epilimnetic temperature (± standard deviation) in Big Twin was 21.5 °C (±0.05) during the June sampling, 25.8 °C (±0.38) during the July sampling, and 23.6 °C (±0.45) during the August sampling. The metalimnion, or middle layer of rapid temperature change, extended to 8 m, 9 m, and 8 m during the June, July, and August samplings, respectively.

Little Twin was thermally stratified during all 2022 samplings (Figure 1). The epilimnion extended to 4 m, 3 m, and 4 m during the June, July, and August samplings, respectively. The average epilimnetic temperature (± standard deviation) in Little Twin was 22.3 °C (±0.05) during the June sampling, 26.3 °C (±0.68) during the July sampling, and 24.4 °C (±0.50) during the August sampling. The metalimnion extended to 10 m, 9 m, and 10 m during June, July, and August samplings, respectively.

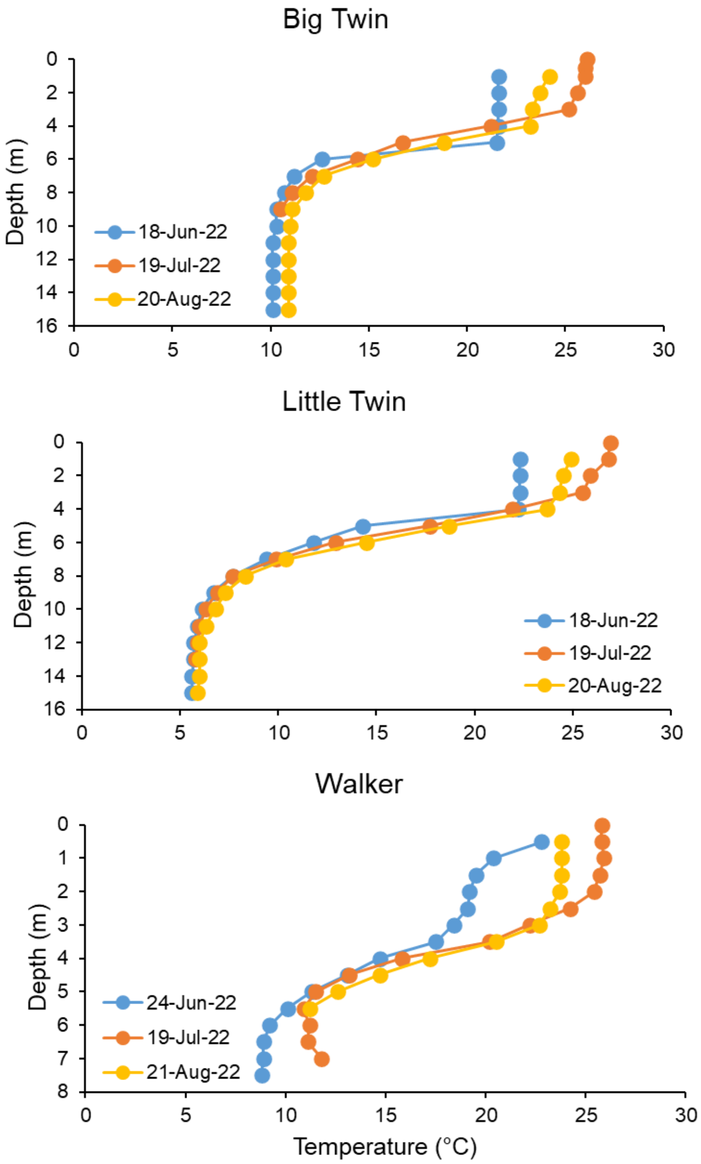


Figure 1: Temperature depth profiles in TWCWC lakes during the summer of 2022. Note different sampling dates and Y axis scales among panels.

Walker was thermally stratified during the June and July samplings and partially stratified (i.e., no hypolimnion was delineated) during the August sampling (Figure 1). The epilimnion extended to 3.5 m, 2 m, and 3 m during the June, July, and August samplings, respectively. The average epilimnetic temperature (± standard deviation) in Walker was 19.5 °C (±1.63) during the June sampling, 25.7 °C (±0.19) during the July sampling, and 23.5 °C (±0.46) during the August sampling. The metalimnion extended to 5 m and 5.5 m during the June and July samplings and extended to the deepest sampling point (5.5 m) during the August sampling.

Thermal stratification of deep lakes is expected in the Pocono region as the surface water is heated by the sun and the deeper water remains cool. Thermal stratification breaks down in the fall as surface waters cool and lakes “turnover”, or the layers mix. Partial stratification can occur in more shallow lakes, as seen in Walker during the August sampling.

B. Dissolved Oxygen

Big Twin was oxygenated through the epilimnion during all 2022 samplings (Figure 2). Dissolved oxygen (DO) concentration declined through the metalimnion during all samplings. Average DO concentration in the hypolimnion, or deep water, was 1.25 mg/L during the June sampling, 0.08 mg/L during the July sampling, and 0.10 mg/L during the August sampling. The depth at which DO concentration was below 2 mg/L, the threshold for oxygen depletion, was 10 m, 5 m, and 5 m during the June, July, and August sampling, respectively.

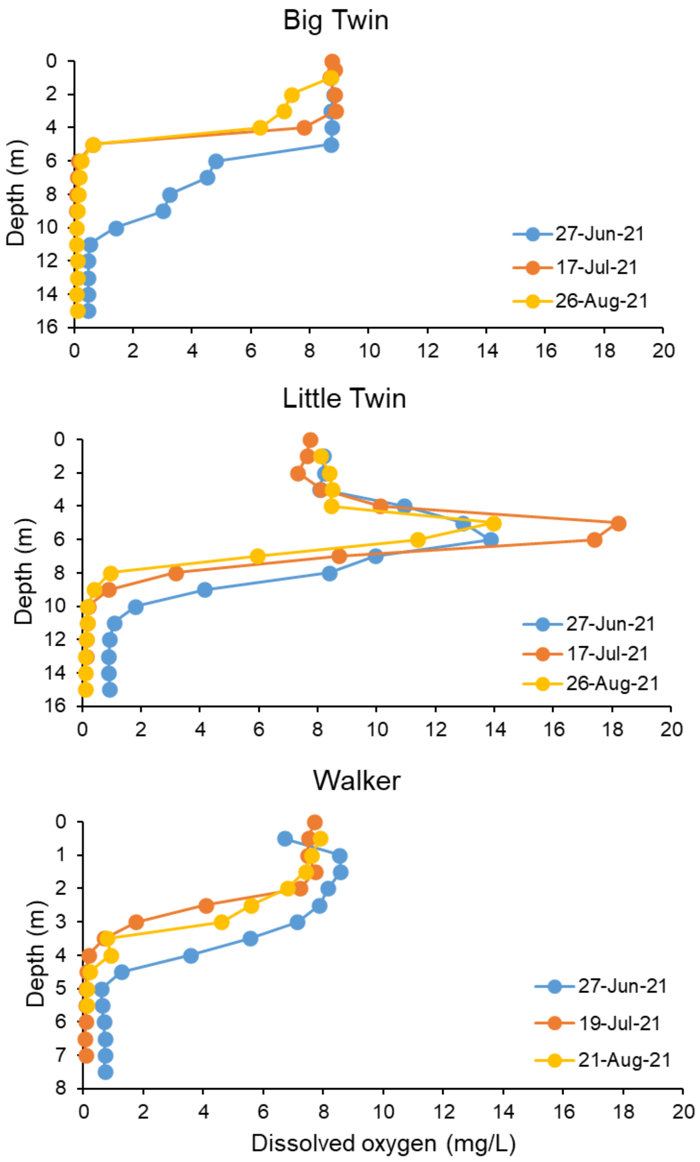


Figure 2: Dissolved oxygen depth profiles in TWCWC lakes during the summer of 2022. Note different sampling dates and Y axis scales among panels.

Little Twin was also oxygenated through the epilimnion during all 2022 samplings (Figure 2) with maximum dissolved oxygen (DO) concentration in the metalimnion (peak DO concentration at 5-6 m) on all dates. DO concentration declined at depths below these maxima. Average DO concentration in the hypolimnion was 1.03 mg/L during the June sampling, 0.31 mg/L during the July sampling, and 0.14 mg/L during the August sampling.

As in the Twin lakes, Walker Lake was oxygenated through the epilimnion and DO concentration declined through the metalimnion during all 2022 samplings (Figure 2). Average hypolimnetic DO concentration was 0.71 mg/L during the June sampling and 0.08 during the July sampling. A hypolimnion was not delineated during the August sampling, but the DO concentration at the deepest sampling point was 0.1 mg/L.

The DO profiles observed in Twin and Walker lakes are typical. DO concentration is often high in the epilimnion due to diffusion of oxygen across the surface of the lake as well as the abundance of algae in this warm, typically well-lit layer. Algae produce oxygen as a biproduct of photosynthesis. DO peaks in the metalimnion (sometimes referred to as metalimnetic oxygen maxima) can occur when algae congregate in the middle depths. This is common in clear water lakes, such as Little Twin, where metalimnetic waters still have plenty of light for photosynthesis but less of the harmful ultraviolet wavelengths.

Oxygen depletion is common in the hypolimnion (as seen in all three TWCWC lakes) where decomposition of organic matter in the water and lake sediments removes oxygen and the lack of light prohibits photosynthesis. The hypolimnion often remains hypoxic until thermal stratification breaks down and the lake layers mix.

C. Conductivity

Conductivity in Big Twin was generally stable through the epilimnion, decreased slightly in the metalimnion, and increased through the deeper waters (Figure 3). Conductivity ranged from 52.6-74.0 µS/cm during the June sampling, from 62.0-75.8 µS/cm during the July sampling, and from 71.7-105.4 µS/cm during the August sampling. Conductivity in the hypolimnion was greater during the August sampling by approximately 25 µS/cm compared to the June sampling. The highest conductivity was generally recorded near the sediments. The relatively

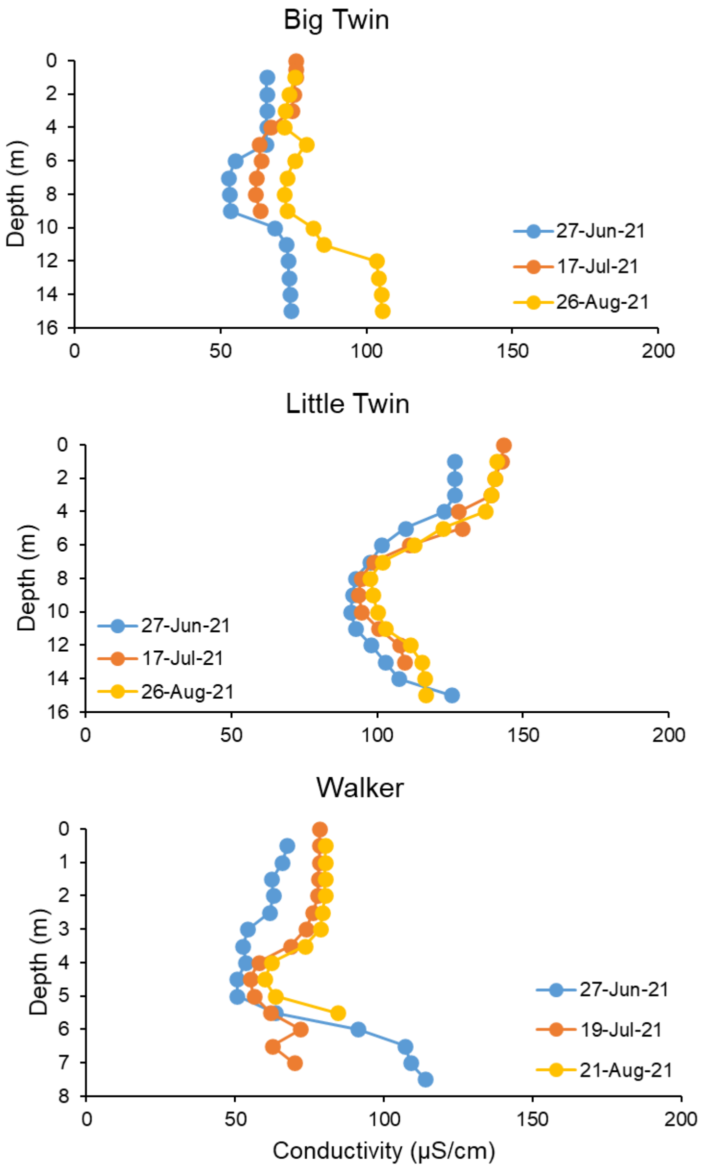


Figure 3: Conductivity depth profiles in TWCWC lakes during the summer of 2022. Note different sampling dates and Y axis scales among panels.

constrained range during the July sampling may be due to the more shallow profile.

Conductivity in Little Twin was also stable through the epilimnion but was greater than that of Big Twin by ~60 µS/cm (Figure 3). Conductivity in the metalimnion was less than that of the epilimnion and hypolimnion in Little Twin and epilimnetic conductivity was generally greater than the hypolimnion. Conductivity ranged from 90.8-126.5 µS/cm during the June sampling, from 93.6-143.2 µS/cm during the July sampling, and from 97.5-141.1 µS/cm during the August sampling.

Conductivity of Walker was generally stable in the epilimnion, decreased in the metalimnion, and increased in the deep waters (Figure 3). Conductivity ranged from 50.5-113.9 µS/cm during the June sampling, from 55.0-78.4 µS/cm during the July sampling, and from 59.8-84.4 µS/cm during the August sampling.

Conductivity is a measure of the amount of ions, or charged particles, in the water which come from dissolved compounds. Lake conductivity responds to several factors including underlying geology, runoff, point-source inputs, precipitation, evaporation, and in-lake productivity. Increased conductivity near the sediments in some 2022 TWCWC profiles may be a result of the increased biological activity at the water sediment interface or in extreme cases, due to the probe contacting the sediments.

D. pH

pH in Big Twin ranged from 6.33-8.30 during the June sampling, from 6.43-732 during the July sampling, and from 6.28-8.07 during the August sampling (Figure 4). pH in Big

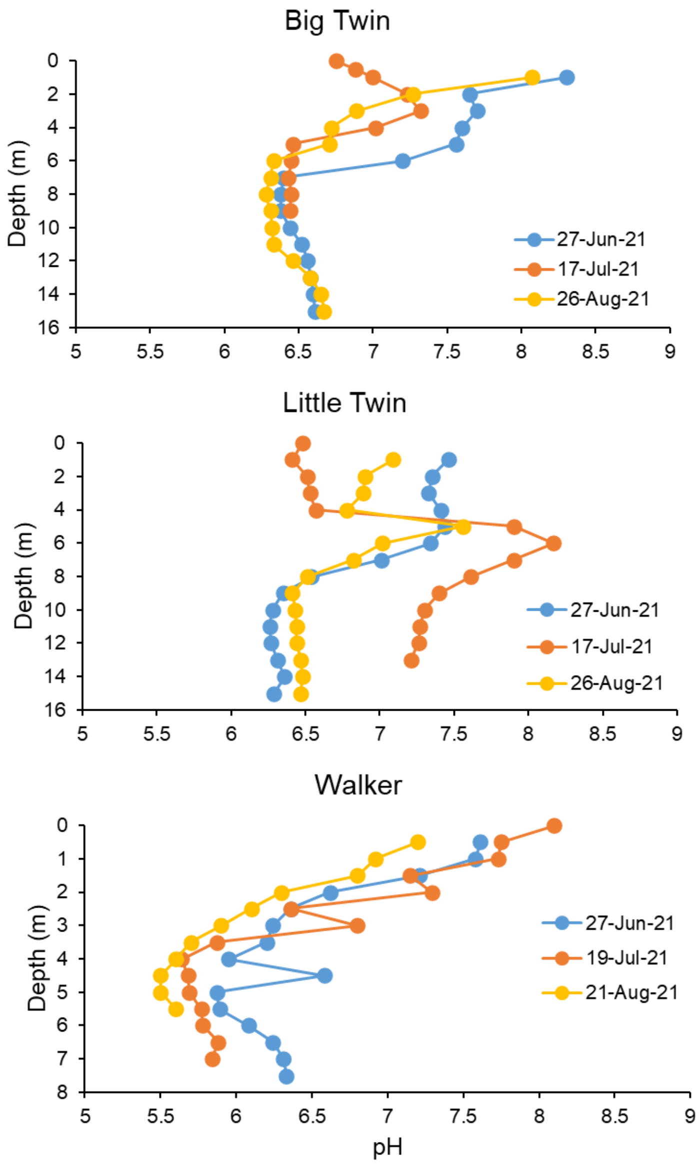


Figure 4: pH depth profiles in TWCWC lakes during the summer of 2022. Note different sampling dates and Y axis scales among panels.

Twin decreased through the epilimnion and metalimnion and stabilized in the deeper waters during the June and August sampling. During the July sampling, pH increased through the epilimnion before decreasing through the metalimnion and stabilizing in the hypolimnion.

pH in Little Twin ranged from 6.30-7.45 during the June sampling, from 6.41-8.17 during the July sampling, and from 6.41-7.56 during the August sampling (Figure 4). pH in Little Twin was generally fairly stable through the epilimnion, peaked and declined through the metalimnion, and stabilized through the hypolimnion.

pH in Walker ranged from 5.87-7.61 during the June sampling, from 5.64-8.10 during the July sampling, and from 5.50-7.20 during the August sampling (Figure 4). pH in Walker generally declined through the epilimnion and upper metalimnion and stabilized through the deeper waters.

pH is a measure of the acidity of water with a logarithmic scale ranging from 0 (very acidic) to 14 (very basic). Freshwater ecosystems are usually pH neutral, typically ranging from 6-9[1]. pH in the TWCWC lakes was generally within this range, although Walker tended to be more acidic (lowest pH was 5.5). Several factors affect water pH, including geology, precipitation, runoff, point-source inputs, and carbon dioxide. Carbon dioxide, a biproduct of decomposition, forms carbonic acid in water. Decomposition in the hypolimnion can contribute to the declining pH through depth in stratified lakes[2]. This was seen in some TWCWC profiles.

III. Water Transparency

A. Secchi depth

Secchi depth is a measure of water transparency and is defined as the depth at which an 8-inch diameter black and white disk lowered straight down into the water disappears from view. Lakes with clear water have deeper Secchi depths than those with more murky or dark water. Several factors influence water transparency such as the amount of suspended particles (including algae) and the amount and color of dissolved compounds. Secchi depth can be used to calculate Carlson's Trophic State Index (TSI) according to the following equation[3]:

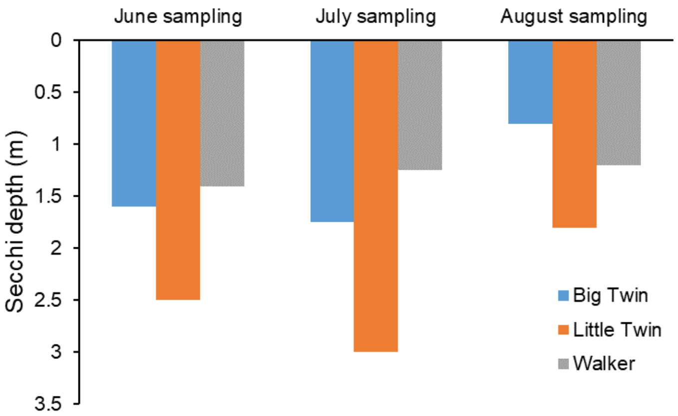


Figure 5: Secchi depth during the summer 2022 samplings of TWCWC lakes. Note orientation of Y axis.

$$TSI_{secchi} = 60 - 16.41 \times \ln(Secchi\ depth)$$

Secchi depth in Big Twin was 1.5 m, 1.75 m, and 0.8 m during the June, July, and August samplings, respectively (Figure 5). TSI_{Secchi} of Big Twin across these samplings was 53.2, 51.9, and 63.2, respectively, classifying Big Twin as eutrophic during all of the 2022 samplings.

Secchi depth in Little Twin was consistently the most clear TWCWC lake in 2022, with Secchi depths of 2.5 m, 3 m, and 1.8 m during the June, July, and August sampling, respectively (Figure 5). TSI_{Secchi} of Little Twin across these samplings was 46.3, 44.2, and 51.5, respectively, classifying Little Twin as mesotrophic during the June and July samplings and eutrophic on the August sampling.

Walker was the least clear TWCWC lake on all 2022 sampling dates except for August (Figure 5). Secchi depth in Walker was 1.4 m, 1.25 m, and 1.2 m during the June, July, and August sampling, respectively. TSI_{Secchi} of Walker across these samplings was 55.2, 56.8, and 57.4, respectively, classifying Walker as eutrophic (Table 5).

Table 5: Trophic classification description

TSI	**Secchi depth (m)**	**Chla (µg/L)**	**TP (µg/L)**	**Classification**	**Description**
<40	>4	0-2.6	0-12	Oligotrophic	Low primary production, clear, low nutrient concentration
40-50	2-4	2.6-7.3	12-24	Mesotrophic	Intermediate production, aquatic plants
50-70	0.5-2	7.3-56	24-96	Eutrophic	High productivity, low transparency, excess nutrients
70-100	<0.5	>56	96+	Hypereutrophic	Very high productivity, frequent blooms, excess nutrients

B. Light attenuation

Water transparency can be measured directly as light attenuation. Dissolved and particulate material affect the rate at which light intensity attenuates with depth. Light intensity declines exponentially with depth allowing for the calculation of a vertical extinction coefficient (k), or the rate of attenuation, and the depths at which there remains 10% and 1% of surface irradiance ($Z_{10\%}$ and $Z_{1\%}$, respectively). These parameters are commonly measured for the wavelengths of light used for photosynthesis (between 400-700 nm, or photosynthetically active radiation; PAR). Note that k and Z are inversely related: as attenuation rate increases, the depths at which 10% or 1% surface irradiation remains decrease.

Light profiles were measured in TWCWC lakes during the July 2022 sampling. Little Twin was the clearest of the TWCWC lakes at this time ($k = 0.88$, $Z_{10\%} = 2.62$, $Z_{1\%} = 5.23$), followed by Big Twin ($k = 0.97$, $Z_{10\%} = 2.38$, $Z_{1\%} = 4.76$), and Walker ($k = 1.66$, $Z_{10\%} = 1.38$, $Z_{1\%} = 2.77$).

IV. Chlorophyll Results

Chlorophyll *a* (chla) is a pigment found in algal cells and is used as a proxy for algal abundance and lake productivity. PLEON measured chla concentration in a sample collected from 0.5 m and in a composite sample (Appendix I).

Chla concentration at 0.5 m in Big Twin ranged from 2.35 µg/L to 9.30 µg/L over the 2022 samplings (Figure 6). The lowest epilimnetic chla concentration occurred in June with a slight increase in July. Epilimnetic chla concentration in August was over 3x that of the earlier months. The composite sample collected on 26 August had more chla than the surface sample in June but less in July and August, suggesting algae was concentrated in the epilimnion during the later months.

Chla concentration at 0.5 m in Little Twin ranged from 0.07 µg/L to 4.33 µg/L over the 2022 samplings (Figure 6). As in Big Twin, algal abundance in the epilimnion increased during the August sampling, with chla concentration more than 3x that of the earlier months. However, chla concentration in Little Twin was approximately 5 µg/L less than Big Twin during the August sampling. Chla concentration at 0.5 m was greater than that of the composite sample during the August sampling, suggesting a near-surface bloom.

Walker was the least productive of the TWCWC lakes during both the June and August 2022 sampling (Figure 6). Chla

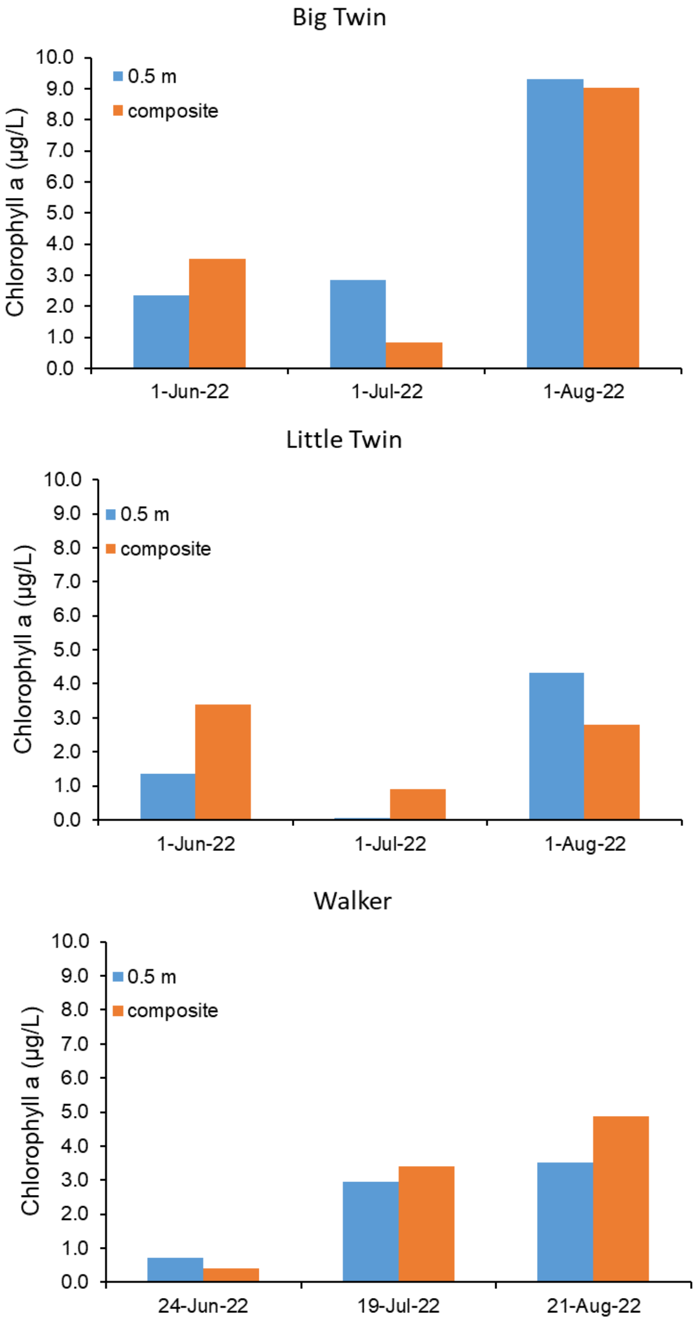


Figure 6: Chlorophyll concentration in TWCWC lakes during 2022. Bars represent single samples.

concentration at 0.5 m in Walker ranged from 0.72 µg/L to 3.53 µg/L in 2022 and increased over the course of the summer. The chla concentration at 0.5 m was less than that of the composite samples in July and August, suggesting more algal biomass in the mid-depths compared to the near-surface during these months.

TSI can be calculated from chlorophyll *a* concentrations measured at 0.5 m according to the following equation[3]:

$$TSI_{chlorophyll} = 30.6 + 9.81 \times \ln\left(\text{chlorophyll a}\,\frac{\mu g}{L}\right)$$

The $TSI_{chlorophyll}$ of Big Twin was 39.0, 40.8, and 52.5 during the June, July, and August sampling, respectively, classifying Big Twin as oligotrophic during June, mesotrophic during July, and eutrophic during August (Table 5).

The $TSI_{chlorophyll}$ of Little Twin was 33.6, 3.91, and 45.0 during the June, July, and August sampling, respectively, classifying Little Twin as oligotrophic during June and July and mesotrophic during August (Table 5).

The $TSI_{chlorophyll}$ of Walker was 27.3, 41.3, and 43.0 during the June, July, and August sampling, respectively, classifying Walker as oligotrophic during June and mesotrophic during July and August (Table 5).

V. Nutrient Results

A. Total nitrogen

Total nitrogen (TN) concentration in samples collected from 0.5 m in Big Twin ranged from 0.29 mg/L to 0.79 mg/L in the summer of 2022 (Figure 7). TN concentration during

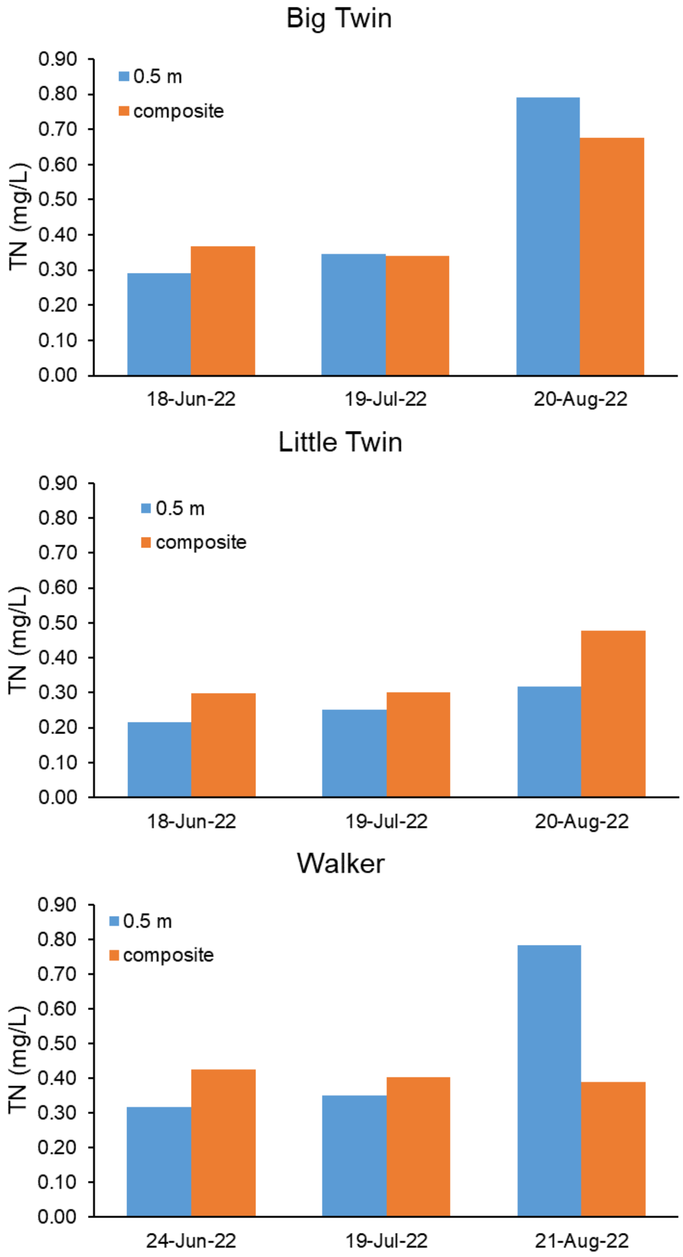


Figure 7: Total nitrogen concentration in TWCWC lakes during 2022. Bars represent single samples.

the August sampling was more than 2x that during June and July. Composite sample data suggest that TN was concentrated in the mid-depths during June but in the surface waters during August.

TN concentration in samples collected from 0.5 m in Little Twin ranged from 0.22 mg/L to 0.32 mg/L during 2022 with a slight increase from month to month (Figure 7). Composite samples had more TN that the surface samples, suggesting that TN was concentrated in the mid-depths.

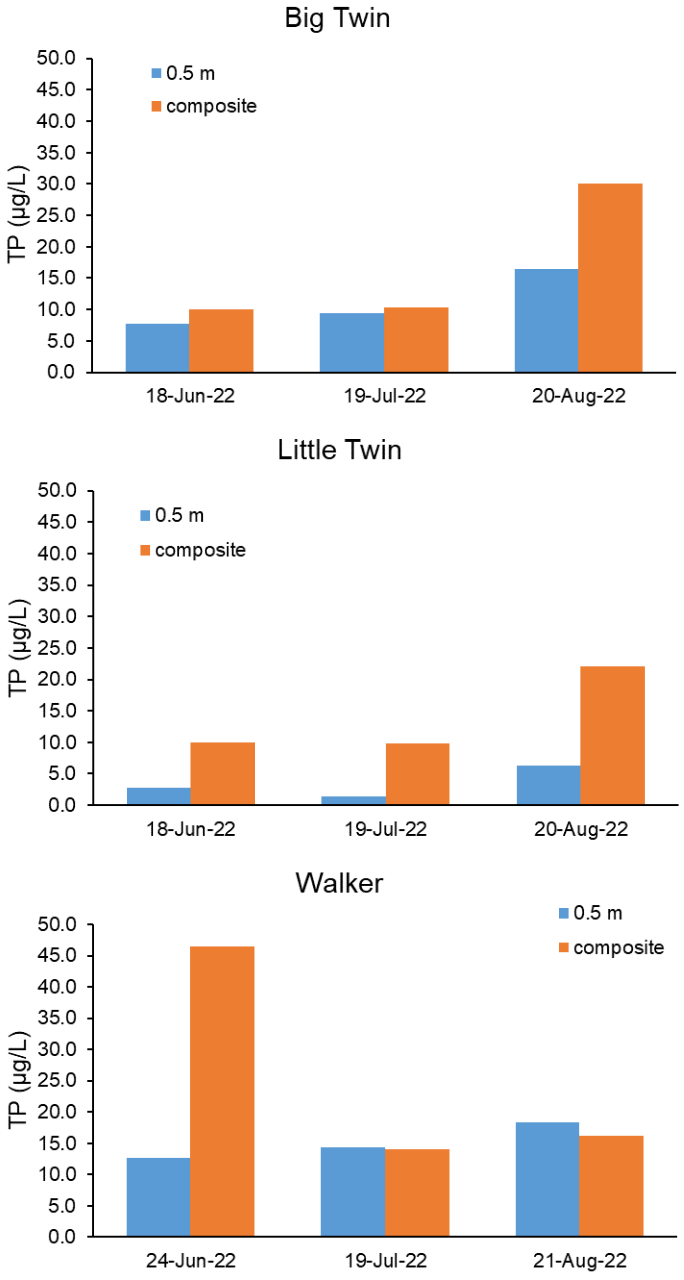


Figure 8: Total phosphorus concentration in TWCWC lakes during 2022. Bars represent single samples.

TN concentration in samples collected from 0.5 m in Walker ranged from 0.32 mg/L to 0.78 mg/L during the summer of 2022 (Figure 7). TN concentration during the August sampling was more than 2x that of the June and July samplings. Composite sample data suggest that TN was concentrated in the surface waters during the August sampling.

Nitrogen is an essential nutrient for algae and other aquatic life. Elevated concentrations of nitrogen can be a sign of eutrophication, or nutrient enrichment, of lakes. TN concentrations in TWCWC lakes were below the 3 mg/L threshold used by Penn State Extension to indicate nitrogen pollution[1].

B. Total phosphorus

Total phosphorus (TP) concentration in samples collected from 0.5 m in Big Twin ranged from 7.78 µg/L to 16.5 µg/L during the 2022 samplings (Figure 8). The greatest epilimnetic TP concentration occurred during the August sampling and was approximately 7 µg/L greater than that of the

July sampling. TP concentration in the August composite sample was approximately 3x greater than that collected in July.

TP concentration in samples collected from 0.5 m in Little Twin ranged from 1.39 µg/L to 6.24 µg/L across all 2022 samplings (Figure 8). As in Big Twin, the greatest epilimnetic TP concentration occurred during the August sampling. TP concentration in the composite samples was also greatest in August and composite samples contained more TP than the surface samples in all months.

Walker was generally the most phosphorus-rich TWCWC lake (Figure 8). TP concentration in samples from 0.5 m in Walker ranged from 12.7 µg/L to 18.4 µg/L during the 2022 samplings and gradually increased from month to month. TP concentration in the composite sample was quite high during the June sampling (46.5 µg/L) but were similar to surface samples during the other months.

Like nitrogen, phosphorus is an essential nutrient for aquatic life and is often considered to be the primary nutrient limiting algal growth in lakes. Elevated concentrations of phosphorus can be a sign of eutrophication in lakes and can fuel algal blooms. Epilimnetic TP concentrations were below the 25 µg/L threshold for nutrient pollution suggested by Penn State Extension[1] during all 2022 samplings. However, TP concentration in composite samples in Big Twin and Walker occasionally exceeded this threshold.

Algae uptake of phosphorus can influence TP concentrations, particularly in the surface and metalimnetic waters. Phosphorus is also liberated from sediments under anoxic conditions, which can increase TP concentration in deep waters. This likely explains instances of TP concentration in composite samples exceeding that of surface samples in TWCWC lakes. Lake mixing and precipitation can affect nutrient profiles.

TSI can be calculated from TP concentration at 0.5 m as[3]:

$$TSI_{TP} = 4.15 + 14.42 \times \ln\left(\mathrm{TP}\frac{\mathrm{\mu g}}{\mathrm{L}}\right)$$

TSI_{TP} of Big Twin was 33.3, 36.0, and 44.0 during the June, July, and August samplings, respectively. TSI_{TP} classified Big Twin as oligotrophic during June and July and as mesotrophic during August (Table 5).

TSI_{TP} of Little Twin was 18.8, 8.76, and 30.1 during the June, July, and August sampling, respectively. TSI_{TP} classified Little Twin as oligotrophic during all three months (Table 5).

TSI_{TP} of Walker was 40.3, 41.9, and 45.5 during the June, July, and August sampling respectively. TSI_{TP} classified Walker as mesotrophic during all three months (Table 5).

C. Dissolved organic carbon

Walker generally had more dissolved organic carbon (DOC) than the Twin Lakes (Figure 9). DOC concentration in surface waters increased over the summer in the Twin lakes. DOC concentration from 0.5 m depth ranged from 3.97 mg/L to 5.36 mg/L in Big Twin, from 2.93 mg/L to 3.45 mg/L in Little Twin, and from 3.14 mg/L to 4.94 mg/L in Walker during the summer of 2022. DOC concentration in Walker was greatest during the August sampling but least during the July sampling. There was slightly more DOC in surface samples compared to composite samples in the Twin Lakes while this varied in Walker.

DOC in lakes includes soluble organic compounds from runoff, biproducts of decomposition, and molecules synthesized within the water column[4]. DOC concentration is affected by the frequency and intensity of precipitation as well as watershed soil chemistry and structure.

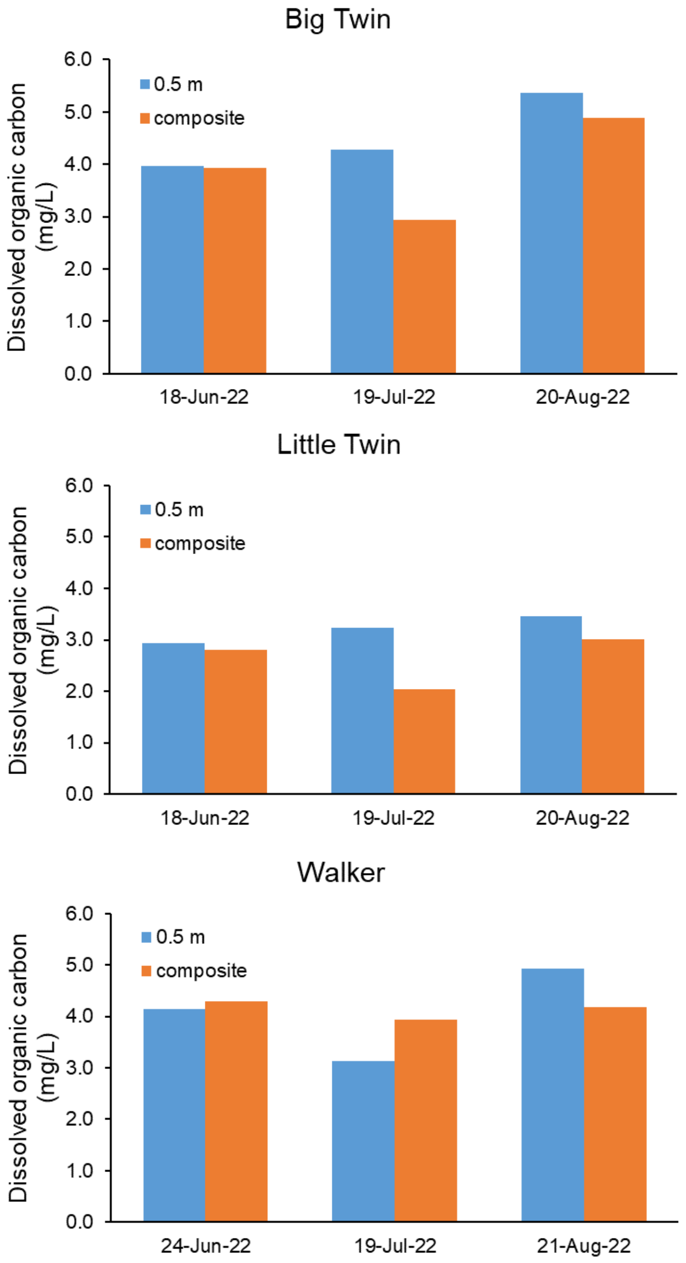


Figure 9: Dissolved organic carbon concentration in TWCWC lakes during 2022. Bars represent single samples.

VI. Plankton Communities

A. Zooplankton

Zooplankton are microscopic animals and key components of lake food webs. Zooplankton samples were collected from Big and Little Twin on 19 July 2022. Walker was not sampled for zooplankton.

Zooplankton numbers in both lakes was dominated by rotifers, which made up 80% and 75% of zooplankton density in Big and Little Twin, respectively (Table 6). Rotifers eat detritus, bacteria, algae, and protozoans. Rotifers are small in size and made up 16% and 17% of zooplankton biomass in these lakes, respectively.

Cladocerans made up 10% and 15% of zooplankton density in Big Twin and Little Twin, respectively. Copepods were similarly abundant (10% and 15%, respectively). These groups are larger organisms (together making up 84% and 83% of biomass in these lakes, respectively) that eat algae and are important food for fish.

Community richness is the number of taxa present while diversity accounts for both the number and distribution of individuals among taxa. Average zooplankton richness in Big Twin and Little Twin was 10.5 and 9, respectively and average diversity (Shannon-Wiener Index) was 0.77 and 0.63, respectively.

Table 6: Zooplankton community in the Twin Lakes on 19 July 2022 (averages of 2 samples).

	BIG TWIN				**LITTLE TWIN**			
	Density (#/L)	**(%)**	**Biomass (µg/L)**	**(%)**	**Density (#/L)**	**(%)**	**Biomass (µg/L)**	**(%)**
PROTOZOA	**0**	**0%**	**0**	**0%**	**0**	**0%**	**0**	**0%**
ROTIFERA	**373**	**80%**	**30**	**16%**	**134**	**75%**	**18**	**17%**
Ascomorpha	14		1		0		0	
Asplanchna	0		0		3		7	
Conochilus	70		3		8		0	
Keratella	178		16		104		9	
Polyarthra	104		9		19		2	
Trichocerca	5		0		0		0	
COPEPODA	**47**	**10%**	**93**	**49%**	**18**	**10%**	**43**	**41%**
Copepoda-Cyclopoida								
Cyclops	10		24		2		6	
Mesocyclops	21		26		2		3	
Copepoda-Calanoida								
Nauplii	16		43		13		34	
CLADOCERA	**49**	**10%**	**65**	**35%**	**26**	**15%**	**44**	**42%**
Bosmina	37		36		14		14	
Ceriodaphnia	11		28		12		30	
Diaphanosoma	1		1		0		0	
OTHER	**0**	**0%**	**0**	**0%**	**0**	**0%**	**0**	**0%**
TOTAL	**468**		**188**		**177**		**105**	

B. Phytoplankton

Phytoplankton, or algae, are the base of planktonic food webs and help regulate oxygen dynamics in lakes. Phytoplankton were sampled from the Twin lakes on 19 July 2021.

Cyanophyta (cyanobacteria) were the numerically dominant group in both Twin Lakes, making up 97% and 99% of phytoplankton density in Big Twin and Little Twin, respectively. Other groups of algae were in low abundance (Table 7).

The Cyanophyta community in the Twin Lakes was composed of filamentous forms. In Big Twin, *Dolichospermum* was the only genus identified. The Little Twin community consisted of *Aphanizomenon*, *Dolichospermum*, *Limnothrix*, and *Planktothrix*. These genera are capable of producing toxins that can be harmful to humans and pets.

Average phytoplankton taxonomic richness in Big Twin was 11 and average diversity (measured using the Shannon-Wiener Index) was 0.08. Planktonic richness in Little Twin was 11 and diversity was 0.26.

Table 7: Phytoplankton community in the Twin Lakes on 19 July 2022 (averages of 2 samples).

	Big Twin				Little Twin			
	Density (cells/ml)	**(%)**	**Biomass (µg/ml)**	**(%)**	**Density (cells/ml)**	**(%)**	**Biomass (µg/ml)**	**(%)**
BACILLARIOPHYTA	**249**	**2%**	**205**	**5%**	**195**	**<1%**	**162**	**3%**
Centric Diatoms	44		53		21		25	
Araphid Pennate Diatoms	205		153		174		136	
CHLOROPHYTA	**212**	**1%**	**473**	**11%**	**21**	**<1%**	**84**	**1%**
Flagellated Chlorophytes	0		0		0		0	
Coccoid/Colonial Chlorophytes	50		20		0		0	
Desmids	163		453		21		84	
CHRYSOPHYTA	**5**	**<0.1%**	**14**	**<1%**	**121**	**<1%**	**364**	**6%**
Flagellated Classic Chrysophytes	5		14		121		364	
CRYPTOPHYTA	**17**	**<1%**	**27**	**<1%**	**0**	**0%**	**0**	**0%**
CYANOPHYTA	**15997**	**97%**	**3199**	**71%**	**29,101**	**99%**	**5,017**	**86%**
Filamentous Nitrogen Fixers	15997		3199		24,989		4,976	
Filamentous Non-Nitrogen Fixers	0		0		4,112		41	
EUGLENOPHYTA	**6**	**<0.1%**	**2**	**<0.1%**	**0**	**0%**	**0**	**0%**
PYRRHOPHYTA	**16**	**<1%**	**575**	**13%**	**26**	**<1%**	**217**	**4%**
TOTAL	**16502**		**4497**		**29,465**		**5,843**	

VII. PTOX Cyanobacteria Screen

Cyanobacteria (sometimes called blue-green algae) are a common group of photosynthetic bacteria often classified as algae. Some cyanobacteria are capable of producing toxins that can be harmful to wildlife, pets, and humans. Cyanobacteria are the algae most commonly responsible for harmful algal blooms, or HABs, in freshwater ecosystems. Potentially toxigenic (PTOX) cyanobacteria genera can be identified using a microscope.

PLEON collected a sample from the shores of Big Twin Lake and from the swampy inlet to Big Twin Lake on 26 May 2022. Samples were collected from wrist depth and were shipped to Greenwater Laboratories for microscopic analysis (Appendix I).

PTOX cyanobacteria were not observed in either sample (but see Section VI for description of phytoplankton community later in the summer; PTOX cyanobacteria were dominant at this time).

VIII. Historical Context: Twin and Walker Lakes Over Time

A. Description of historical dataset

PLEON began monitoring the Twin and Walker lakes in 2019. Data from 2014-2018 were provided by the TWCWC in the form of yearly "state of the lake" reports by FX Browne.

B. Chemical profiles over time

Chemical profiles in Big Twin and Little Twin are incomplete for much of the dataset as the TWCWC probe did not extend to the bottom of these lakes until 2021. Prior to 2021, complete depth profiles exist for these lakes in July of 2019 and 2020. All Walker Lake profiles are complete. Appendix II shows July data since 2014 as examples of typical summer profiles. The descriptions in this section include all summer profiles from 2014-2022.

TWCWC lakes were generally stratified in the summer months (June, July, August) from 2014-2022. Surface temperature in Big Twin and Little Twin, while variable, significantly increased over this time period (linear regression, $p \leq 0.04$, $r^2 \geq 0.47$; Figure 10). Surface temperature in Walker Lake was more variable over this time period with no statistically significant increase.

The TWCWC lakes were generally deplete of oxygen in the hypolimnion during the summer months. Note that data for the

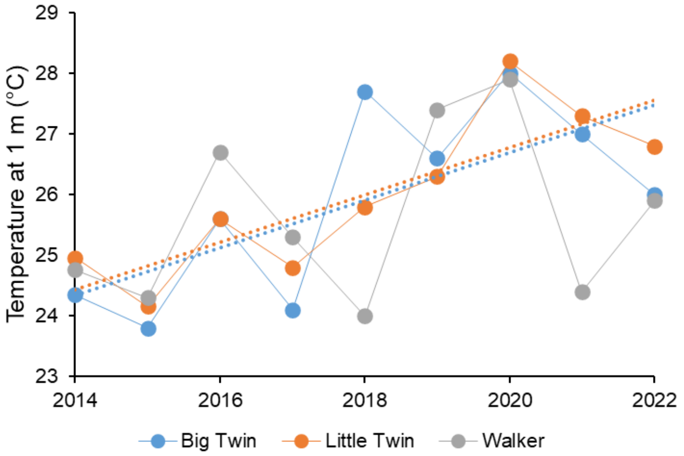


Figure 10: Average water temperature at 1 m during July in TWCWC lakes since 2014. Dotted lines show significant linear increase in temperature in the Twin lakes over time.

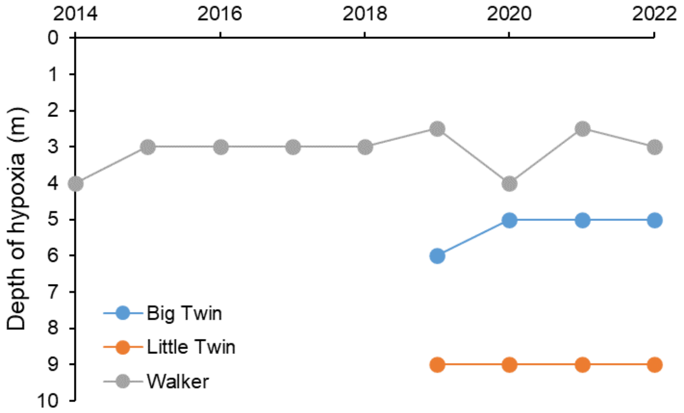


Figure 11: Depth of oxygen depletion (<2 mg/L) during July profiles in TWCWC lakes over time. Note orientation of Y axis.

Twin Lakes does not include hypolimnetic oxygen until 2019. Since 2019, the depth at which DO concentrations was less than 2 mg/L (the threshold for oxygen depletion) was deepest in Little Twin and most shallow in Walker (Figure 11). Metalimnetic oxygen maxima were common in Little Twin during the summer months and occurred occasionally in Big Twin as well.

Conductivity in Little Twin was consistently greater than that of Big Twin and Walker across the dataset, with an average conductivity of 150.2 µS/cm compared to 85.7 µS/cm and 87.3 µS/cm in the other lakes, respectively (averages include all depths in June, July, and August of all years).

pH in Walker Lake was generally lower than that of Big Twin and Little Twin across the dataset, with an average pH of 6.69 compared to 7.04 and 7.28 in the other lakes, respectively (averages include all depths in June, July, and August of all years). However, this may be due to missing hypolimnetic data in the Twin lakes; pH tended to be lower in the deep water.

C. Water transparency over time

Secchi depth in the TWCWC lakes has been measured since 2003, allowing for a robust temporal analysis of water clarity in these lakes. Little Twin was the clearest lake with an average summer Secchi depth of 3.6 m, followed by Big Twin with an average summer Secchi depth of 2.6 m, and Walker with an average Secchi depth of 1.7 m (averages include all readings in June, July, and August from 2003-2022; Figure 12). Secchi depth in Walker decreased over the 9-year dataset (linear regression, $p < 0.01$, $r^2 = 0.61$). Secchi depth in Big Twin and Little Twin was the most shallow in 2022.

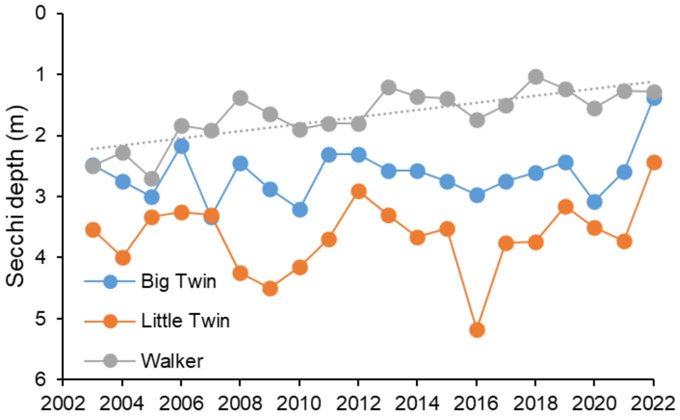


Figure 12: Average summer (June, July, August) Secchi depth in TWCWC lakes since 2003. Note orientation of the Y axis. Dotted line shows statistically significant decline in Secchi depth in Walker over time.

Light attenuation parameters have been measured in TWCWC lakes in July since 2019. Over this time, Little Twin was the most transparent (*k* ranged from 0.4-1.0), followed by Big Twin (*k* ranged from 0.8-1.0) and Walker (*k* ranged from 1.4-2.0; Figure 13). Transparency in Little Twin decreased from 2020 to 2022.

D. Chlorophyll a over time

Chla concentration has been measured in TWCWC lakes since 2014. Over this time, average summer (June, July, August) chla concentration at 0.5 m has ranged from 1.82 µg/L to 10.2 µg/L in Big Twin, from 0.71 µg/L to 5.5 µg/L in Little Twin, and from 4.19

µg/L to 11.6 µg/L in Walker (Figure 14). Chla concentrations were on the low end of those ranges from 2019 to 2021 in the Twin lakes. However, chla concentration has been increasing since 2019 in Little Twin and Walker and chla concentration in Big Twin increased dramatically in 2022 compared to recent years.

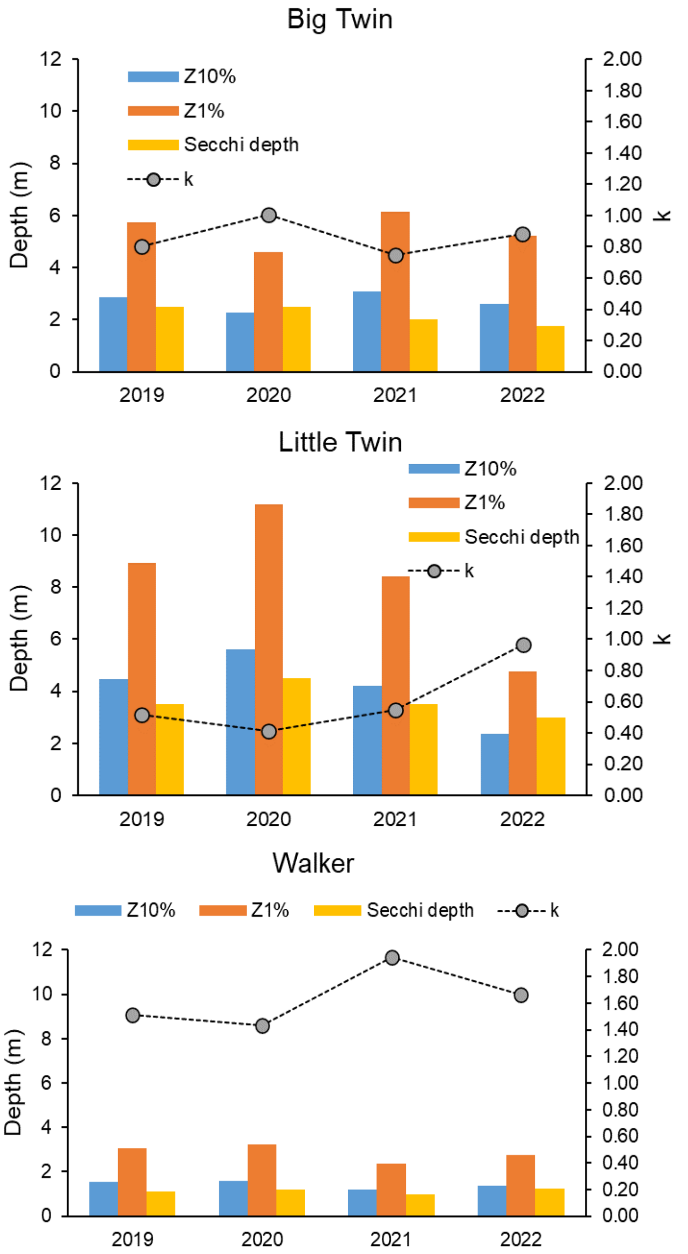


Figure 13: Water transparency during July sampling in TWCWC lakes from 2019-22. Note orientation of Y axes.

E. Nutrients over time

TN concentration has been measured in TWCWC lakes since 2019. Average summer (June, July, August) TN concentration measured in samples collected from 0.5 m ranged from 0.22 mg/L to 0.49 mg/L in Big Twin, from 0.17 mg/L to 0.94 mg/L in Little Twin, and from 0.37 mg/L to 0.78 mg/L in Walker (Figure 14). The greatest TN concentration occurred in 2020 in all three lakes. TN concentration declined in 2021 and 2022 in Little Twin and Walker but was more variable in Big Twin.

TP concentration has been measured in TWCWC lakes since 2014. Average summer (June, July, August) TP measured in samples collected from 0.5 m ranged from 3.54 µg/L to 25.0 µg/L in Big Twin, from 3.5 µg/L to 29.0 µg/L in Little Twin, and from 11.2 µg/L to 28.0 µg/L in Walker (Figure 14). Summer TP concentration generally declined over the 8-year period in all lakes. This decline is statistically significant in Big Twin (linear regression, $r^2 = 0.54$, $p = 0.02$) but not Little Twin or Walker.

DOC concentration was quantified in August of 2020 and in June, July, and August 2021 and 2022 in all three lakes. Over this time, DOC concentration in Big Twin has increased and DOC concentration in Walker has decreased (Figure 14). However, three data points is not enough to determine if these are persistent trends.

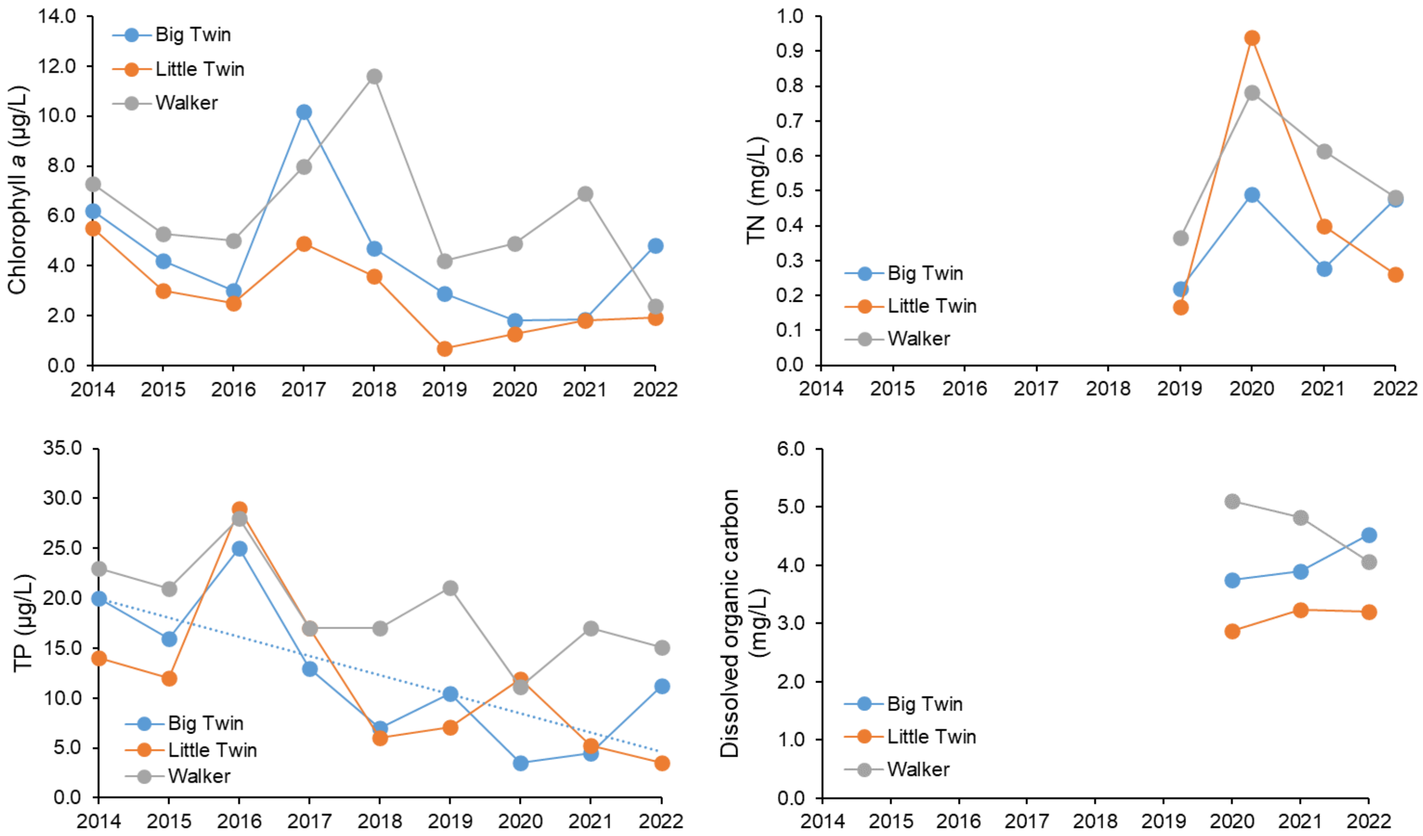


Figure 14: Chlorophyll, total nitrogen, total phosphorus, and dissolved organic carbon concentration in TWCWC lakes from 2014-2022. Dashed line shows linear decline in TP concentration over time in Big Twin Lake.

F. Trophic status over time

Big Twin and Walker were generally mesotrophic since 2014 and Little Twin was generally oligo-mesotrophic (Figure 15). $\text{TSI}_{\text{Secchi}}$ was typically greater than $\text{TSI}_{\text{chlorophyll}}$ and TSI_{TP} in all three lakes, particularly since 2018 when $\text{TSI}_{\text{chlorophyll}}$ and TSI_{TP} began declining. However, these metrics increased in the Twin lakes in recent years.

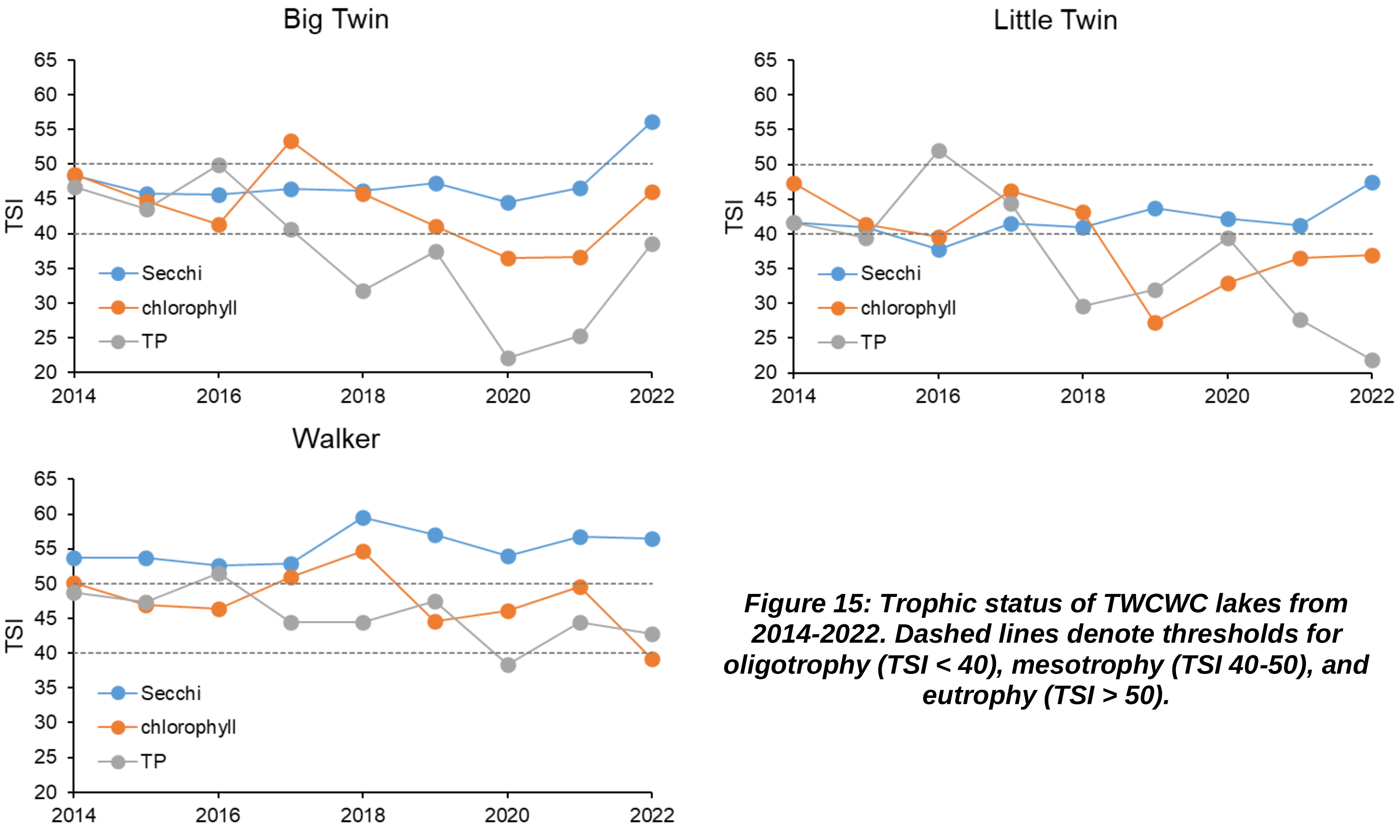


Figure 15: Trophic status of TWCWC lakes from 2014-2022. Dashed lines denote thresholds for oligotrophy (TSI < 40), mesotrophy (TSI 40-50), and eutrophy (TSI > 50).

G. Zooplankton over time

PLEON has quantified plankton abundance and biomass in Big Twin and Little Twin since 2019 (Appendix I).

Zooplankton density ranged from 116 to 540 individuals/L in Big Twin and from 128 to 341 individuals/L in Little Twin across the 4-year PLEON dataset (Figure 16). Zooplankton were generally less abundant in Little Twin compared to Big Twin. Zooplankton communities in both lakes were dominated by rotifers across all years.

Average zooplankton richness ranged from 10.5-12 in Big Twin and from 9-16.5 in Little Twin over the 4-year dataset (Figure 16). Zooplankton diversity ranged from 0.72-0.84 in Big Twin and from 0.72-0.95 in Little Twin. Average zooplankton length ranged from 0.16 mm to 0.19 mm in Big Twin and from 0.15 mm to 0.27 mm in Little Twin.

H. Phytoplankton over time

Phytoplankton density increased dramatically in 2022 compared to previous years in both Twin lakes (by 4.5x in Big Twin Lake and by 11x in Little Twin Lake; Figure 17). This increase was driven by an increase in the abundance of Cyanophyta, or cyanobacteria.

Phytoplankton diversity in both Twin lakes declined from 2020 to 2022 (Figure 17).

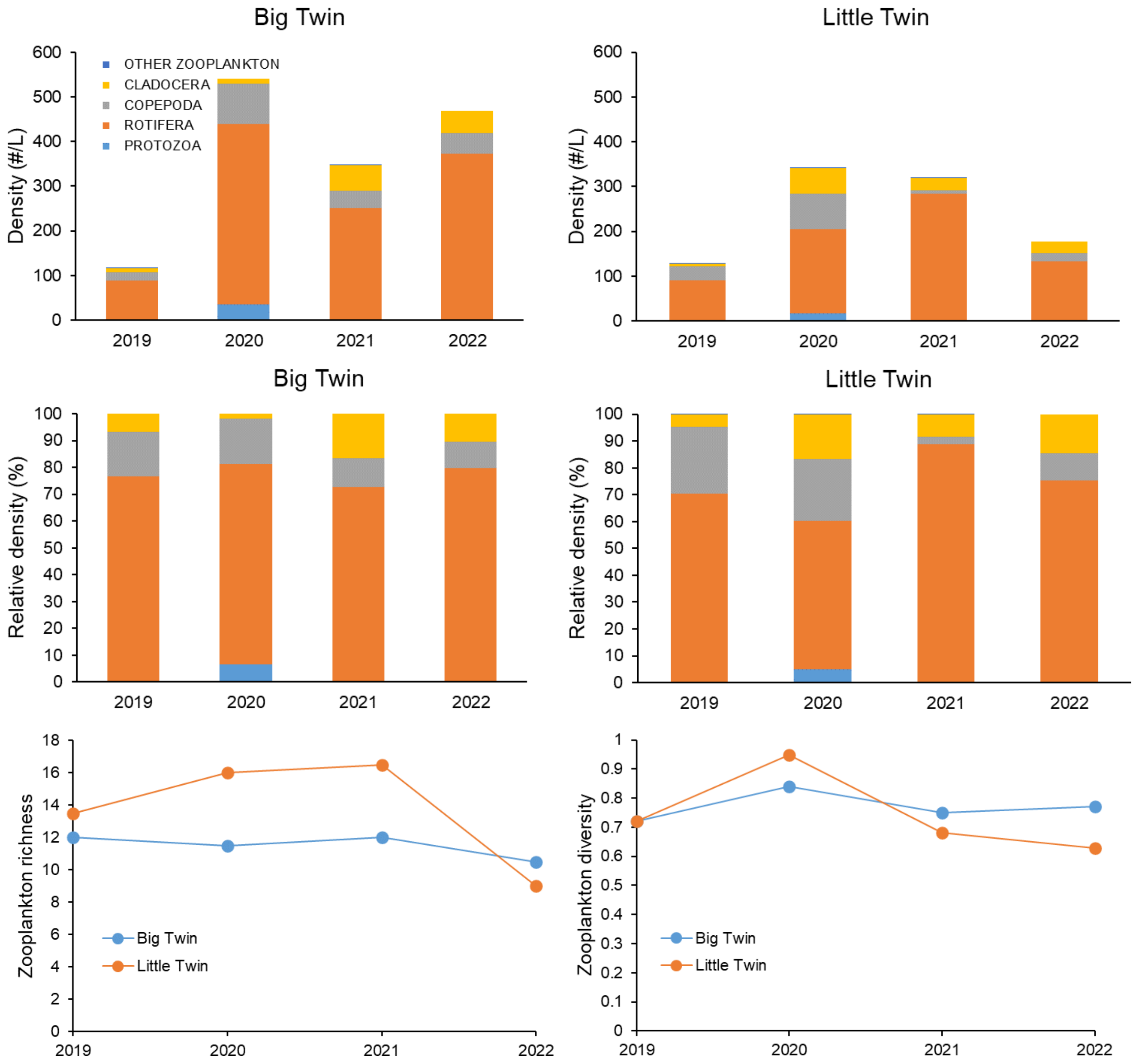


Figure 16: Zooplankton communities in the Twin Lakes from 2019-2022. Samples were collected in July of each year. Bars and symbols are averages of two replicates.

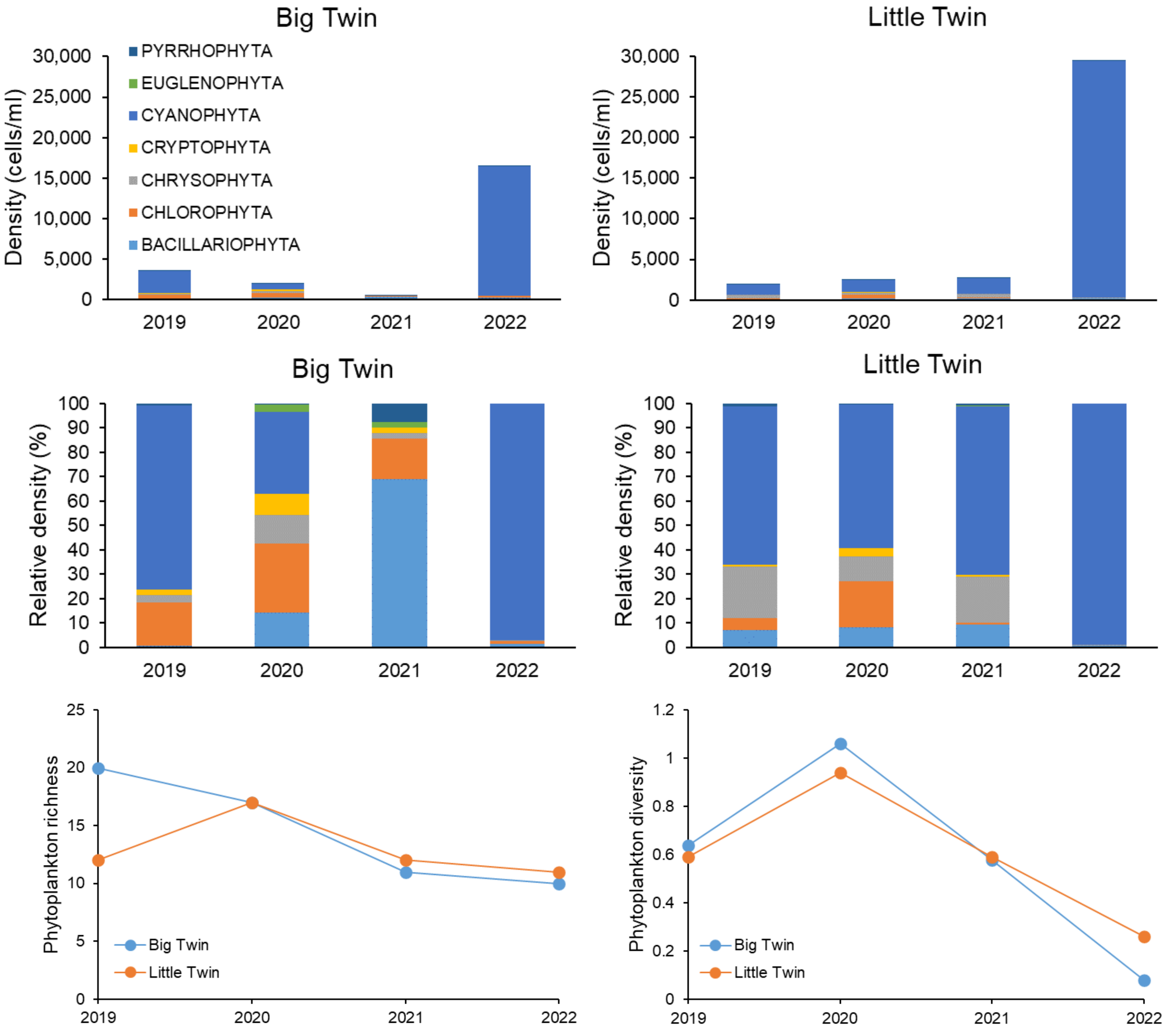


Figure 17: Phytoplankton communities in the Twin Lakes from 2019-2022. Samples were collected in July during each year. Bars and symbols are averages of two replicates.

I. Cyanobacteria and cyanotoxins over time

Samples from all three lakes were screened for potentially toxigenic (PTOX) cyanobacteria in 2019 and 2020, as part of the Pocono Lakes HABs Survey (Lauren Knose, Miami University), and by the PA Harmful Algae Bloom Task Force monitoring program, respectively. Single samples from Big Twin Lake were screened through

PLEON in 2021 and 2022. All PTOX screens and cyanotoxin analysis (2020 and 2021 only) were conducted by Greenwater Laboratories. As previously described, in-depth phytoplankton community analysis has been conducted on samples collected from the Twin Lakes since 2019.

Cyanobacteria communities have been variable over the three-year dataset in all three TWCWC lakes, as reflected in the comprehensive community analyses (Twin lakes only) and the PTOX screens.

Cyanobacteria abundance in Big Twin were variable over the 4-year dataset, making up 0%-97% of the phytoplankton communities depending on the year (Figure 17). When present, 100% of the cyanobacteria communities in Big Twin were composed of genera thought to be capable of producing cyanotoxins (Figure 18). Two visible cyanobacteria blooms have been sampled along the shores of Big Twin Lake in the past 4 years, both containing *Dolichospermum* (Table 8). Note that the bloom sampled in 2021 occurred in June along the shoreline while the analysis of the phytoplankton community collected at center lake in July contained no cyanobacteria.

Cyanobacteria made up 59-99% of the phytoplankton communities in Little Twin over the 4-year dataset (Figure 17). Cyanobacteria communities were composed entirely of potentially toxigenic taxa each year, with the exception of 2022 when a large proportion of the community was composed of *Spirulina* (Figure 18). PLEON (and collaborations) has not observed visible cyanobacteria blooms in Little Twin, but PTOX screens (Table 8) and detailed analyses of phytoplankton communities suggest blooms are possible.

PLEON (and collaborations) has not observed visible cyanobacteria blooms in Walker, but PTOX screens have contained potentially toxigenic taxa in 2019 and 2020 (Table 8).

Table 8: Results of PTOX screens of TWCWC lakes.

		Program	**Location**	**Observations**	**PTOX genera**	**Toxins***
Big Twin	17 Jul 2019	*Knose Survey*	*Center (0.5 m)*	*No bloom visible*	*Dolichospermum*	Testing not recommended
	24 Aug 2020	*PA HABs Task Force*	*Deiner dock (wrist)*	*Visible bloom along the shoreline day of collection*	*Dolichospermum*	<MDL
	2 Jun 2021	*PLEON*	*East dock (wrist)*	*Shoreline bloom visible days before.*	*Dolichospermum*	Testing declined
	26 May 2022	*PLEON*	*Shoreline (wrist)*	*Appearance of filamentous algae*	*None found*	—
Little Twin	24 July 2019	*Knose Survey*	*Center (0.5 m), Dock (wrist)*	*No bloom visible*	*Dolichospermum*	Testing not recommended
	24 Aug 2020	*PA HABs Task Force*	*Dock (wrist)*	*No bloom visible*	*Dolichospermum* *Aphanizomenon/Chrysosporum*	<MDL
Walker	15 July 2019	*Knose Survey*	*Center (0.5 m) Dock (wrist)*	*No bloom visible*	*Aphanizomenon*	Testing not recommended
	24 Aug 2020	*PA HABs Survey*	*Dock (wrist)*	*No bloom visible*	*Chrysosporum*	<MDL

**MDL=minimum detection limit*

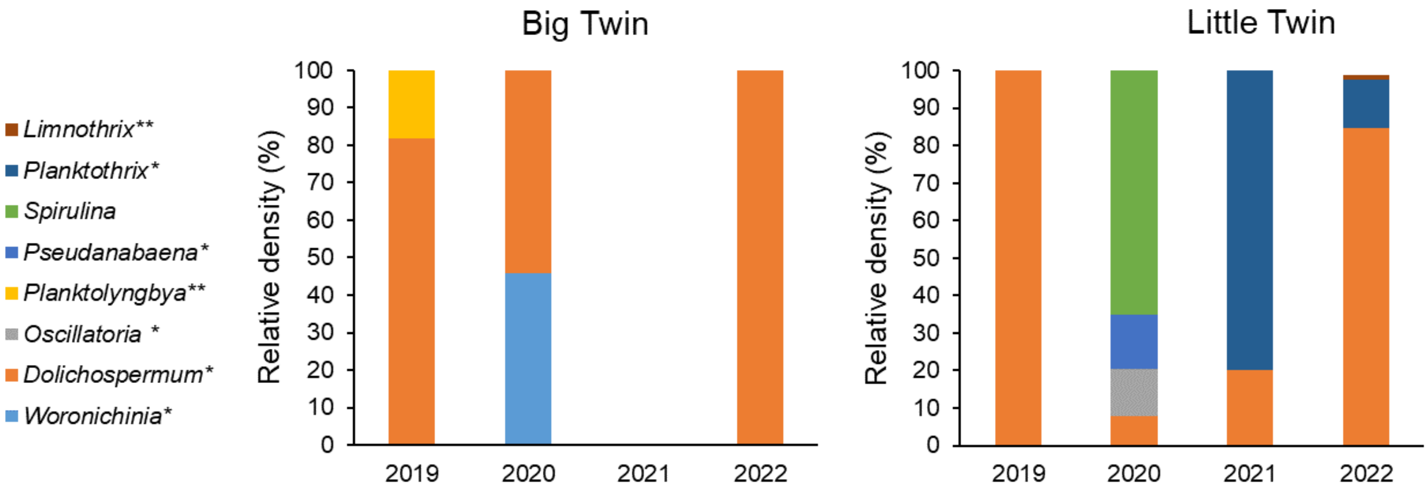


Figure 18: Relative abundance of cyanobacteria genera found in detailed phytoplankton community analysis in 2019-2022 as a percentage of the total cyanobacteria density. Bars are averages of replicate samples. No cyanobacteria were found in 2020 samples collected from Big Twin.

IX. Twin and Walker Lakes in the Context of the Poconos

A. Description of PLEON Lakes

The PLEON dataset consists of 21 lakes in Pike, Wayne, and Monroe Counties. Lakes range from ~29,000-1,130,000 m^2 (mean of ~363,000 m^2) in surface area, ~900-7,800 m (mean of ~3,140 m) in shoreline and ~2-23 m (mean of ~8 m) in depth (Figure 19). Big Twin has the largest surface area of PLEON lakes and is above the average shoreline and maximum depth. Little Twin has below average surface area and shoreline but is deeper than the average maximum depth. Walker is slightly above average in surface area and shoreline and is shallower than the PLEON average.

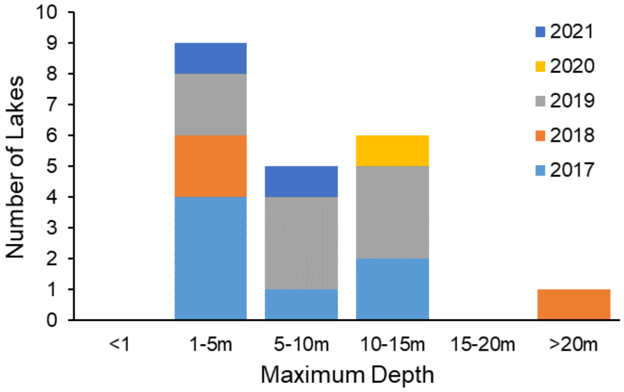


Figure 19: Maximum depth of PLEON lakes. Years refer to the first PLEON sampling year. Not all lakes are sampled every year.

B. Water transparency

The Secchi depth of the 15 PLEON lakes monitored in the summer of 2022 ranged from 0.8 m to 5 m with an average of 2.1 m (Figure 20). The average summer Secchi depth of Big Twin and Walker were more shallow than the PLEON average while the summer Secchi depth in Little Twin was deeper than the PLEON average.

C. Lake productivity

Lake productivity, as measured by chla concentration at 0.5 m depth, was assessed in 15 PLEON lakes during the summer months (June, July, August) in 2022. Chla concentration in these lakes ranged from 0.09 µg/L to 16.07 µg/L with an average of 3.97 µg/L (Figure 20). Average summer chla concentration in Big Twin was above the PLEON average while summer chla concentration in Little Twin and Walker were below the PLEON average.

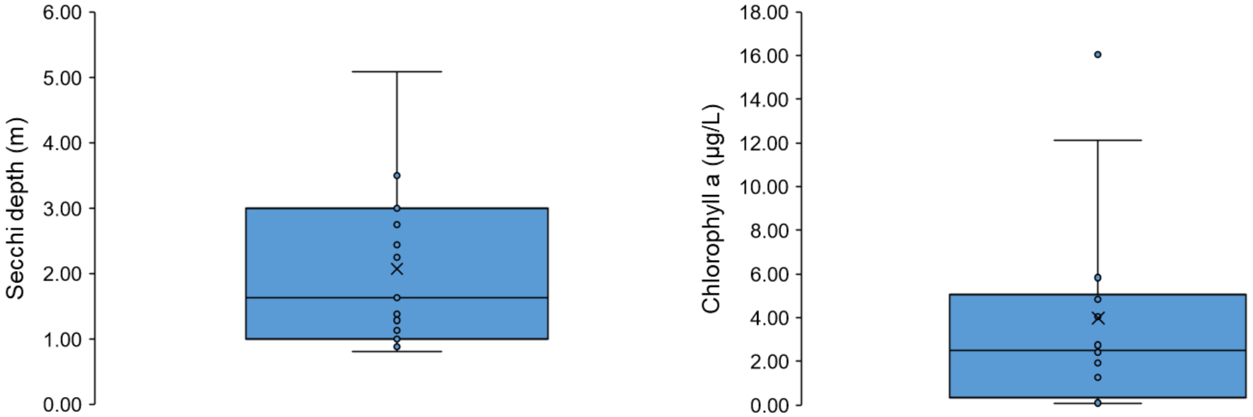


Figure 20: Average summer (June, July, August) Secchi depth (left) and chlorophyll a concentration at 0.5 m (right) across 15 PLEON lakes monitored in 2022. Lines within boxes are medians and X symbols are means. Upper and lower box boundaries denote the 75th and 25th percentile, respectively while upper and lower whiskers are the maximum and minimum values, respectively. Circles represent a single measurement from a lake or an average if the lake was sampled more than once during the summer.

D. Nutrient concentration

Total nitrogen (TN) and total phosphorus (TP) concentration was quantified at 0.5 m depth in 15 PLEON lakes during the summer months (June, July, August) of 2022. Average summer TN concentration ranged from 0.02 mg/L to 0.48 mg/L in these lakes, with an average concentration of 0.26 mg/L (Figure 21). TN concentration in Big Twin and Walker were greater than the PLEON average while TN concentration in Little Twin was equal to the PLEON average.

Average summer TP concentration ranged from 1.81 µg/L to 37.1 µg/L across PLEON lakes in 2022, with an average of 14.2 µg/L (Figure 21). TP concentration in the Twin Lakes was less than the PLEON average and greater than the PLEON average in Walker.

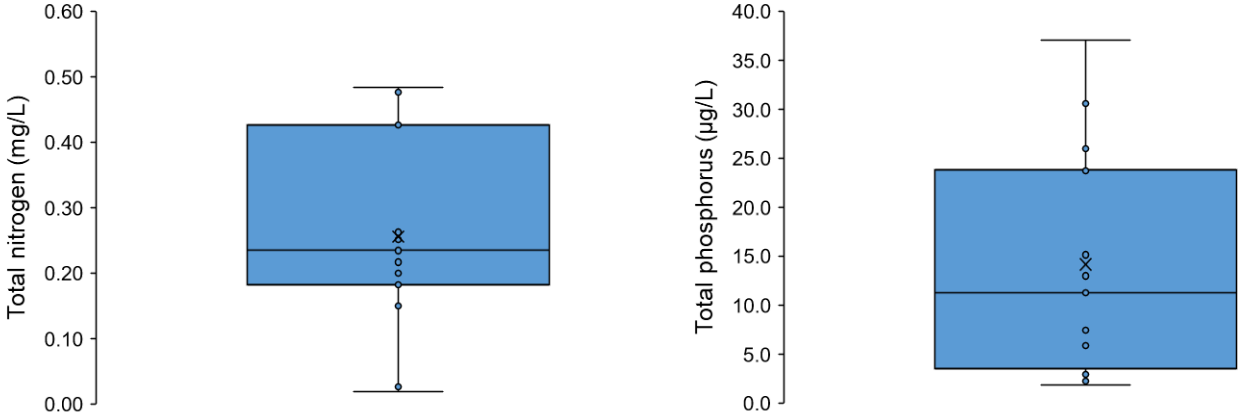


Figure 21: Average summer (June, July, August) total nitrogen concentration (left), and total phosphorus concentration (right) across 15 PLEON lakes monitored in 2022. Lines within boxes are medians and X symbols are means. Upper and lower box boundaries denote the 75th and 25th percentile, respectively while upper and lower whiskers are the maximum and minimum values, respectively. Circles represent a single measurement from a lake or an average if the lake was sampled more than once during the summer. Nutrient concentrations were quantified from 0.5 m depth.

E. PTOX Cyanobacteria

Since 2017, PLEON has collected 207 samples for PTOX screening as a part of its formal monitoring program. These samples were collected from 19 lakes during months ranging from May through September. This count includes samples collected from different locations within the same lake on the same day. Samples include collections from 0.5 m, surface grabs, and composite samples and include pelagic, shore and near-shore environments. All samples were screened by Greenwater Laboratories.

Ten (possibly 11, some specimens are difficult to identify) PTOX cyanobacteria genera have been found in PLEON samples to date (Figure 22). The most commonly found genera are *Dolichospermum*, followed by *Aphanizomenon* (or *Aphanizomenon*-like). *Chrysosporum*, *Woronichinia*, and *Microcystis* were also common. 46 of the samples (or 22%) did not have PTOX taxa present. Two lakes within the dataset have been consistently free of PTOX taxa, but note

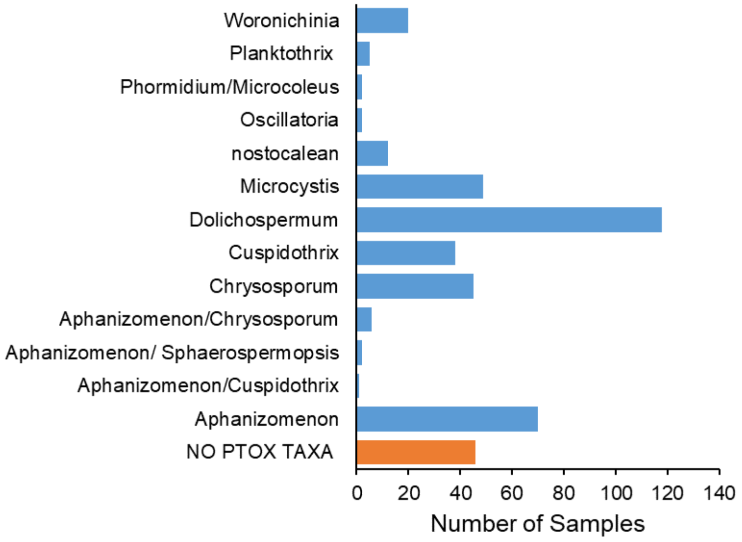


Figure 22: Potentially toxic (PTOX) cyanobacteria genera found in samples collected from PLEON lakes since 2017. PTOX screens were conducted by Greenwater Laboratories.

that these lakes were among the lakes sampled the least frequently.

Dolichospermum, *Aphanizomenon*, and *Chrysosporum* species have been found in PTOX samples from TWCWC lakes. *Woronichinia*, *Planktothrix*, and *Oscillatoria* species have been found in phytoplankton community analysis of Big Twin and Little Twin (Appendix I), along with species of *Pseudanabaena*, *Spirulina*, *Limnothrix,* and *Planktolyngbya*, genera not found in the PLEON PTOX database to date.

Based on the results of the PTOX screens, Greenwater Laboratories has recommended quantifying microcystin/nodularin concentration in 34% of the samples and quantifying cylindrospermopsin, anatoxin-a, and/or saxitoxin concentration in 24% of the samples. Cyanotoxin quantification is an opt-in service; to date, between 62% and 77% of the recommended analyses have been conducted, depending on the toxin.

Microcystin/nodularins, cylindrospermopsin, and saxitoxin have been detected in PLEON lakes (Table 9). Microcystin/nodularins are hepatotoxins, cylindrospermopsin is a hepatotoxin and a nephrotoxin, and saxitoxin is a potent neurotoxin[5]. The US Environmental Protection Agency recommends microcystin and cylindrospermopsin magnitude thresholds of 8 µg/L (or ng/mL) and 15 µg/L in recreational waters[6]. The Lake Erie Harmful Algal Bloom Monitoring and Response Strategy recommends a Recreational Use Advisory when saxitoxin concentration is 0.8 µg/L or above[7]. Commonwealth of Pennsylvania does not have recommended thresholds at this time.

To date, cyanotoxins have not been detected in TWCWC lakes. Note that TWCWC declined the cyanotoxin testing recommended by Greenwater Laboratories in 2021.

Table 9: Samples tested for cyanotoxins from PLEON lakes since 2017. Cyanotoxin analyses were conducted by Greenwater Laboratories.

Toxin	**# recommended for testing**	**# tested**	**# ≥ MDL***	**Mean concentration (ng/mL)**	**Range (ng/mL)**
microcystins/nodularins	70	54	20	9.4	0.16-129
cylindrospermopsin	50	31	1	0.07	-
anatoxin-a	49	33	0	-	-
saxitoxin	50	34	3	0.33	0.15-0.45
homoanatoxin-a	1	1	0	-	-

**MDL = minimum detection limit*

X. What it all Means: Emerging Concerns for Twin and Walker Lakes

Several findings from the Twin and Walker lakes 2022 monitoring program should be highlighted:

1. There was a late summer algal bloom in both Twin Lakes in 2022.

Chlorophyll a concentration in the epilimnion of Big Twin increased by more than 3x from July to August. While there was more chlorophyll in Big Twin compared to Little

Twin during the August sampling, the magnitude of change from July to August in Little Twin was much larger; there was over 60x more chlorophyll in Little Twin from July to August. There was also a higher algal count in both Twin lakes in 2022 compared to recent years.

There was also a marked decrease in the clarity of both Big Twin and Little Twin from July to August in 2022. Secchi depth declined by nearly 1 m in Big Twin and more than 1 m in Little Twin over that time. The fact that this decline coincided with a substantial increase in algal abundance in both lakes suggests that algae impacted water clarity.

Nutrients, particularly phosphorus, can fuel algal production. Indeed, total phosphorus concentration in both Twin lakes increased from July to August, coinciding with the increase in algal abundance. This increase occurred in the epilimnion (1.5x increase in Big Twin and >4x increase in Little Twin) and in the composite samples (3x increase in Big Twin and >2x increase in Little Twin). These data suggest that phosphorus availability may have contributed to the late summer algal blooms in these lakes.

Phosphorus can enter lakes from several sources, including surface and subsurface runoff. Septic system leakage and near-shore fertilization can increase phosphorus runoff into lakes. Another common source of phosphorus is regeneration from the sediments when oxygen concentrations are low (below 2 mg/L). The hypolimnion of both Twin lakes are commonly anoxic during the summer months, so regeneration is likely occurring. The importance of regeneration to the phosphorus budget of the Big Twin and Little Twin is not known.

2. Long-term trends in TWCWC lakes do not show strong correlations between algal abundance and water clarity or between algal abundance and phosphorus availability.

The coincident changes in water clarity, algal abundance, and phosphorus availability observed from July to August 2022 in the Twin lakes described above seems to suggest that these variables are affecting each other (increases in phosphorus fuel algal abundance which decreases water clarity). However, when these variables are averaged over the summer and compared over the several-year dataset, the connections appear to weaken (Figure 23). Average summer Secchi depth was not significantly correlated with average summer chlorophyll a concentration in any of the TWCWC lakes. Similarly, chlorophyll a concentration was not significantly correlated with total phosphorus concentration in TWCWC lakes over time.

There are several explanations for this. First, phosphorus could be an important but not sole driver of algal abundance. Other factors such as nitrogen availability, temperature, and light (particularly in Walker) could also be playing a role, weakening the correlation over time. The importance of phosphorus in algal abundance regulation may be stronger during certain points of the summer and this relationship is weakened when several timepoints are averaged together. Similarly, algal abundance may be an important but not sole influence on water clarity.

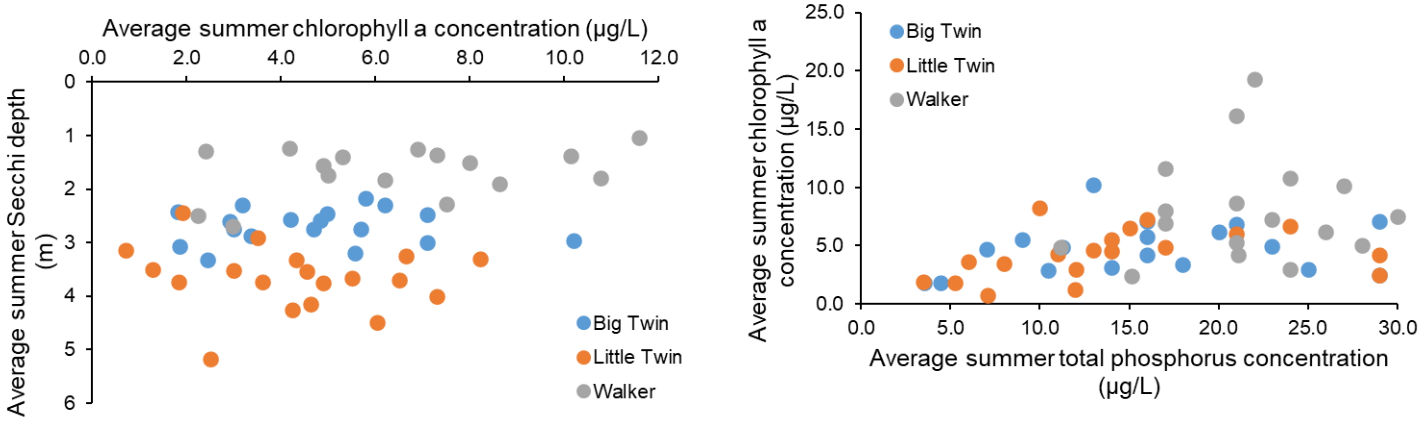


Figure 23: Relationship between average summer chlorophyll concentration and Secchi depth (left) and between average summer total phosphorus concentration and chlorophyll concentration (right) in TWCWC lakes from 2003-2022. Pearson correlations were not statistically significant (p>0.16).

3. There is a long-term decline in water clarity of Walker Lake.

There is a different story in Walker Lake. Water clarity (as determined by average summer Secchi depth) has been declining since 2003. As described above, there does not appear to be a tight relationship between algal abundance and water clarity in Walker Lake. Other factors that can influence water clarity include suspended and dissolved compounds. There are not enough dissolved organic carbon data to make any conclusions as of now.

4. The potential for cyanobacteria blooms remains a concern in all three lakes.

Periodic screening for potentially toxigenic (PTOX) cyanobacteria has detected several PTOX cyanobacteria genera in all three TWCWC lakes, including *Dolichospermum*, *Aphanizonmenon*, and *Chrysosporum*. PTOX cyanobacteria counts were high enough to prompt toxin analysis according to the PA DEP HABs Task Force protocols in August of 2020 in all 3 lakes. It is important to note that no bloom while there was noticeable algae on the surface of Big Twin, *no visible bloom was present in Little Twin or Walker when these samples were taken*. PTOX cyanobacteria counts from a sample collected from Big Twin in June of 2021 also prompted Greenwater Laboratories to recommend cyanotoxin testing.

Phytoplankton community analysis since 2019 in Big Twin and Little Twin also show the potential for cyanobacteria blooms in these lakes. While variable over time, several potentially toxigenic genera have been found in these lakes.

It is important to note that algae results presented in this report pertain only to the sampling date and time. Algal communities are very dynamic and their abundance can change quickly, sometimes in a matter of hours. More information about harmful algae blooms (HABs), tips for identification, and other resources can be found on the PLEON HABs webpage. TWCWC may want to consider a HABs monitoring and response plan in 2022.

Report of 2022 PLEON Sampling: Twin and Walker Lakes

APPENDICES

APPENDIX I: Description of Field Sampling Methods

A. Physical Profiles

Temperature, dissolved oxygen, conductivity, and pH were measured using a handheld YSI Professional Plus multiparameter instrument fitted with a polarographic dissolved oxygen probe and a pro series pH probe. Probes were calibrated in early June 2022 and periodically through the summer. Probes were lowered through the water column starting at the surface (probes just under water, “0 m”). Readings were recorded in the field every 0.5-1 m.

Secchi depth was taken from the shady side of the boat using a Secchi Disk standard to freshwater sampling.

Light profiles were taken by lowering the sensor through the water column suspended off the side of the boat to avoid boat-shadow using a LiCOR spherical quantum sensor (model LI-193).

B. Chlorophyll

Chlorophyll a pigment was extracted from phytoplankton using the method developed by Robert Moeller and currently used by the Williamson Lab. Water samples were collected from the epilimnion, metalimnion (when appropriate), and hypolimnion (determined by temperature profile collected on the same day) using a Van Dorn bottle. Two replicate samples were collected from each depth. Samples were kept cold until filtered. For each replicate, a known volume was filtered onto a glass fiber filter with nominal pore size of 0.7 µm using a vacuum pump. Filters were frozen until extraction. Pigments were extracted from filters with 10 ml of a 5:1 acetone:methanol solution. The extraction took place over 48 hours at -20°C with a 2-minute heating step (60°C) after 24 hours. Chlorophyll concentration of the extractant was determined via fluorometry (Turner Designs 10AU fluorometer) and corrected for phaeophyton according to EPA method 445.0.

C. Nutrients

Two replicate water samples were collected using a Van Dorn horizontal water sampler from the epilimnion, metalimnion (if applicable), and hypolimnion. Water samples were collected in acid washed bottles and kept cold until return to the lab. A 60 ml subsample of each replicate was frozen at -20°C until analysis for total nitrogen (TN) and total phosphorus (TP) concentration.

Total nutrient samples were digested using an alkaline persulfate oxidizing reagent and heating at 80°C for 16-24 hours. This process simultaneously converts ammonium, inorganic nitrogen (excluding N_2), and organic nitrogen to nitrate (NO_3^-) and inorganic and organic phosphorus to orthophosphate (PO_4^{-3}).

NO_3-N concentration of the digested samples was quantified via cadmium reduction using a discrete autoanalyzer (AQ300, SEAL Analytical) at Lafayette College.

PO_4-P concentration of the digested samples was quantified via the ascorbic acid colorimetric method using a discrete autoanalyzer (AQ300, SEAL Analytical) at Lafayette College.

D. Dissolved organic carbon (DOC)

40-ml subsamples of water samples were filtered through ashed GF/F filters (Whatman, 0.7 um pore size). Subsamples were stored in ashed, amber glass vials and kept cold until analysis for DOC at the Global Change Limnology Laboratory at Miami University of Ohio.

E. PTOX screening and cyanotoxin analysis

PLEON sends PTOX samples to GreenWater Laboratories for PTOX screening. Samples are kept cold in the field and sent to GreenWater Laboratories within 30 hours. GreenWater Labs provides the following description of the screening process:

“A one mL aliquot of each sample was prepared using a Sedgewick Rafter cell. The samples were scanned at 100X for the presence of potentially toxigenic (PTOX) cyanobacteria using a Nikon Eclipse TE200 inverted microscope equipped with phase contrast optics. Higher magnification was used as necessary for identification and micrographs.”

Cyanotoxins were analyzed by Greenwater Laboratories using Enzyme-Linked Immunosorbent Assay (ELISA; microcystin-nodularins and saxitoxins) or Liquid chromatography mass spectrometry/mass spectrometry (LC-MS/MS; anatoxins and cylindrospermopsin) according to laboratory-specific protocols.

F. Zooplankton and phytoplankton community analysis

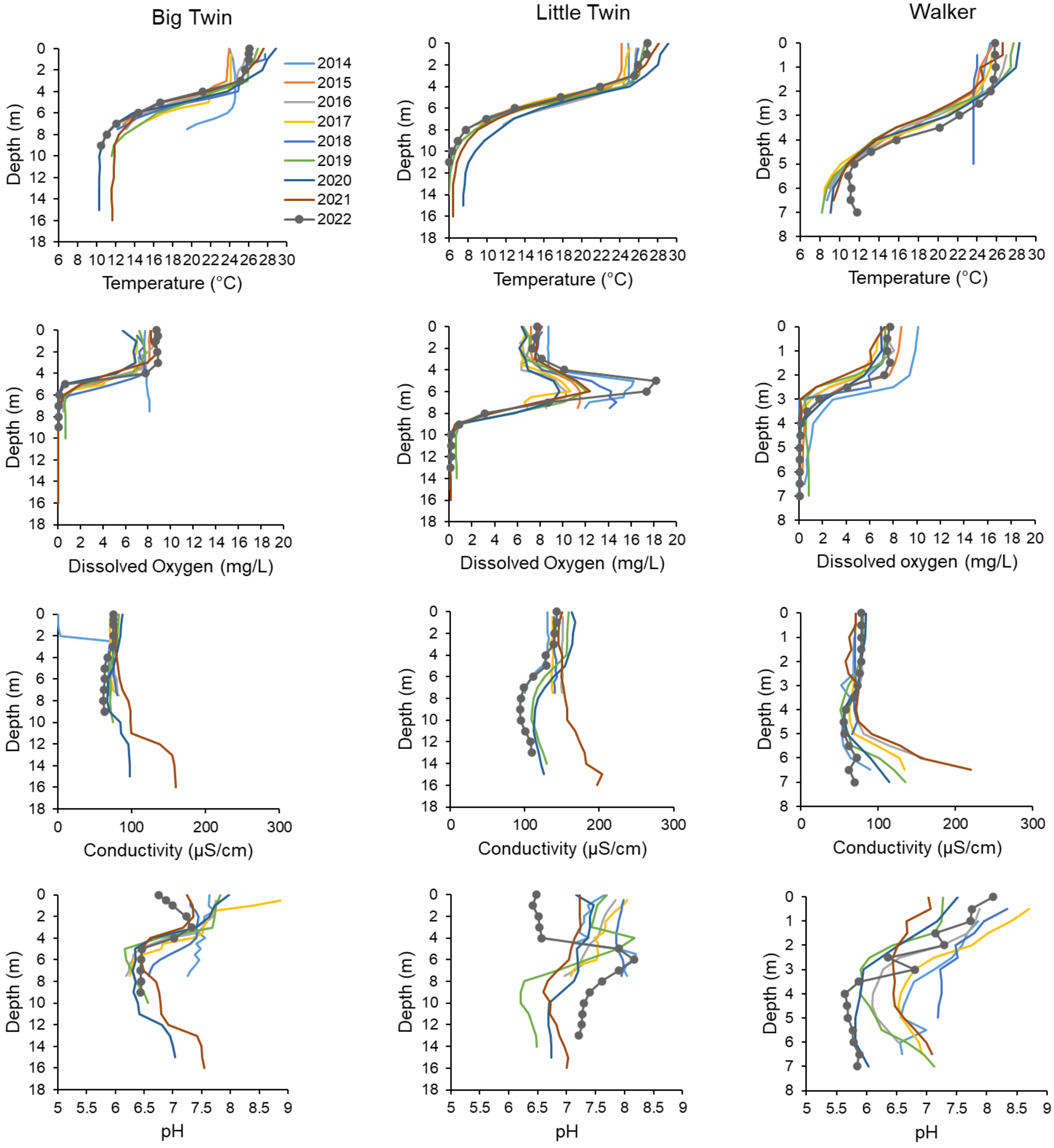
Phytoplankton communities were sampled by collecting water from 5 depths through the epilimnion and metalimnion using a Van Dorn bottle. Water from all depths was composited and gently homogenized. Two 250-ml samples were screened through a 153-µm mesh to remove large debris. Samples were preserved with Lugols iodine.

Zooplankton samples were collected using a Wisconsin-style tow net with a 0.2 m diameter and 48 µm mesh. Vertical tows were collected from mid to lower metalimnion through the surface with one tow per sample. Two replicate samples were collected and preserved with Lugols Iodine.

Zooplankton and phytoplankton enumeration was done by Ken Wagner of Water Resources. Briefly, samples are concentrated (typically to a factor of 10 to 30) and counted using a Palmer-Maloney style counting chamber viewed at 40X for zooplankton and at 400X for phytoplankton.

Appendix II: July profiles

July profiles from 2014-2022 in TWCWC lakes. Note differences in Y axes among lakes.



Appendix III: Literature Cited

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7. Lake Erie harmful algal bloom monitoring response strategy. July 2017. https://seagrant.psu.edu/sites/default/files/PA%20Lake%20Erie%20HAB%20Response%20Strategy%207-24-2017_0.pdf

Appendix IV: Glossary

Anatoxin-a: A neurotoxin produced by some cyanobacteria, including members of the genera *Microcystis*, *Aphanizomenon*, *Planktothrix*, and *Cylindrospermum*. Considered dangerous for humans and pets.

Carlson’s trophic state index: An index designed by R. E. Carlson in 1977 that ranks lakes on a scale of 0-100. The index is based on algal biomass and can be calculated using Secchi depth, chlorophyll concentration, or phosphorus concentration.

Conductivity: the ability of a solution to conduct electricity (also called specific conductance). Dissolved materials increase the conductivity of water so this variable can indicate the amount of dissolved solids. Sea water, for example, has a conductivity of 50,000 µS/cm.

Cyanobacteria: a group of photosynthetic bacteria commonly found in freshwater phytoplankton communities. Some taxa are capable of fixing nitrogen from the atmosphere. Some taxa produce secondary metabolites that are toxic to humans.

Cylindrospermopsin: a liver and kidney toxin produced by some cyanobacteria.

Dissolved oxygen: The amount of oxygen gas dissolved in water. This variable is important because oxygen is required for respiration by lake organisms. Dissolved oxygen enters water via diffusion at the water surface and through the process of photosynthesis, of which oxygen is a waste product.

Epilimnion: The surface layer of a thermally stratified lake. The epilimnion is mixed by waves and wind; therefore the temperature is fairly uniform throughout this layer. The lower boundary of the epilimnion is determined by a rapid change in temperature. This layer is typically more oxygenated than the lower layers.

Eutrophic: trophic state describing productive lakes. Eutrophic lakes are typically high in nutrients with low transparency.

Hypereutrophic: trophic state describing highly productive lakes. Hypereutrophic lakes have extreme levels of excess nutrients and have very low transparency.

Hypolimnion: the deep waters of a thermally stratified lake. The hypolimnion consists of cold water that does not mix with the warmer epilimnion. This layer can be depleted in oxygen due to the absence of photosynthesis.

Mesotrophic: trophic state describing lakes with intermediate productivity. Mesotrophic lakes have intermediate levels of nutrients and intermediate transparency.

Metalimnion: the middle layer of a thermally stratified lake defined by the rapid change in temperature with depth. This is the transition layer between the epilimnion and hypolimnion.

Metalimnetic Oxygen Maximum: elevated dissolved oxygen concentration that can develop in the metalimnion, often due to a concentration of phytoplankton that are producing oxygen through photosynthesis.

Microcystin: a group of toxins produced by some cyanobacteria genera including *Microcystis* and *Planktothrix.* Microcystins are liver toxins that can be harmful to humans and pets.

Oligotrophic: trophic state describing lakes with low productivity. Oligotrophic lakes are nutrient poor and have high transparency.

pH: a measure of hydrogen ions on a logarithmic scale from 0-14. Values above 7 are considered basic and values below 7 are considered acidic. Lake organisms have specific pH tolerances.

Photosynthetically Active Radiation (PAR): wavelengths of light that are used in the process of photosynthesis. Range from 400-700 nm.

Potentially Toxic (PTOX) Cyanobacteria: cyanobacteria groups that are known to have the capability to produce toxins that are harmful to humans and pets.

Richness: Richness refers to the number of different types or taxa of organisms within a group that are found in a given area. For example, there may be 5 different types of fish in a lake. Richness is often used as a measure of biological diversity.

Saxitoxin: a neurotoxin produced by some cyanobacteria genera including *Aphanizomenon* and *Planktothrix.* Exposure can be harmful to humans and pets.

Secchi depth: a standardized value of water transparency measured using a flat disk with black and white quadrants called a Secchi disk. Secchi depth is positively correlated with transparency.

Shannon-Wiener Index: an index of biological diversity that takes into account both the number of taxa as well as their relative abundance. The index ranges from 0 (least diverse or a diversity of one) to one.

Vertical Extinction Coefficient (*k*): The rate at which light attenuates with depth. Different wavelengths of light have different coefficients. Dependent on dissolved and particulate matter in lake water that may reflect or absorb light.

Appendix V. Greenwater Laboratory Reports

Included as separate files:

Lacawac Sanctuary PTOX Cyanobacteria Screen 220526 (Big Twin)